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H. Chemical Biology and Drug Discovery [H-1]

Role of ER-mitochondria contacts in autophagy and atherosclerosis

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ER and mitochondria interact with each other to form a contact site, called MAM (Mitochondria Associated ER Membranes) and it is involved in many physiological activities. Increased MAM formation promotes calcium transport to mitochondria resulted in several diseases. Here, we found that CTS, an active principle of Danshen(*Salvia miltiorrhiza*), alleviates atherosclerosis through autophagy induction in *apoE*$^{-/-}$ mouse. To explore the mode of actions of CTS, DARTS-LC-MS/MS analysis was performed to identify the target protein of CTS. As a result, proteins belonging to the GRP family which is known to play an important role in MAM formation, were identified. Among them, CTS-BP showed high resistance to proteolysis through conformational change by direct binding to CTS. Proximity ligation assays, TEM imaging and mitochondrial calcium influx demonstrate that CTS disrupts the interaction between ER and mitochondria. Further biological validation of binding of CTS to CTS-BP was conducted with knockdown of the target gene resulting in autophagy induction. Collectively, this study provides new insights into the possible role of MAM complex in autophagy regulation and small molecules targeting MAM complex could be new therapeutic agents for anti-atherosclerosis via autophagy induction.

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H. Chemical Biology and Drug Discovery [H-2]

Target identification and validation of a natural disaccharide and its therapeutic effect in Nonalcoholic steatohepatitis

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Autophagy is a main process for modulating cellular homeostasis and a natural disaccharide is known for autophagy inducer. Especially, clearance of excessive lipid accumulation through autophagy, also known as lipophagy, has been implicated as a potential strategy for regulating lipid metabolism disorders such as NASH (Nonalcoholic steatohepatitis). However, therapeutic effect of a natural disaccharide (TRE) in NASH and its molecular mechanism remains to be uncovered. Here, to address the mode of actions of TRE, we identified the target protein of TRE through a label free target identification method called DARTS with LC-MS/MS analysis. The binding protein of Tre (TBP) was validated through CETSA and *in silico* docking analysis. Moreover, we found that interaction of TRE and TBP regulates the metabolite transportation and AMPK signaling pathway. Autophagy activation through AMPK alleviates lipid accumulation and adipocyte differentiation in 3T3-L1. Notably, lipid degradation via autophagy was also observed in mouse model of NASH. In conclusion, these results demonstrate a possible role of TBP in the activity of TRE to inhibit the adipogenic process by enhancing autophagy and reducing metabolite transport.

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H. Chemical Biology and Drug Discovery [H-3]

Label-free compound based chemical proteomics reveals YCGEP as the functional target of an anti-cancer agent in human hepatoma cells

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Many studies have been attempted to develop new agents that target EGFR mutant or regulate downstream players in various carcinomas. A new small molecule, YCGE, was discovered through cell-based screening to inhibit cancer cells bearing EGFR mutation. Previous studies have shown that YCGE effectively inhibits anchorage-independent 3D growth of sphere-forming cells transfected with EGFR mutant cDNA. However, the underlying mechanism remains to be uncovered. In this study, we investigated the target protein of YCGE by combination of DARTS with LC-MS/MS using label-free YCGE as a bait and HepG2 cell lysates as proteome pool. As the result, YCGEP was identified as one of binding proteins of YCGE that is responsible for biological activity of the compound. The interaction between YCGEP and YCGE was validated through DARTS and CETSA methods. Additionally, genetic knock-down of YCGEP was validated in regard with its link to cell proliferation. Collectively, YCGEP is identified as a biologically relevant target of YCGE to address an anti-cancer activity of the compound and these results provide insights into a role of YCGE as a downstream player of EGFR mutant.

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H. Chemical Biology and Drug Discovery [H-4]

A natural autophagy inducing compound ameliorates non-alcoholic fatty liver disease (NAFLD)

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Autophagy has been highlighted as a critical regulator of cellular homeostasis, dysregulation of which is associated with diverse diseases. Particularly, the autophagy of cytoplasmic lipid droplets is known as lipophagy. A link between non-alcoholic fatty liver disease(NAFLD) and lipophagy related mechanism remains to be addressed. To explore the role of autophagy in lipid regulation, we identified a natural compound (ACA) as a novel anti-NAFLD agent. Notably, ACA suppressed lipid accumulation and attenuated the expression of adipogenesis related factors without showing cell toxicity. Moreover, ACA activates autophagic degradation *in vitro* and exhibits anti-NAFLD effect *in vivo*. To investigate the mode of action for ACA, we applied a label free small molecule target identification method called DARTS with LC-MS/MS analysis. Target protein of ACA (TPA) was identified and validated using DARTS and CETSA methods. Collectively, these results demonstrated that ACA exhibits anti-NASH activities via binding to TPA resulted in dissociation of mTORC1 complex for the inhibition of lipidosis and amelioration of steatosis.

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H. Chemical Biology and Drug Discovery [H-5]

Exploring autophagy and mitochondria associated ER membranes in UQCRB-overexpressing cells

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Ubiquinol-cytochrome c reductase binding protein (UQCRB), subunits of mitochondrial Complex III, has been implicated as a crucial regulator in hypoxia-induced angiogenesis through mitochondria-derived reactive oxygen species (mROS). Recently, we reported that UQCRB overexpression activates autophagy via regulating mROS level. Notably, increasing level of mROS caused by UQCRB overexpression can release Ca2+ by activation of lysosomal TRPML1 channels. This activation triggers TFEB nuclear translocation and lysosome biogenesis leading to autophagy flux. Treating UQCRB inhibitor A1938 inhibited autophagy flux and triggered cell death. Recently, ER and Mitochondria interacted and formed contact site MAM (Mitochondria Associated ER Membranes), which is responsible for primary region of Ca2+ transfer and autophagy. Here, we investigated that whether UQCRB overexpression affects MAM formation. To analyze the distance between ER and mitochondria, we performed the TEM imaging and proximity ligation assay. As the result, MAM formation is increased in UQCRB overexpression cell line than normal HEK293. Further studies will focus on the link between mROS, autophagy and MAM, provide new insights into the role of mROS in MAM complex and autophagy.

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H. Chemical Biology and Drug Discovery [H-6]

Proteome profiling through CETSA and LC-MS/MS reveals the target proteins of small molecules

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Target identification of small molecule is important step to understand the underlying mechanism of biological activity of the compound and to explore the function of target proteins in biological system. Here, we established a new strategy to analyze target proteins that directly bind to small molecules based on label-free target identification methods. We used 6 different small molecules from FDA approved drugs and natural products. To investigate the target proteins, CETSA-LC-MS/MS analysis was conducted. Cellular Thermal Shift Assay (CETSA) is a label free small molecule-based target identification method by monitoring thermal degradability change resulting from binding of protein and small molecule. Using Tandem Mass Tag (TMT) labeling based MS analysis, protein pools obtained from CETSA have been quantitated. We analyzed the characteristics of target candidates using STRING database. As the result, two FDA approved drugs for hypertension demonstrated direct drug binding to known targets and provided new target protein candidates related to other pathologies. In addition, CESTA profiling of other 4 compounds having unknown target protein provided multiple binding proteins candidates. Collectively, this CETSA-LC-MS/MS analysis of small molecules and their targetome profiling provided new target proteins of small molecules and demonstrate great potential to explore underlying mechanism of small molecules.

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H. Chemical Biology and Drug Discovery [H-7]

Target identification of a natural cholesterol regulating compound

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Lipids are water-insoluble biological macromolecules that are essential for maintaining cellular structure, function, signaling and energy storage. In particular, cholesterol plays an important role in the formation of biological membranes. Disruption of cholesterol homeostasis leads to the development of cardiovascular disease (CVD), and neurodegenerative diseases, etc. Using the 658 natural product library, we screened molecules that efficiently suppressed cholesterol levels by filipin staining and cholesterol concentration assay and then identified a hit compound, Cory. Cory is a unique bioactive compound extracted from *Corydalis bungeana Turczc*, whose mechanism remains unclear. In this study, we confirmed that Cory significantly reduced cholesterol in 3T3-L1 adipocytes by filipin staining. To investigate the mode of action of Cory, we performed CETSA-LC-MS/MS to reveal the target protein of Cory. We obtained candidate target proteins that are stable to thermal degradation upon ligand binding. Further studies will focus on investigating the biological activity of the target protein in 3T3-L1 cells. Overall, our study will demonstrate the potential of Cory as a new cholesterol inhibitor and elucidate its mechanism of action.

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H. Chemical Biology and Drug Discovery [H-8]

Perturbation of intracellular organelle networks using small molecules

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To maintain cellular homeostasis, cellular organelles continuously communicate with other organelles. These communications are observed in diverse organelles, including the ER(endoplasmic reticulum), mitochondria, lysosomes, Golgi apparatus, peroxisomes, and more. Since abnormal organelle interactions can cause various diseases such as metabolic syndrome, cancer, and neurodegenerative disorders, the demand to regulate these interactions is increasing. Genetic regulation has been mainly used to unveil organelle crosstalk. Recent advances in applying chemical biology to explore biological events, however, have enabled to regulate organelle interactions using small molecules. Here, we investigated the effect of small molecules on the ER-mitochondria interactions using a proximity ligation assay. As the result, we found that autophagy-inducing compounds decrease the distance between the ER and mitochondria. Moreover, these small molecules induce contact changes in mitochondria-peroxisomes, mitochondria-lysosomes, ER-lysosomes, and ER-Golgi apparatus. Collectively, we found that small molecules inducing autophagy affect organelle interactions for their biological activities. In addition, our results suggest that when the interaction of a specific organelle is affected by a small molecule, the interaction of others could be affected in a cascade manner resulting in systemic changes in organelle communication.

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H. Chemical Biology and Drug Discovery [H-9]

Compound Y protects the kidney from senescence and fibrosis by inhibiting AT1R

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Regardless of extensive efforts in therapeutic development, only few effective treatments are able to treat renal fibrosis. Here, we found that compound Y has protective effects on the senescence and fibrosis induced by kidney injury and aging. Using cisplatin- and adenine-induced kidney injury models, we showed that Compound Y highly attenuated kidney injury, senescence, and fibrosis *in vivo*. In addition, the protective effects of Compound Y were confirmed in naturally aged mice (23 to 24 months old). To elucidate the mechanism of action, we perform in silico molecular docking of Compound Y, and found that it binds directly to AT1R, by doing so blocking the binding of Ang II. Compound Y subsequently inhibits Gαi2 signaling, hence downregulates ROS production and DNA damage. Further, the level of senescence -associated secretory phenotype (SASP) factors were partially rescued by Compund Y, resulting in lower levels of senescence . In conclusion, our findings reveal that Compound Y provides a significant protection against kidney injury and aging-related fibrosis and may be a novel pharmacological therapeutics candidate for the disease.

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H. Chemical Biology and Drug Discovery [H-10]

Synthesis and biological evaluation of cilengitide derivatives on TGF-β1-induced epithelial-to-mesenchymal transition in human non-small cell lung cancer cells

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Epithelial-to-mesenchymal transition (EMT) is an important process leading to inva1siveness of cancer cells and poor prognosis in non-small cell lung cancer (NSCLC) progression. Cilengitide (cyclo[RGDf(NMe)V]), a cyclic RGD pentapeptide, has been shown to enhance the inhibitory effect of epidermal growth factor receptor (EGFR) inhibitors on TGF-β1-induced mesenchymal marker expression and invasion by NSCLC A549 cells. In this study, we synthesized cilengitide and derivatives and eval1uated their biological effects on TGF-β1-induced EMT phenotype marker expression and invasion in human NSCLC cells. Among the synthesized derivatives, R-1 (cRGDwV) and R-7 (cRGDyV) were found to be the most effective in inhibiting the growth of NSCLC cells. These cilengitide derivatives showed an inhibitory effect on the TGF-β1-induced EMT process and invasion through inhibition of Smad or non1Smad signaling pathways in NSCLC A549 cells. Through this study, we demonstrated that cilengitide derivatives containing the RGD sequence and hydrophobic amino acids, such as cilengitide, exhibit inhibitory effects on NSCLC cell growth and EMT inhibition. In addition, the potential of these peptides as a drug that can be used to inhibit metastasis of various cancers accompanying the EMT process was suggested.

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H. Chemical Biology and Drug Discovery [H-11]

Anticancer potential of PRMT1 inhibitor furamidine against glioblastoma stem cells

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Protein arginine methyltransferases (PRMTs) regulate various cellular functions such as DNA repair, gene expression, and signal transduction. Aberrant expression of PRMTs has been implicated in cancer development and other pathological processes. However, it is still unclear whether PRMTs play a role in the maintenance of glioblastoma stem cells (GSCs), a major cause of poor prognosis in glioblastoma treatment. To investigate the involvement of PRMTs in GSC growth, we evaluated the anticancer activity of the PRMT1 inhibitor furamidine on GSCs. Furamidine effectively suppressed the proliferation and tumorsphere formation by inducing cell cycle arrest and apoptosis in U87MG-derived GSCs. The inhibitory effect of furamidine on GSC growth was associated with the downregulation of key GSC markers, including CD133, Integrin a6, ALDH1A1, Sox2, Oct4, and Nanog. In addition, combined treatment of furamidine and the calcium/calmodulin-dependent protein kinase II (CaMKII) inhibitor berbamine more potently inhibited GSC growth compared to single-compound treatments. Our results therefore suggest that targeting PRMT1 may be a promising strategy to combat GSCs.

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H. Chemical Biology and Drug Discovery [H-12]

Cyclophilin A inhibitors suppress gastric cancer stem cells by downregulating CypA/CD147 axis

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Gastric cancer stem cells (GCSCs) are a highly self-renewing and multilineage differentiating subset of gastric cancer cells that drive tumor initiation, metastasis, drug resistance, and recurrence. Therefore, targeting GCSCs could be an effective treatment strategy for advanced or metastatic gastric cancer. In this study, we investigated the effects of natural cyclophilin A (CypA) inhibitors, including 23-demethyl 8,13-deoxynargenicin (C9) and cyclosporin A (CsA), on the growth of MKN45-derived GCSCs. C9 and CsA effectively suppressed cell proliferation by inducing cell cycle arrest at the G0/G1 phase and promoted apoptosis by activating the caspase cascade in MKN45 GCSCs. In addition, C9 and CsA potently inhibited tumor growth in the MKN45 GCSC-transplanted chick embryo chorioallantoic membrane model. Furthermore, the two compounds significantly downregulated key GCSC biomarkers and CypA/CD147-mediated AKT and MAPK signaling pathways. Notably, silencing of CypA demonstrated that CypA/CD147 axis plays a critical role in the growth and metastasis of GCSCs. Collectively, our findings suggest that the natural CypA inhibitors C9 and CsA could be novel anticancer agents used to combat GCSCs by targeting the CypA/CD147 axis.

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H. Chemical Biology and Drug Discovery [H-13]

Bufotalin suppresses proliferation and metastasis of triple-negative breast cancer cells by downregulating EGFR pathway

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Triple-negative breast cancer (TNBC) is a highly aggressive form compared to other breast cancer subtypes and has a poor prognosis. TNBC lacks hormone receptors (estrogen, progesterone) and expresses low levels of HER2, making TNBC unresponsive to hormonal or targeted therapies. Therefore, there is a need to develop effective therapies for patients with TNBC. Bufotalin is a bufadienolide isolated from the skin and parotid venom glands of the toad *Bufo gargarizan* and has numerous pharmacological properties, including antimicrobial, antiparasitic, and antitumor activities. This study is the first to demonstrate that bufotalin inhibits the proliferation and metastasis of MDA-MB-231 and HCC1937 TNBC cells. Bufotalin potently inhibited the proliferation of TNBC cells by promoting cell cycle arrest and apoptosis. Furthermore, bufotalin effectively suppressed the migration and invasion of TNBC cells by regulating the expression of key epithelial-mesenchymal transition biomarkers, matrix metalloproteinases, and integrin α6. Notably, the anticancer effect of bufotalin on TNBC cells was associated with the downregulation of epidermal growth factor receptor (EGFR)-mediated downstream signaling pathway. Collectively, our results suggest that the natural compound bufotalin may have the antiproliferative and antimetastatic activities against TNBC cells through inhibition of EGFR pathway.

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H. Chemical Biology and Drug Discovery [H-14]

Anticancer activity of ethyl acetate extract of Hovenia dulcis Thunb. in Huh7-derived liver cancer stem cells

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Liver cancer stem cells (LCSCs) contribute to the initiation, metastasis, therapy resistance, and recurrence of hepatocellular carcinoma (HCC). Accordingly, the exploration of potential anticancer agents targeting LCSCs can provide a new therapeutic strategy to overcome HCC treatment failure. Natural products are a rich source of anticancer drugs. *Hovenia dulcis* Thunb. (HDT), a hardy tree found in Asia, possesses various biological activities, including antifatigue, antidiabetic, neuroprotective, hepatoprotective, and antitumor. However, the therapeutic effect of HDT to eliminate LCSCs has not yet been evaluated. Herein, we investigated the anticancer effect and underlying molecular mechanism of ethyl acetate extract of HDT (EAHDT) against LCSCs. Our results demonstrated that EAHDT effectively inhibited the proliferation and tumorsphere formation of Huh7-derived LCSCs. EAHDT-induced cell cycle arrest in the G0/G1 phase, apoptosis, and necroptosis in Huh7 LCSCs. Furthermore, EAHDT downregulated key cancer stemness-related biomarkers and c-Met signaling pathways in Huh7 LCSCs. In conclusion, this study suggests that EAHDT may be a new natural product drug candidate for the treatment of HCC by targeting LCSCs.

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H. Chemical Biology and Drug Discovery [H-15]

The Real-time Stability Evaluation of National Biological Reference Standards for Viral and Bacterial Vaccines in Korea

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The National Institute of Food and Drug Safety Evaluation (NIFDS) has been developing national reference standards (NRS) for biologics on the basis of guidelines of WHO. The stability tests are performed to evaluate and maintain the stability of NRSs. In the present study, the stability test of 3 NRSs were evaluated. Also, the regression analysis was performed to identify the trends. An estimated mean potency of stability tests, lower and upper control limit for each NRS were as follows; Bordetella Pertussis Vaccine (for potency test) 120 units/vial (56-224), Bordetella Pertussis Vaccine (for detoxification test) 3.27 HSU/vial (3.20-6.40), Live Varicella Vaccine (3rd) 4.69 log10PFU/0.5mL (4.17-5.27). The results showed all of the mean potencies were distributed within the control limit, while the regression analysis showed stable trends. In conclusion, the present study demonstrated the values of NRSs were maintained stable. The NIFDS's advanced system for NRSs is expected to contribute to supply high-quality biological products in Korea. This research was supported by a grant (21171MFDS184) from Ministry of Food and Drug Safety in 2022.

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H. Chemical Biology and Drug Discovery [H-16]

Immunogenicity Risk Assessment of Antiviral Drug Candidate in Rat and Rabbit Models

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Over the past few years, there has been active research into biopharmaceuticals such as protein drugs, antibody therapeutics, and cell-based therapies, which have demonstrated remarkable therapeutic efficacy in clinical outcomes. However, these drugs have a highly immunogenic nature, and although processes such as humanization have been implemented to prevent immunogenicity upon administration, the issue of immunogenicity remains unresolved and is still a challenge to be addressed. Immunogenicity can be alter the pharmacokinetics and pharmacodynamics properties of drugs in unintended ways, reducing their efficacy and potentially causing serious side effects. Therefore, it is crucial to evaluate the impact of immunogenicity in the drug development process. In this study, a validated bridging Enzyme-Linked Immunosorbent Assay (ELISA) method was used to evaluate the anti-drug antibodies (ADA) response following intravenous administration of an antibody-based antiviral drug candidate in pregnant rats and rabbits. The results showed that no ADA was observed in any of the samples. Our findings will contribute to a better understanding of the immunogenicity of biopharmaceuticals and provide insights for further drug development.

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H. Chemical Biology and Drug Discovery [H-17]

Anti-cancer AUTOTAC induced targeted degradation of mutant p53 aggregates via the autophagy-lysosome system

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The tumor suppressor p53 has various functions including senescence, cell cycle arrest, DNA repair, and apoptosis in oncogenic stress. Known as a guardian of the genome, p53 is the most frequently mutated protein in pan-human cancers, especially in the late stage. However, mutant p53 ($p53^{MUT}$) leads to catastrophic effects. The prion-like property of $p53^{MUT}$ induces itself to aggregate, leading to the loss of normal functions while gaining oncogenic functions. Evaluated chemicals targeting $p53^{MUT}$ are mostly irreversible and require high dose intake for functional modulation of $p53^{MUT}$, which may cause increased toxicities. Here we suggest a novel therapeutic approach for the $p53^{MUT}$ aggregates targeted degradation. A chimeric compound AUTOTAC consists of a target-binding ligand that selectively binds to $p53^{MUT}$ aggregates and autophagy-targeting ligands that recognize p62, an adaptor protein for selective autophagy. Our results showed that the oncogenic function of $p53^{MUT}$ is reduced by selectively targeting and degrading aggregates using AUTOTAC. AUTOTAC induced in vitro and in vivo degradation and dissociation of the $p53^{MUT}$ aggregates and reduced size of $p53^{MUT}$ pancreatic cancer cell-originated tumors. These results insist new vision for therapeutic anti-cancer treatment via selective targeting of $p53^{MUT}$ aggregates.

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H. Chemical Biology and Drug Discovery [H-18]

Targeted degradation of α-synuclein aggregates in Parkinson's disease using the AUTOTAC technology

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There are currently no disease-modifying therapeutics for treating Parkinson's disease (PD). Although extensive efforts were undertaken to develop therapeutic approaches aiming to transiently relieve or delay the symptoms of PD, untreated alpha-synuclein aggregates can stimulate the recurrence of symptoms and even further progression of PD. Targeted protein degradation (TPD) has drawn attention as a therapeutic modality to selectively target alpha-synuclein, however, no TPDs have yet shown to degrade alpha-synuclein aggregates. Here, we employed the AUTOTAC (Autophagy-Targeting Chimera) technology to develop a disease-modifying therapeutic agent that enables the targeted degradation of alpha-synuclein aggregates by the autophagy-lysosome system. We show that PD-AUTOTAC induces p62-dependent macroautophagy to selectively target alpha-synuclein aggregates, alleviating synucleinopathy. such a degradation efficacy also led to the reduced cell-to-cell transmission of alpha-synuclein species *in vitro*. Extending these, the oral administration of PD-AUTOTAC showed therapeutic efficacy in mice injected with alpha-synuclein preformed fibrils, which decreased the formation of aggregates and associated immune responses. The *in vivo* degradative efficacy also correlated with the mitigation in the progression of motor deficits. Our results demonstrate that the AUTOTAC technology enables the development of disease-modifying drugs for PD.

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H. Chemical Biology and Drug Discovery [H-19]

Ginger-derived compounds regulate anti-asthmatic effects by suppressing allergic responses mediated T-helper 2 cell in vivo.

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6-Shogaol (SHO) and 6-gingerol (GIN), naturally derived compounds of ginger, have been found to have anti-allergic effects on dermatitis-like skin lesions and rhinitis. Although SHO and GIN have demonstrated a potential in various inflammatory diseases, their efficacy and mechanism in asthma have not been largely examined. Therefore, the present study demonstrated the anti-asthmatic effects of SHO and GIN on the T-helper (Th) 2 cell-mediated allergic response pathway in an ovalbumin (OVA)-induced asthma mouse model. The asthma mouse model was established with an intraperitoneal (i.p.) injection of 50 μg OVA and 1 mg aluminum hydroxide with or without an i.p. injection of SHO and GIN (10 mg/kg) before treatment with OVA. SHO and GIN inhibited eosinophilia in the bronchoalveolar lavage fluids and H&E-stained lung tissues. Both factors also decreased mucus production in periodic acid-Schiff-stained lung tissues and the levels of Th2 cytokines in these tissues. GIN attenuated oxidative stress by upregulating the expression levels of anti-oxidative proteins. It was found that SHO and GIN effectively suppressed the allergic response in the mouse model by inhibiting eosinophilia and Th2 cytokine production. It was suggested that SHO and GIN can inhibit lung inflammation and epithelial cell remodeling.

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H. Chemical Biology and Drug Discovery [H-20]

Radix Bupleuri inhibits IL-1β induced Inflammation by Downregulating NF-κB Signaling Pathway in Human Umbilical Vein Endothelial Cells

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Inflammation of vascular endothelial is a key process in the development of atherosclerosis. *Radix Bupleuri* (RB) have been used for a long time for prevention and treatment of various diseases. Phytochemical researches demonstrated that RB exhibited many biological properties including anti-cancer, anti-microbial, anti-viral, immunomodulatory effects and anti-inflammation. However, it remains unknown whether RB could modulate vascular endothelial inflammation. Here, we investigated the role of RB in interleukin-1β (IL-1β)-stimulated human umbilical vein endothelial cells (HUVECs). IL-1β stimulation induced vascular cell proliferation and migration. The expression of intercellular adhesion molecule 1 (ICAM-1) protein greatly increased in IL-1β-stimulated HUVECs. Our data showed that RB significantly suppressed vascular cell proliferation, migration and the expression of ICAM-1. In addition, RB blocks IL-1β-induced oxidative stress and tight junction disruption in HUVECs. Moreover, RB decreased the phosphorylation of the nuclear factor- κB (NF-κB) p65 subunit and nuclear translocation. The findings of this study demonstrate that RB prevents IL-1β-induced vascular inflammation and has the potential to protect against the early progression of atherosclerosis.

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H. Chemical Biology and Drug Discovery [H-21]

KM-C inhibits cell proliferation through induction of apoptosis in ovarian cancer cells

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Ovarian cancer (OC) is the eighth leading female-related cancer incidence and mortality worldwide. Despite the development of many treatments for OC, it is necessary to develop new diagnostic and therapeutic agents to increase the survival rate of OC. Here, we studied the anticancer efficacy of KM-C, which is known to have antibacterial and antifungal effects, in OC cells. First, KM-C reduces proliferation and induces apoptosis in OVCAR8, SKOV3, and A2780 cells in a dose- and time-dependent fashion. Moreover, KM-C significantly increases ROS and regulates p53, PUMA, BAX, p21, which are known to be important proteins for apoptosis, in OC cells. Our results suggest that KM-C is a novel anti-proliferative agent and has potential as a new therapeutic agent for OC.

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H. Chemical Biology and Drug Discovery [H-22]

Effect of Natural Minerals containing Chitooligosaccharides on Fatty Acid Synthesis and β-oxidation in Adipocytes

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To determine effect of natural minerals containing chitooligosaccharides (COS) on fatty acids synthesis and β-oxidation, we performed glycerol-3-phosphate dehydrogenase (GPDH) activity assay and RT-PCR in differentiated or mature 3T3-L1 adipocytes with the treatment of natural minerals or natural minerals containing COS. In differentiated 3T3-L1 adipocytes, both natural minerals and natural minerals containing COS treatment inhibited GPDH activity and the expressions of genes related to fatty acids synthesis such as SREBP 1c, FAS, and aP2. On the other hands, the expressions of genes related to β-oxidation such as CPT 1, MCAD, ACO, and UCP 2 were increased in mature 3T3-L1 adipocytes. Furthermore, the phosphorylation of AMPK also was increased by natural minerals and natural minerals containing COS treatment in both differentiated and mature 3T3-L1 adipocytes. Taken together, these data suggest that natural minerals containing COS is more effective than natural minerals alone in inhibitory effect on fatty acids synthesis and stimulatory effect on β-oxidation and AMPK activation ("This research was a part of the project titled 'Global multi-combination product development and export utilizing deep seawater extracted minerals (FDA notification)' funded by the Ministry of Oceans and Fisheries, South Korea.")

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H. Chemical Biology and Drug Discovery [H-23]

Assessment of Physico-chemical Stability of Hepatotropic Viruses for Hospital Environments

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Emerging and re-emerging viruses are a high global threat and require a swift and efficient response after identification. This poses an important timeframe to establish basic infection models and safety guidelines especially in the context of an epidemic/pandemic.

Hepatitis B Virus (HBV) and Hepatitis D Virus (HDV) are major public health problems. Together they represent 296 million chronic cases, with the risk of developing life-threatening liver cirrhosis/cancer. Virus inactivation conditions need to be addressed as fast as possible, since prevention of nosocomial and occupational transmission is the key to slowing down virus spread. To this end, we developed software assisted end-point dilution assays to set up an infection model, and to test physico-chemical treatments to inactivate viral pathogens, including HBV and HDV.

In this report, we tested the stability of HBV and HDV against thermal treatment, disinfection with commonly used alcohols in hospitals and treatment with the most common WHO-recommended hand-antiseptics. Our systematic investigation revealed that different alcohols and commercially available hand antiseptics had a virucidal effect against HBV and HDV.

This methodology is virtually adaptable to new viral threats and would enable us to provide valuable information on disinfection guidelines to hospitals in the early phase of new pandemics.

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H. Chemical Biology and Drug Discovery [H-24]

Hederagenin selectively inhibits SHP-1 and alleviates type-2 diabetes in 3T3-L1 and C2C12 cell lines.

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Diabetes is identified as the most progressive metabolic disorder worldwide. SHP-1 is a non-receptor protein tyrosine kinase that is highly expressed in type-2 diabetes and inhibits the phosphorylation of AMPK, a key marker for glucose transport across the plasma membrane. The knockdown of SHP-1 has been linked to the activation of AMPK and suppression of adipogenesis, making it a promising drug target for treating diabetes and obesity. In the present study, we investigated the effects of Hederagenin (HG), a natural compound extracted from medicinal plants, on SHP-1, with the goal of identifying a potential drug for treating diabetes and obesity by inhibiting SHP-1. Hence, SHP-1 was overexpressed and purified using an *E. coli* system and affinity chromatography respectively. Further, we analyzed its inhibition using a substrate, 6,8-fluoro-4-methylubelliferyl (DiFMUP). Out of 1033 natural drug compounds screened, HG showed therapeutic effects against SHP-1, with an IC50 value of 13.7 μM. HG also increased glucose uptake through the activation of AMPK in C2C12 and 3T3L1 cells, as well as induced insulin-dependent activation of AKT in 3T3L1 cells. These findings suggest that Hederagenin has strong potential as a therapeutic agent for treating type-2 diabetes.

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H. Chemical Biology and Drug Discovery [H-25]

Quercetagitrin shows antidiabetic effect by targeting protein tyrosine phosphatase associated with type 2 diabetes mellitus

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Protein tyrosine phosphatase (PTP) is a crucial enzyme that regulates various physiological functions, such as ligand-mediated receptor signaling and cell cycle events, through the dephosphorylation of tyrosine residues on intracellular proteins. Among members of the PTP family, PTPN9 has been linked to type 2 diabetes mellitus (T2DM). Previously, we identified compounds that inhibit PTPN9 by screening 658 species of natural products and demonstrated that their inhibition enhances glucose uptake and insulin sensitivity. Expanding our natural product screening to include 1033 species, we discovered quercetagitrin extracted from marigold, which inhibits PTPN9. Our study shows that quercetagitrin stimulates glucose uptake and attenuates palmitate-induced insulin resistance through AMP-activated protein kinase (AMPK) phosphorylation and serine/threonine kinase Akt activation in C2C12 myotubes and 3T3-L1 adipocytes. These results suggest that quercetagitrin could be a potential antidiabetic drug.

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H. Chemical Biology and Drug Discovery [H-26]

A bioluminescence cellular assay to follow Dengue virus nonstructural protein NS1 folding and secretion amenable to high-throughput drug screening

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Dengue virus (DENV) is transmitted by mosquitos and cause a variety of symptoms in human, from mild fever to severe hemorrhage and shock. Without effective vaccine nor specific treatment, the number of cases reported and deaths has been dramatically increasing worldwide.

DENV is an enveloped single-stranded positive-sense RNA virus. The genome is translated into three structural and seven non-structural (NS) proteins. NS1 protein exists in various oligomeric forms depending upon its location and plays various functions during viral infection. NS1 dimers are required for intracellular viral replication, while the secreted hexamers induces systemic inflammation and subsequent vascular leakage. The importance of NS1 for viral amplification and pathology development makes it a valuable drug target.

Here, we describe the establishment of a bioluminescent assay using recombinant DENV NS1 fused with donor and acceptor proteins to monitor NS1 biogenesis from folding to oligomers formation and secretion into the extracellular space. The native DENV NS1 produced upon viral infection is integrated into the recombinant bioluminescent oligomers, showing that the latter are structurally close which allows for tracking through native protein folding and secretion pathways. This robust assay is amenable to miniaturization and high-throughput screening that may uncover novel compounds for drug discovery.

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H. Chemical Biology and Drug Discovery [H-27]

Effects of Poly I:C treatment on particulate matter exposed human primary lung epithelial cells

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Recently, airborne particulate matter (PM) has been suggested to be associated with various pulmonary disorders. However, most of these studies focused on the cytotoxic effects of PM. Therefore, it is necessary to confirm the biological effects of the interaction between PM exposure and viral infection on the lungs in terms of cellular stress and toxicity.

Here, we investigate the effects of PM under 10 μm (PM_{10}) and diesel PM (DPM) on polyinosinic–polycytidylic acid sodium salt (poly I:C) treatment in human primary bronchial epithelial cells (BEAS-2B) by evaluating SG formation. As a result, SG formation was observed in BEAS-2B cells treated with poly I:C, and the number of SG-positive cells and SGs were increased by addition of PM_{10} or DPM. Moreover, immunoblotting analysis revealed that phosphorylation of eIF2α, which is essential for SG-related signaling, was increased by poly I:C and PM. Thus, under stressful conditions such as viral infection, cytotoxicity is further increased by PM, promoting lung toxicity.

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H. Chemical Biology and Drug Discovery [H-28]

Identification of beta-boswellic acid targeting SHP-2 for the treatment of diabetes.

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The protein tyrosine phosphatase SHP-2 plays a crucial role in regulating cell signaling, and its malfunctioning is associated with various diseases such as diabetes. This study aimed to find new compounds that target SHP-2 selectively and explore their potential in treating diabetes. To achieve this, SHP-2 was overexpressed in Escherichia coli and then purified using affinity chromatography. The activity of SHP-2 was assessed using a surrogate substrate called 6,8-difluoro-4-methylumbelliferyl (DiFMUP). In previous study, we found that knockdown SHP-2 in C2C12 cells resulted in increased phosphorylated AMPK, which is a vital regulator in glucose transport. After screening a library of 1,033 natural drug compounds against SHP-2, we identified beta-boswellic acid found in Boswellia serrata is the most effective inhibitor with an IC50 value of 6.3 μM. Our results demonstrate that beta-boswellic acid selectively inhibits SHP-2 and enhances glucose uptake by increasing the phosphorylation of AMPK and AKT. These findings indicate that beta-boswellic acid has the potential to treat type 2 diabetes by selectively targeting SHP-2 and promoting glucose uptake.

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H. Chemical Biology and Drug Discovery [H-29]

A novel pyridine-based DYRK1A inhibitor rescues down syndrome-related phenotypes

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DYRK1A is a significant pathogenic factor in Down syndrome (DS). Thus, inhibition of DYRK1A is considered as a therapeutic strategy to modify the disease. Here, we identified pyridine-based compound as a novel DYRK1A inhibitor through two-step screening approach using structure-based virtual screening of ~ 320,000 KCB chemical library (1st step) and cell-based NFAT-RE promoter assay (2nd step). Pyridine-based compound potently inhibited the kinase activity of DYRK1A *in vitro* (IC_{50} = 6 nM) and suppressed DYRK1A-mediated hyperphosphorylation of Tau in cells, which was stronger than those of other DYRK1A inhibitors. Pyridine-based compound rescued neurological and phenotypic defects of DS-like *Drosophila* model. Moreover, oral administration of pyridine-based compound suppressed Tau hyperphosphorylation in the brain of DYRK1A TG mice. In the open field test, pyridine-based compound (1 mg/kg) ameliorated the anxiety-like behavior of DYRK1A TG mice. Together, our results demonstrate that pyridine-based compound as a novel DYRK1A inhibitor rescues DS phenotypes in cells and *in vivo* and also suggest its therapeutic potential for the treatment of DYRK1A-related diseases including DS and Alzheimer's disease (AD).

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H. Chemical Biology and Drug Discovery [H-30]

Antioxidant and Anti-amyloid Activities of Kalopanax pictus fermented

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This study was to investigate antioxidant and anti-amyloid activities of the extract from Kalopanax pictus fermented with Hericium erinaceum mycelium (KP-HE). Antioxidant activity of KP-HE evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, 2,2′-azino-bis(3-ethylbenzothia- zoline-6-sulfonic acid) (ABTS) radical and peroxyl radical scavenging assays. KP-HE showed 80% DPPH radical scavenging activity at 500 mg/mL, 94.34% ABTS radical scavenging activity at 100 mg/mL, and 53.12% peroxyl radical scavenging activity at 100 mg/mL. KP-HE was shown to significantly inhibited DNA strand breakage induced by peroxyl radical. KP-HE also prevented peroxyl radical-mediated human serum albumin and neurofilament-L modification. β-Amyloid (Aβ) aggregates have been proposed as a pivotal event in the pathogenesis of Alzheimer's disease (AD). Thoflavin-T assays showed that KP-HE significantly inhibited Aβ1-42 aggregation in a concentration-dependent manner. Our results suggest that KP-HE should be viewed as a novel neuroprotective strategy for AD and related diseases.

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H. Chemical Biology and Drug Discovery [H-31]

Anti-Oxidation and Anti-Inflammatory effect of Bakuchiol

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This study demonstrated the protective effects of bakuchiol on oxidative modification of biomacromolecules and inflammatory responses. Antioxidant activity of bakuchiol was investigated by DPPH radical and ABTS radical scavenging assays. To confirm the effect of bakuchiol on oxidative modification of DNA, Oxidative modification of calf thymus DNA induced by peroxyl radical. As the result, various concentrations of bakuchiol effectively inhibit oxidative modification of DNA, also proteins and enzymes. We observe the anti-inflammatory function of bakuchiol in cells. RAW 264.7 mouse macrophage cells were treated with various concentrations of bakuchiol, and the cell viability was confirmed through MTT assay. The viability was maintained and improved, and cell death occurred at high concentrations. And then, we confirm anti-inflammatory effect. Inflammatory response of cells induced by LPS and treated with bakuchiol. As a result, NO production and iNOS was suppressed as the concentration of bakuchiol increased. In conclusion, it suggested that bakuchiol could be used in the development of cosmetics and medicines as an effective antioxidant and anti-inflammatory component.

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H. Chemical Biology and Drug Discovery [H-32]

Antioxidative Effect of Astaxanthin in melanoma

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We demonstrated that astaxanthin has antioxidant and anti-aging effects through in vitro experiments and whitening experiments in B16F10 melanoma. Antioxidant activity was mesured by 1,1-diphenyl-1-picrylhydrazyl radical (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical(ABTS) scavenging assays. As a result, astaxanthin showed high levels of radical scavenging activity in DPPH and ABTS assays. Astaxathin effectively protect DNA and bovine serum proteins, Cu, Zn-SOD, Catalase from oxidative damage and deformation. Astaxanthin effectively inhibited Tyrosinase and Hyaluronidase, thus showing postive results for skin whitening and immune response, respectively. Astaxanthin inhibiting tyrosinase in melanoma cells, it exhibited an inhibitory effect on intracellular melanin production in a non-toxic concentration. These results suggest that astaxanthin can be applied as an effective natural antioxidant and anti-aging material.

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H. Chemical Biology and Drug Discovery [H-33]

A novel NAMPT inhibitor that targets NAPRT-deficient cancer cells and alleviates CIPN

Minjee Kim[1#], Hyeyoung Kim[1,4#], Bu-Gyeong Kang[5#], Jooyoung Lee[3#], Hwanho Lee[1], Jane Jung[1,4], Myung Joon Oh[1,2], Yeojin Sung[1], Yunji Lee[1], Sun-Shin Cha[5*], Hosung Jung[1,4*], Hyun Seok Kim[1,2,3*]

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Exploiting synthetic lethal (SL) relationships between a pair of proteins has shown promise in discovering anti-cancer drugs. NAMPT, a key enzyme in the NAD+ salvage pathway, has gained attention as a potential target due to its significance in cancer cell survival and SL relationship with NAPRT, the rate-limiting enzyme of the NAD+ priess-handler pathway. However, the clinical use of NAMPT inhibitors is challenging since NAD+ is also essential in normal cells. Here, we discovered a novel NAMPT inhibitor, A4276, with enhanced specificity against NAPRT-deficient cancer cells. A drug derivatives screening approach was used to select an agent with a broad therapeutic window between the NAPRT-negative and NAPRT-positive lung cancer cell lines. A4276 conferred cytotoxic effects on NAPRT-deficient cancer cells by binding to NAMPT and inhibiting its enzymatic function. Of particular note, we validated that depletion of NAPRT also indicates EMT-subtype cancer. A4276 was effective against NAPRT-deficient EMT subtype-cancer in multiple tumor types. Taken together with the fact that A4276 alleviates the progression of chemotherapy-induced peripheral neuropathy (CIPN), the study reveals the great therapeutic potential of A4276 as a single agent, and in combination with commonly used chemotherapeutic agents by targeting NAPRT-deficient EMT-subtype cancers and abrogating CIPN.

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H. Chemical Biology and Drug Discovery [H-34]

Chemical Compositions, Antioxidant and Protective Effects against Oxidative Stress of The Mixture Extracts from Ganoderma lucidum and Cordyceps militaris

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Ganoderma lucidum and *Cordyceps militaris* are two important medicinal mushrooms with significant pharmacological activities. In this study, we first combined the extracts of these two mushrooms into a mixture and investigated its composition using LC-QTOF MS and HPLC-DAD. The antioxidant capacity was then evaluated based on its ability to scavenge 2,2-diphenyl-1-picrylhydrazyl free radicals (DPPH), with Trolox used as an analytical standard. A protective effect of the mixture was subsequently evaluated against H_2O_2-induced oxidative stress on human dermal fibroblasts. The results showed that the mixture contained a total of 94 signature compounds, belonging to different bioactive classes: phenolic acid, flavonoids, triterpenes, and nucleosides. Among them, the contents of major compounds like adenosine and cordycepin were 0.53 mg/g and 0.14 mg/g, respectively. The free radical scavenging activity of the product was equivalent to 45.00 ± 7.41 µmol of Trolox per gram of the combined extract. The mixture showed protective effects against oxidative stress; At a concentration of 20 µg/ml, the mixture reduced 20% of the rate of cell death caused by 3.12 µM of H_2O_2.

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H. Chemical Biology and Drug Discovery [H-35]

Development of LC-MS/MS analysis method for high throughput screening of protein covalent compounds and verification through Interlaboratory study

Sang Hyun Bae[1*], Hyeon Jun Lee[2], Minhee Kang[2], Young Ho Jeon[2], Jong Suk Lee[3], Jin Young Kim[3], Haejin Kwon[4], Hyun-Mee Park[4], Yeji Do[5], Hazara Begum Mohammad[5], Min-Sik Kim[5], Yoonha Park[6], Je-Hyun Baek[6], Kwang Hoe Kim[7], Jong Hwan Shin[7], Dong Geun Kim[8], Hoi Keun Jeong[8], Ju Yeon Lee[1,9*], Jin Young Kim[1,9*]

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Our study focuses on the development and verification of a LC-MS/MS method for efficiently screening covalent binding between electrophilic compounds and nucleophilic residues in proteins, particularly cysteine in a protein. To assess the covalent bond formation, we introduced cysteine in the druggable pocket of MDM2 as target protein and selected 30 electrophilic inhibitors from a cysteine-focused compounds library, divided into three groups of 10 compounds each. We incubated each group of 10 compounds with MDM2(M62C) and digested the mixture with trypsin followed by LC-MS/MS analysis. We calculated the relative binding of the compounds to the protein and were able to rank the compounds in order. To verify the method's reproducibility, we conducted an interlaboratory study involving seven laboratories, which performed LC-MS/MS analysis using the same procedures. By comparing the results obtained from each laboratory, we evaluated the universal applicability of our LC-MS/MS method for high-throughput screening of covalent binding between electrophilic compounds and nucleophilic residues in proteins. Our findings suggest that this method can be a useful tool for such screenings in various laboratories.

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H. Chemical Biology and Drug Discovery [H-36]

Seongsanamide B Inhibits γ-Irradiation-Induced Cancer Cell Metastasis in Human Lung Cancer Cells

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Lung cancer is the most common cause of cancer-related death, due to its high rate of metastasis. Despite recent improvements in diagnostic technologies, approximately 70% patients with non-small cell lung cancer (NSCLC) are diagnosed at an advanced stage with metastatic disease. Here, we explored the ability of seongsanamide B, a bicyclic peptide from Bacillus safensis KCTC 12796BP, to improve the efficacy of radiotherapy in NSCLC. Seongsanamide B suppressed cell migration and invasion caused by γ-irradiation in NSCLC cells. Furthermore, seongsanamide B suppressed γ-irradiation-induced Bcl-XL expression and its downstream signaling molecules, such as SOD2 and phosphorylated Src, by blocking phosphorylated STAT3 into the nucleus. Additionally, seongsanamide B significantly modulated γ-irradiation-induced E-cadherin and vimentin. Therefore, seongsanamide B may have a potential to overcome IR-induced metastasis caused by radiotherapy in patients with NSCLC.

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H. Chemical Biology and Drug Discovery [H-37]

Chemical and biological profiles of the sprout of gamma-irradiated mutant wheat (Triticum aestivum)

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Wheat (Triticum aestivum Linn.; Poaceae) is one of the most popular food crops worldwide and its grain is energy-rich owing to the high carbohydrate content. Recently, the use of other wheat parts, such as sprout and bran, has attracted significant interest as a rich source of bioactive phytochemicals that are beneficial to human health. In this study, a new colored wheat line (PL18) was developed via gamma-irradiated mutation breeding from the original cultivar (Woori-mill × D-7; PL01). The chemical profiles of the new colored wheat line (PL18), the original cultivar (PL01), and the cirtified cultivar (Geumgang; PL26) were systematically investigated via HPLC-DAD-ESIMS, and revealed fourteen characteristic peaks that differed markedly in content between these wheat sprout samples. In addition, the contents of three standard compounds (isoschaftoside, isoorientin, and isoscoparin) in these wheat sprout samples were determined. The biological properties of ethanol extract taken of them was also examined using the DPPH and ABTS radical scavenging assay and the measurement of LPS-induced NO production in RAW264.7 cells, and PL18 extract showed the greatest antioxidant and anti-inflammatory activities. Therefore, a new colored wheat line (PL18) has high usefulnee for sprouts and may contribute to the development of new cultivars and functional foods.

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H. Chemical Biology and Drug Discovery [H-38]

Policosanol profiles and adenosine 5'-monophosphate-activated protein kinase (AMPK) activation potential of the sprout of Oriental wheat (Triticum turgidum ssp. Turanicum)

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Oriental wheat (Khorasan wheat; *Triticum turgidum* ssp. *turanicum*; Poaceae) is a tetraploid wheat species known as superfood with a high-fiber and high neutrient density. Adenosine 5'-monophosphate-activated protein kinase (AMPK), a heterotrimetric enzyme is responsible for regulating the metabolism of glucose, cholesterol, and hepatic lipids. AMPK activation has been shown to inhibit the synthesis of fatty acid and cholesterol and promote insulin secretion, thus has beneficial effects in treating metabolic desease. Policosanols are reported as a health promoting aliphatic alcohol known as low-density lipoprotein cholesterol-lowing agent. To explore the functional properties of Oriental wheat, we investigated the policosanol profiles and AMPK activation potential of the sprout of five Oriental wheat which were distributed from the Rural Development Administration's Genebank (IT308416, IT308447, IT308132, IT311253, and IT330600). Nine policosanols in the hexane extracts of Oriental wheat sprouts were analyzed using gas chromatograpy coupled with mass spectrometry. Their properties were also examined on AMPK phosphorylation in the human hepatoma (HepG2) cell line. Among them, the extract of IT308416 induced dose-dependent activation of AMPK phosphorylation via activating AMPK. Therefore, these results may promote the potential application of Oriental wheat for the funtional foods.

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H. Chemical Biology and Drug Discovery [H-39]

Identification of molecular target for suppressing desmoid tumors

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Desmoid tumors, known as aggressive fibromatosis, are derived from connective tissues and local invasion is usually observed. Although intensive efforts have been performed to investigate the novel anti-cancer agents in desmoid tumors, effective clinical management for treating desmoid tumors have not been developed yet. Also, the molecular mechanisms involved in the tumorigenesis of desmoid tumors have not been discovered. In this study, given the frequent mutations of Wnt components and loss of function mutations in p53 in desmoid tumors, we developed the mouse models harboring *apc* mutation with/without p53 knockout, $apc^{1638n/+}$, $apc^{1638n/+}/p53^{-/-}$, respectively. Interestingly, we established two primary cells derived from desmoid tumors in $apc^{1638n/+}$, $apc^{1638n/+}/p53^{-/-}$. Next, we have screened 3120 chemicals by using FDA-approved chemical library, which is safe and target specificities are confirmed. Halofuginone hydrobromide (HH), targeting TGFβ signaling pathway, shows the most significant growth inhibition effects on $apc^{1638n/+}$, $apc^{1638n/+}/p53^{-/-}$. Especially, HH also shows dramatic anti-cancer effects on colorectal cancer cells and cancer organoids derived from colorectal cancer patients. Taken all, targeting TGFβ signaling pathway is the effective strategy for treating the desmoid tumors and also colorectal cancer patients.

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H. Chemical Biology and Drug Discovery [H-40]

Improving autophagy flux by TFEB activation with Ceria-Zirconia antioxidant nanoparticles attenuated kidney injury in cellular and animal models of Fabry disease

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Objectives: Fabry disease (FD) is a lysosome storage disease (LSD) characterized by significantly reduced intracellular autophagy function. Phospholipid–polyethyleneglycol-capped Ceria-Zirconia antioxidant nanoparticles (PEG-CZNPs) have been reported to enhance autophagy flux. We accessed the action mechanisms of PEG-CZNPs in autophagy regulation and checked the effect on chronic kidney injury in cellular and animal models of FD.

Methods: PEG-CZNPs were synthesized using a non-hydrolytic sol-gel reaction method. HK-2 cells were transfected with α-galactosidase A (α-GLA) shRNA for permanent cellular model of FD. For in-vivo study 4-week-old male B6;129-Glatm1Kul/J mice were treated for 48 weeks with 10mg/kg of PEG-CZNPs twice per week via intraperitoneal injection.

Results: TFEB translocated to the nucleus by treatment with PEG-CZNPs. Autophagy flux was enhanced by PEG-CZNPs treatment. Autophagy flux significantly decreased after knockdown of TFEB with PEG-CZNPs treatment. TFEB dephosphorylation was independent of both mTOR and ERK but GSK3ß signaling pathway showed massive impact on TFEB dephosphoryation by PEG-CZNPs.

Conclusions: These results suggested PEG-CZNPs promote autophagy flux through TFEB signaling pathways, showed the beneficial effect on renal fibrosis in cellular and animal models of FD. It thus provided a new insights of the potential therapeutics

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H. Chemical Biology and Drug Discovery [H-41]

Sirt6 activation ameliorates inflammatory bone loss in ligature-induced periodontitis in mice

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Periodontitis is an inflammatory disease caused by microorganisms that induce the destruction of periodontal tissue. Inflamed and damaged tissue produces various inflammatory cytokines, which activate osteoclasts and induce loss of alveolar bone and, eventually, tooth loss. Sirt6 expression suppresses inflammation and bone resorption; however, its role in periodontitis remains unclear. We aimed to identify the protecive effect of Sirt6 on bone loss against ligature-induced periodontitis. To understand the role of Sirt6 in periodontitis, we compared periodontitis with ligature placement around the maxillary left second molar in control (C57BL/6J) mice to Sirt6 overexpressing Tg (Sirt6Tg) mice and analyzed phenotypes using micro-CT. MDL801, a Sirt6 activator, was used as a therapy for periodontitis through oral gavage. As results, Sirt6Tg was observed to resist bone loss against ligature-induced periodontitis. Furthermore, pro-inflammatory cytokines and osteoclast numbers induced by periodontitis surgery were significantly reduced in Sirt6Tg-ligated mice. Consistent with this observation, we confirmed that bone loss was significantly reduced when MDL801, a Sirt6 activator, was included in the ligation mouse model. In concolution, **o**ur findings demonstrate that Sirt6 activation prevents bone loss against ligature-induced periodontitis. Thus, a Sirt6 activator may provide a new therapeutic approach for periodontitis.

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H. Chemical Biology and Drug Discovery [H-42]

Identification of SARS-CoV-2 inhibitor targeting PLpro using virtual screening of a natural compound database

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The global community faces a grave threat from the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and a lack of effective antiviral medicine makes this a worldwide health emergency. Two necessary cysteine proteases mainly control replication of SARS-CoV-2. One of them is a papain-like protease (PLpro), which disrupts host-immune detection by deubiquitinating host-protein substrates, regulates viral replication, thus representing a promising target for developing antiviral drugs. In this study, we identified possible inhibitors of PLpro through virtual screening of a natural compound database with molecular dynamics (MD) simulation. Using the virtual screening approach, consisting of three layers of molecular docking approaches, 54 hits were identified from the database, from which four compounds were further selected based on ADME/T and binding energy analysis. The binding stability and dynamics of these selected hit with PLpro were further evaluated by MD simulation, which revealed UNPD111909 as a potent hit that showed higher binding energy than the known inhibitor, 5-amino-2-methyl-N-[(1R)-1-naphthalen-1-ylethyl]benzamide (GRL0617). Interestingly, binding mode of UNPD111909 in the active site was the same as GRL0617, along with high intermolecular interaction. Our data provide structural insights and a molecular basis for designing new potent PLpro inhibitors for anti-covid therapy.

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H. Chemical Biology and Drug Discovery [H-43]

Bioanalytical Characterization of Antibody-Drug Conjugates: Preclinical Pharmacokinetic Study of Ado-Trastuzumab Emtansine (T-DM1) in Rats

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Antibody-drug conjugates (ADCs) are monoclonal antibodies covalently bound to cytotoxic drugs by a linker. They are designed to selectively bind target antigens and present a promising cancer treatment that with reduced systemic toxicity. Ado-trastuzumab emtansine (T-DM1) is an ADC that approved by US FDA for the treatment of HER2-positive breast cancer. T-DM1 is a combination of trastuzumab and DM1. We optimized four analytical methods for quantification of T-DM1 in rats: 1) enzyme-linked immunosorbent assay (ELISA) to quantify the total trastuzumab levels in all drug-to-antibody ratios (DARs), 2) ELISA to quantify the conjugated trastuzumab levels in all DARs except DAR 0, 3) LC–MS/MS analysis to quantify the levels of released DM1, and 4) bridging ELISA to quantify the level of anti-drug antibodies (ADAs) of T-DM1. The bioanalytical samples from rats injected intravenously with T-DM1 (20 mg/kg, single dose) were analyzed using these optimized methods. Based on these analytical methods, we evaluated the quantification, pharmacokinetics, and immunogenicity of T-DM1. This study provides valuable insights into the characterization of bioanalysis systems using T-DM1, for future investigation on the efficacy and safety of ADC development.

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H. Chemical Biology and Drug Discovery [H-44]

Crataegus pinnatifida Root extract has Anti-obesity effect and prevents non-alcoholic fatty liver disease in High-fat diet-induced obese C57BL/6 mouse.

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In previous studies, we found that *Crataegus pinnatifida* root extract (CRE) has anti-obesity effect through inhibition of adipocyte differentiation and oxidative stress reduction effect caused by lipid droplet *in vitro*. As a mechanism for this effects, inhibition of SREBP-1 translocation from the cytoplasm to the nucleus due to CRE-induced AMPK phosphorylation was proposed.

As a follow-up study, we present the anti-obesity and non-alcoholic fatty liver disease (NAFLD) preventive effects of CRE *in vivo*. In a high-fat diet-induced obese mouse model, when CRE was administered for 12 weeks, it was confirmed that body weight, adipose tissue weight, and adipocyte size in WAT were decreased. In addition, it was confirmed that CRE decreases blood triglyceride and cholesterol level through plasma profiling.

CRE was also effective in preventing NAFLD, a complication induced by obesity, which was confirmed by suppressing lipid accumulation in liver and reducing AST, ALT levels through histological analysis and plasma profiling.

Therefore, through this study, we suggest the anti-obesity effect and preventive effect of non-alcoholic fatty liver disease of CRE.

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H. Chemical Biology and Drug Discovery [H-45]

Regulation of AMPK-Hippo/YAP pathway by Spatholobi Caulis and its active compounds has an antioxidant effect in vitro and in vivo

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Enhanced oxidative stress in the organ has been shown to lead to excessive ROS production and mitochondrial dysfunction leading to cell and tissue damages. Although Spatholobi Caulis (SC) has been reported to have beneficial effects, the scientific studies about the effects on the oxidative stress and the underlying mechanisms are insufficient. This study investigated the antioxidant effects and mechanisms of SC in cells as well as in mice. Oxidative stress insults including iron and acetaminophen induced an increase in ROS production, impairment of mitochondrial membrane potential and consequent apoptosis, which was markedly blocked by SC. More importantly, SC activated AMP-activated protein kinase (AMPK)-related proteins as well as Hippo/Yap pathways, which were related with its effects. These results provide the potential of SC as an anti-oxidant. This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (number: HF20C0212) and (number: HF21C0061), and by the National Research Foundation of Korea (NRF) grant funded by the Korea Government [MSIP] (NRF-2022R1A2C1092168).

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H. Chemical Biology and Drug Discovery [H-46]

Bioconverted extract of Sophorae Fructus stimulates immune response in RAW264.7 cell

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Bioconversion is a process that manufactures metabolites contained in natural products through biotechnology such as enzyme treatment and microbial fermentation. *Sophorae fructus*, known as the fruit of *Sophora japonica*, has various bioactivities such as antioxidant, immune modulation effects, which is used in traditional oriental medicine. However, the stimulation of immune response effect of bioconverted extracts of *S. fructus* is not well known. In this study, *S. fructus* extracts was bioconverted using the enzyme from *Aspergillus kawachii*. To evaluate whether the bioconversion of *S. fructus* could influence the stimulation of immune response, we used RAW264.7 murine macrophage cell line. When RAW264.7 cells were treated with bioconverted *S. fructus* extract (BSFE), the level of nitric oxide synthesis and the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) increases but not in non-bioconverted *S. fructus* extract (SFE). Also, we observed the increased expression of pro-inflammatory cytokines when RAW264.7 cells were treated with BSFE. However, further studies should be needed, such as analysis of component changes by biological transformation and molecular level signaling pathway of BSFE-induced immuno-stimulation. In summary, we suggest that BSFE may provide a hopeful source of an immune-enhancing agent.

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H. Chemical Biology and Drug Discovery [H-47]

Microencapsulation of Potential Oxygen Release Drug Systems and their Application for Tissue Regeneration

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This study addressed the hypothesis that tissue regeneration can be enhanced by controlled oxygen producing system which is modulated by an enzyme, catalase. Various encapsulation methods have unique advantages and disadvantages for specific drug delivery systems. Based on this criterion, hydrogen peroxide microsystems have been developed to be used as oxygen. We confirmed for optimally safe and efficient release of oxygen from a well-known oxidant H_2O_2, catalase immobilization onto alginate backbone was employed using EDC/NHS chemistry, and also evaluated the capsule as a model of a tissue. As a result, HUVEC cell and cell survival rates improved on supplying oxygen even in hypoxic conditions. In addition, continuous oxygen release produces growth factors and cytokines to enhance the expression of VEGF and HIP-1α genes. These oxygen releasing polymeric system may play an important role in the application of scaffold-based drug delivery system because they provide a solution for oxygen diffusion limitations in the engineering of tissue transplants.

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H. Chemical Biology and Drug Discovery [H-48]

Functionalization of nanocomplex for efficient drug to treat the severe pulmonary disease

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Acute respiratory distress syndrome (ARDS) is a major cause of mortality among patients with irreversible lung disability from SARS-CoV-2, due in part to interference in pulmonary drug delivery caused by accumulated BALF. To address this issue, a zwitterion-functionalized multi-drug nanocomplex (ZnC) was developed, with nitric oxide (NO) to enhance mobility and provide anti-inflammatory properties. Dexamethasone (Dex) was then loaded into the nanocomplex, resulting in the anti-inflammatory mucus permeator (AIM). *In situ* tracking of AIM showed improved alveoli-targeting efficiency and residual time in the alveolar region due to its anti-mucus effect. In an *in vivo* ARDS model, inhalation of AIM resulted in increased anti-inflammatory effects and improved pulmonary function, leading to increased survival rates. This was attributed to the presence of ZnC and the synergy between NO and Dex. Overall, this innovative approach offers a promising solution to the challenges of pulmonary drug delivery in ARDS caused by SARS-CoV-2.

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H. Chemical Biology and Drug Discovery [H-49]

Induction of Apoptosis by JIB-04 mediated by p53- and ER Stress-dependent expression of DR5.

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JIB-04, a pan-inhibitor of histone demethylase, has been shown to induce apoptosis in various cancer cell lines. However, the mechanism by which JIB-04 induces apoptosis is not fully understood. In this study, we investigated the mechanism of JIB-04-induced apoptosis in HCT-116 cells. Treatment with JIB-04 in HCT-116 cells induced caspase-dependent apoptosis, as evidenced by an increase in annexin-V positive cells and cleavage of PARP1 and caspase3. JIB-04 also increased the mRNA and protein levels of DR5. Knockdown of DR5 rescued JIB-04-induced apoptosis, indicating that JIB-04 induces apoptosis through DR5. JIB-04 treatment increased the levels of p53 protein, and inhibition of p53 activation by PFT-α decreased annexin-V positive cells, DR5, and cleaved PARP1. JIB-04 also increased the mRNA and protein expression of ER stress markers. Knockdown of CHOP, a mediator of DR5 transcription under ER stress, decreased JIB-04-induced apoptosis, DR5, and cleaved PARP1. JIB-04 was shown to increase DR5 transcription through the CHOP binding site on the DR5 promoter. Moreover, we found that JIB-04 increases apoptosis through ATF4. Overall, JIB-04 induces apoptosis in HCT-116 cells through the up-regulation of DR5 via p53 and ER stress, and activation of ATF4. This suggests its potential as a therapeutic candidate for cancer treatment.

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L. Infection & Immunology [L-1]

Novel Bispecific Human Antibody Platform Specifically Targeting a Fully Open Spike Conformation Potently Neutralizes Multiple SARS-CoV-2 Variants

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Rapid emergence of new variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has prompted an urgent need for the development of broadly applicable and potently neutralizing antibody platform against the SARS-CoV-2, which can be used for combatting the coronavirus disease 2019 (COVID-19). In this study, based on a noncompeting pair of phage display-derived human monoclonal antibodies (mAbs) specific to the receptor-binding domain (RBD) of SARS-CoV-2 isolated from human synthetic antibody library, we generated K202.B, a novel engineered bispecific antibody with an immunoglobulin G4-single-chain variable fragment design, with sub- or low nanomolar antigen-binding avidity. Compared with the parental mAbs or mAb cocktail, the K202.B antibody showed superior neutralizing potential against a variety of SARS-CoV-2 variants in vitro. Furthermore, structural analysis of bispecific antibody-antigen complexes using cryo-electron microscopy revealed the mode of action of K202.B complexed with a fully open three-RBD-up conformation of SARS-CoV-2 trimeric spike proteins by simultaneously interconnecting two independent epitopes of the SARS-CoV-2 RBD via inter-protomer interactions. Intravenous monotherapy using K202.B exhibited potent neutralizing activity in SARS-CoV-2 wild-type- and B.1.617.2 variant-infected mouse models, without significant toxicity in vivo. The results indicate that this novel approach of development of immunoglobulin G4-based bispecific antibody from an established human

recombinant antibody library is likely to be an effective strategy for the rapid development of bispecific antibodies, and timely management against fast-evolving SARS-CoV-2 variants.

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L. Infection & Immunology [L-2]

Identification a lead small-molecule drug to treat osteoarthritis through a cartilage regeneration mechanism

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Osteoarthritis (OA), primarily characterized by articular cartilage destruction, is the most common form of age-related degenerative whole-joint disease. No disease-modifying treatments for OA are currently available. Although OA is primarily characterized by cartilage destruction, our understanding of the processes controlling OA progression is poor. Here, we report the association of OA with increased levels of osteoclast-associated receptor (OSCAR), an immunoglobulin-like collagen-recognition receptor. In mice, OSCAR deletion abrogates OA manifestations, such as articular cartilage destruction, subchondral bone sclerosis, and hyaline cartilage loss. We found that the inhibition of OSCAR activity attenuated the pathological changes of OA in the subchondral bone and reduced the degeneration of articular cartilage in OA-induced models. Besides, (1) EKs significantly enhance cartilage regeneration in-house mouse experiments, demonstrating a potential as DMOADs (disease-modifying osteoarthritis drugs). (2) Treatments with EK molecules (EKs) attenuates OA pathogenesis caused by experimental OA. In this study, we determined that blocking OSCAR may be the basis of a potential disease-modifying therapy in OA.

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L. Infection & Immunology [L-3]

Colonic inflammation biomarker-ROS responsive thioketal nanoparticle loaded with potent HDAC6 inhibitor, Tubastatin A for the treatment of ulcerative colitis.

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Ulcerative colitis is a common gastrointestinal problem characterized by mucosal injury primarily affecting large intestine. Current treatment options only give symptomatic reliefs. TKNP can releases drug on response to high ROS microenvironment present on UC. TUBA, a potent HDAC6 inhibitor have anti-inflammatory effects. Here, we are developing TKNP-based local/targeted delivery system containing TUBA for treatment of UC.

ROS cleavable thioketal polymer was synthesized by acetal exchange reaction with stepwise polymerization. Nanoparticle was prepared by emulsion solvent evaporation method. Nanoparticle characterization was performed using DLS, FTIR, UV-spectroscopy, and TEM. *In vitro* anti-inflammatory efficacy of the developed formulation was performed on RAW 264.7 cells. Colitis was developed in C57BL/6 mice by feeding with DSS 3% w/v in drinking water for 7 days. Disease severity was measured based on different clinical symptoms.

In vitro drug release showed ROS responsive release of TUBA from TUBA-TKNP. ELISA results showed decreased concentration of pro-inflammatory cytokines on NP treated cell model proving its anti-inflammatory effect. DSS fed mice group showed decrease in body weight, high disease severity index and high myeloperoxidase (MPO) activity indicating the induction of ulcerative colitis.

Our *in vitro* result demonstrated that TUBA-TKNP releasing drug on response to high ROS decreases inflammation.

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L. Infection & Immunology [L-4]

Suppression of Activated Mast Cells by ORAI1 Inhibitor Alleviates Peanut Induced Anaphylaxis and Acute Diarrhea.

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Food allergies are pathological immune responses accompanied by allergic enteritis and anaphylactic symptoms. Allergen-stimulated mast cells (MCs) are known to be closely associated with pathologic mechanisms of food allergy. In this study, using Mcpt5cre-DTA mice, we demonstrate that MCs are critical immune cells mediating responses to peanut induced allergy. Although prophylactic stabilization of MCs is regarded as a viable method for preventing these allergic responses, MCs in their activated state are difficult to suppress. We identified the ORAI1 Ca2+ channel as a pharmacological target for the stabilization of allergen-exposed MCs. An ORAI1 inhibitor treated concurrently with or even after allergen challenge effectively inhibited FcεRI-mediated MC degranulation and de novo synthesis of cytokines. Mechanistically, the ORAI1 inhibitor was found to prevent the association of synaptotagmin2 with the SNARE complex. We furthermore show that the administration of an ORAI1 inhibitor after allergen challenge effectively inhibited allergic acute diarrhea and ameliorated anaphylaxis in an in vivo model of peanut allergy. Our findings reveal that the inhibition of the MC ORAI1 channel in vivo has therapeutic potential for limiting the progression of food allergy-mediated anaphylaxis.

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L. Infection & Immunology [L-5]

MARCH5-dependent NLRP3 ubiquitination is an essential step for NEK7 docking on the mitochondria

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The NLRP3 inflammasome is a global immune-sensor that is activated by a repertoire of endogenous and exogenous stimuli. NLRP3 translocates to mitochondria but whether mitochondria involvement in the inflammasome assembly is unclear. Here, we show that the mitochondrial E3 ligase MARCH5 is a key regulator of NLRP3 inflammasome assembly. Myeloid cell-specific *March*5 conditional knockout (*March5* cKO) mice exhibited an attenuated mortality rate upon LPS or *Pseudomonas aeruginosa* challenge. Macrophages derived from *March5* cKO mice failed to secrete IL-1β and IL-18 after microbial infection. Mechanistically, MARCH5 interacts with the NACHT domain of NLRP3 and promotes K27-linked polyubiquitination of K324 and K430 residues of NLRP3. Ubiquitination-defective NLRP3 mutants neither bind to NEK7, nor form NLRP3 oligomers, but remain binding to MAVS. Accordingly, NLRP3 mutants led to abortive ASC speck formation and diminished IL-1β production. We propose that MARCH5-dependent NLRP3 ubiquitination creates a docking site for NEK7 binding, playing as a fundamental step-wise regulator on the mitochondria.

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L. Infection & Immunology [L-6]

α-Hemolysin plays a crucial role in nullifying acidification of bacteria harboring lysosomes during Uropathogenic E. coli infection in bladder

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Recurrent Urinary tract infections have long been seen as a ramification of persistent uropathogenic Escherichia coli (UPEC) that manage to survive within the bladder epithelial cells (BECs) even after antibiotic treatment. In this study, we have identified that after invading the BECs, UPEC evades expulsion from RAB27b$^+$ vesicles by escaping into the cytoplasm, using the toxin Hemolysin A (Hly A). Following a brief cytoplasmic phase these UPEC were recaptured in LC3A/B$^+$ autophagosomes that in turn mature into LAMP1$^+$ autolysosomes. As they mature, these Lamp1 autolysosomes acidify, thus killing the bacteria and eliminating them. However, it was observed that UPEC containing lysosomes could not acidify due to a failure in recruitment of V-ATPase proton pumps, which was attributed to cytosolic microtubules fragmentation by Hly A. We were able to deduce that bacteria, using Hly A, create a suitable niche for themselves inside the BECs often leading to multiple bouts of persistence infection. Countering measures might include microtubule stabilizer that would aid in elimination of the bacteria thus reducing the bacterial burden and alleviate chances of reoccurrences.

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L. Infection & Immunology [L-7]

Overexpression of cathepsin S aggravates lupus pathogenesis by regulating TLR7 and IFN-alpha.

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Systemic lupus erythematosus (SLE), one of the chronic autoimmune diseases, affects multiple organs with several cytokine-induced major histocompatibility complex (MHC) class II. Recent research has shown that the cysteine protease cathepsin S (CTSS), a member of the cathepsin family, can influence MHC class II expression. Therefore, to research the function of CTSS in SLE, CTSS-overexpressing transgenic (TG) mice were generated. Without any outside stimulation, the TG mice developed exacerbated lupus-like symptoms after eight months. To determine whether the symptoms were similar to SLE symptoms, we treated both wild type (WT) and TG mice with pristane, which is known to cause SLE like symptoms in murine. After treating the mice, we observed elevated monocytes and neutrophils in WT mice with treatment and in TG mice with or without treatment, along with higher toll-like receptor 7 (TLR7) expression, especially in the spleen. Those lymphocytes were attracted by chemokines, which are produced by interferon alpha (IFN-alpha) signaling. In conclusion, overexpression of CTSS in mice promotes TLR7 expression, even increases a cytokine associated with autoimmune reactions, regulates IFN-alpha, and elevates the severity of lupus-like symptoms. Moreover, CTSS-overexpressing TG mice can play an important role in lupus model mouse studies and in therapeutic target studies.

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L. Infection & Immunology [L-8]

Serum amyloid A stimulates γδT cells and neutrophils to secrete IL-17 which affects bone density.

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Serum amyloid A (SAA) is produced in liver and crucial to maintain inflammation. SAA stimulates the release of G-CSF, which causes neutrophilia and produce cytokines such as TNFα, IL-6, and IL-17. γδT cells, ILC3 cells, and neutrophils secrete IL-17, which provokes bone loss and inflammatory diseases including psoriasis, rheumatoid arthritis, and obesity. Bone loss occurs by increasing receptor activator of nuclear factor-κB ligand (RANKL) secretion stimulating osteoclast differentiation. RANKL+ neutrophils were shown to contribute to osteoclastogenesis in vivo. In 6-month-old SAA1 overexpression transgenic (TG) mice, we observed decreased bone mineral density and expression of RANKL, IL-17, and osteoclast-related genes (tnfsf11 and ctsk) were significantly increased. Increased neutrophil as well as higher levels of RANKL and IL-17 expression on surface membranes indicated that neutrophils were heavily involved in bone loss in our TG mice. We concluded that chronic inflammation in 6-month-old TG mice induced an increase in osteoclast-related gene expression accompanied by an increase in IL-17. When SAA1 levels were decreased, inflammatory reactions caused by IL-17 and bone loss might be attenuated. We also showed SAA1-overexpressing TG mice are useful disease model for bone loss.

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L. Infection & Immunology [L-9]

Lithospermum erythrorhizon extract reduces the severity of endotoxin-induced uveitis

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Purpose: Recent studies have reported the anti-inflammatory effect of *Lithospermum erythrorhizon.* We investigated the effect of ethanol extract of *Lithospermum erythrorhizon* (EELE) on endotoxin-induced uveitis (EIU) in rats and mice. In addition, the endotoxin-induced expression of pro-inflammatory cytokines and activation of transcription factor IRF and NFκB were investigated in human monocyte reporter cell lines (THP1) treated with EELE in vitro, to clarify the anti-inflammatory effect.

Methods: EIU was induced in rat and mice by a footpad injection of lipopolysaccharide (LPS). EELE was daily administered for 7 days before LPS injection. After 24 hours, clinical severity and retinal vessel thickness were evaluated. THP1-Blue-ISG and THP1-XBlue cells pretreated with EELE were incubated with LPS for 24 hours. Level of TNFα, IL-6 and IL-8 expression and activation of IRF and NF-κB were investigated.

Results: Clinical severity and retinal vessel thickness were significantly decreased in EELE-treated rat than in control group ($P < 0.001$, $P = 0.0167$). EELE down-regulated the production of TNFα, IL-6 and IL-8. And EELE treatment suppressed the activation of IRF and NF-κB.

Conclusion: Orally administrated EELE alleviated the ocular inflammation. These findings demonstrate that EELE could be an effective agent for the control of endogenous ocular inflammatory disease.

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L. Infection & Immunology [L-10]

In Vitro Screening of Natural Products and Chemical Compounds that Regulate Autophagy for Alleviating Sepsis

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Autophagy is an evolutionarily conserved degradation system that supports metabolic and innate immune homeostasis. In addition, since autophagy is closely related to the innate immune system, which can reduce excessive inflammatory responses, it is important to find regulators of autophagy. However, there is still a lack of clinically applicable medications that modulate autophagy in a precise manner. Here, this study aimed to identify potential novel therapeutic reagents for sepsis by screening natural products and chemical compounds that induce autophagy. We examined 1,679 natural product extracts and 2,423 chemical compounds with the autophagy LC3 HiBiT reporter assay to find novel autophagy inducers. Next, we identified three natural product extracts and fourteen chemical compounds as potential autophagy inducers through the concentration-based verification assays. To determine which natural product extracts and chemical compounds suppress inflammatory reactions, reporter HEK-Blue cell lines expressing TLRs 2, 3, 4, 5, 7, 8, or 9 were tested for their activity. Therefore, our findings suggest that selected natural product extracts and chemical compounds identified through in vitro screening of inducing autophagy have broad inhibitory effects on TLRs and may be effective in modulating excessive inflammatory responses.

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L. Infection & Immunology [L-11]

Deletion of TASL regulates imiquimod-induced psoriasis like inflammation

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TASL (TLR adaptor interacting with SLC15A4 on the lysosome), previously called as CXorf21 is recently defined as an endosomal TLRs signaling adaptor that mediates recruitment and phosphorylation of IRF5. Since TASL is an X-chromosome linked gene escaping X-chromosome inactivation, it has been suspected as a risk factor for autoimmune diseases. Although there are some predictive reports, accurate role of TASL on pathogenesis of autoimmune diseases have not been elucidate, yet. In this study the role of TASL in skin-related autoimmune disease, psoriasis was firstly identified using TASL knockout (KO) mice and IMQ-induced psoriasis model. TASL deficiency attenuated IMQ-induced psoriasis inflammation. Psoriatic lesions of TASL KO mice showed decreased epidermal hyperproliferation and differentiation and immune cell infiltration in dermis compared to wild type mice. In addition, activation of NF-κB and MAPKs which is responsible for psoriasis-associated keratinocyte proliferation and inflammation were reduced in TASL KO mice. Also, TASL deficiency decreased IMQ-induced IRF5 phosphorylation as expected. To evaluate keratinocyte function, TASL knockdown HaCaT cell was used. Calcium differentiated HaCaT cell showed upregulated expression of involucrin, the regulator of epidermal skin barrier. Therefore, it suggests that TASL positively regulates IMQ-induced psoriasis through promoting the NF-κB and MAPKs signaling pathways, as well as IRF5.

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L. Infection & Immunology [L-12]

Protective Effect of Aromadendrin on Ovalbumin-Induced Airway Inflammation in Mice

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Aromadendrin (ARO) exerts anti-inflammatory effects in activated-macrophages by suppressing the secretion of inflammatory molecules, such as cytokines, the expression of inducible nitric oxide synthase (iNOS) and the activation of NF-κB. Based on this effect, we investigated the protective effect of ARO in an experimental models of allergic asthma (AA). In PMA-stimulated A549 airway epithelial cells, the significant upregulation of cytokine/chemokine, such as IL-6, IL-8 and MCP-1 was effectively downregulated by ARO pretreatment. In addition, ARO regulated the PMA-induced activation of NF-KB in A549 cells. In *in vivo* study, ARO reduced the numbers of immune cells (eosinophil/macrophage) and the secretion of Th2 cytokines (IL-4, IL-5 and IL-13)/monocyte chemoattractant protein-1 (MCP-1)/immunoglobluin E (IgE) in an experimental mouse of AA. ARO also attenuated the expression of iNOS and cyclooxygenase-2 (COX-2) in lungs of AA mice. In the histological analysis, the increase in inflammatory cell influx into lung was suppressed by ARO (H&E staining) and the upregulation in mucus secretion was inhibited by ARO (PAS staining). Furthermore, ARO exerted regulatory effect on OVA-induced nuclear factor kappa B (NF-κB) activation in lungs of mice. These results reflect that ARO may be a valuable adjuvant in AA treatment.

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L. Infection & Immunology [L-13]

Digital spatial profiling in lungs of cynomolgous macaques experimentally infected with SARS-CoV-2

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Molecular analysis of SARS-CoV-2 infection in the respiratory organs including lungs is necessary to understand viral pathogenesis and discover clinical biomarkers. Gene expression patterns have been reported in the lungs of mice infected with SARS-CoV-2 using a recently developed spatial transcriptome analysis. However, similar experiments on non-human primates have been challenging to perform because of limited subject-appropriate experimental resources. Here, we reveal gene expression patterns in the lungs of cynomolgous macaques experimentally infected with SARS-CoV-2 using GeoMX ® Digital spatial profiling. The virus infected lungs showed a significant upregulation of genes associated with the inflammatory response, interferon and interleukin signaling, TNF alpha signaling via NFκB, TGF-β signaling, coagulation, complement, hypoxia and apoptosis pathways. The types and amounts of genes expressed in each pathway were different for alveoli, bronchioles, and blood vessels. This study revealed the spatial transcriptome profile of SARS-CoV-2-infected macaques' lungs using the human whole transcriptome atlas probe following histopathological classification of major tissue structures. These findings may assist in facilitating efforts of designing spatial transcriptome analysis in macaque models to aid in understanding the pathogenesis of SARS-CoV-2 variants or in evaluating therapeutics and vaccines against the virus.

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L. Infection & Immunology [L-14]

SINGLE-CELL ANALYSIS REVEALS CHARACTERISTICS OF MYELOID CELLS IN PULMONARY PASC

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In this study, we performed single-cell RNA sequencing (scRNA-seq) from peripheral blood mononuclear cells (PBMC) collected from individuals with pulmonary PASC (PPASC, n=2) and uninfected controls (Control, n=2). Matched control scRNA-seq data from open-sources was integrated to validate our results. Total 9 immune cells were identified based on expression of canonical markers. The proportion of myeloid-lineage-cells (MLC; CD14+/CD16+monocytes and dendritic cells) was increased in PPASC compared to controls. MLC from PPASC were up-regulated (VEGFA, TGF-β, CXCL8, OSM, and IL-1β) associated with fibrosis and inflammation. Similarly, pathway analysis showed that WNT, fibrosis, and cell death pathways were up-regulated, but immune pathways were down-regulated in PPASC. Also, we observed interactive genes (VEGFA, IL-1β, CXCL8, and OSM) among MLC and up regulation of downstream transcription factors and network modules linked to their interactions. Further analysis exploring metabolic analysis in MLC revealed that glycolysis/gluconeogenesis was down-regulated in PPASC. Our study highlighted that MLC were elevated in PPASC. MLC showed gene expression profiles favoring fibrosis, inflammation, and glycolytic suppression, which may serve as pathological drivers of PPASC.

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L. Infection & Immunology [L-15]

Interferon-Gamma Release Assay Combined with Renal Indicators to Improve the Diagnostic Accuracy of LTBI in Hemodialysis Patients

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Tuberculosis (TB), which is caused by *Mycobacterium tuberculosis* (MTB) is still maintaining high infection and mortality rates despite improving medical technologies. And latent TB infection (LTBI) is characterized by an asymptomatic situation of active TB and incessantly reacts to MTB. Currently, two of diagnostic methods are used to detect LTBI, which are the tuberculin skin test (TST) and interferon-gamma (IFN-γ) release assay (IGRA). However, even though IGRA tests id more specific and sensitive than TST, both of those methods could not distinguish LTBI from ATB accurately. Moreover, for patients who are immunosuppressed such as those suffering from hemodialysis (HD) due to chronic renal insufficiency, their IFN-γ level might be lower than the patients who only suffered from LTBI. In this study, 67 HD patients and 96 non-HD patients were enrolled, and their IFN-γ levels were measured by the QuantiFERON-TB Gold In-Tube (QFT-GIT) test. For compensate the lower IFN-γ levels in the IGRA test, three kidney function indicators were selected, which are blood urea nitrogen (BUN), serum creatinine (Cr), and estimated glomerular filtration rate (eGFR). Therefore, using serum Cr and eGFR, and using both of them could improve the previous deficiency of IGRA tests of low sensitivity.

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L. Infection & Immunology [L-16]

Detrimental effects of simulated microgravity on mast cell homeostasis and function

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Exposure to microgravity causes significant alterations in astronauts' immune systems during spaceflight; however, it is unknown whether microgravity affects mast cell homeostasis and activation. Here we show that microgravity negatively regulates the survival and effector function of mast cells. Murine bone marrow-derived mast cells (BMMCs) were cultured with IL-3 in a rotary cell culture system (RCCS) that generates a simulated microgravity (SMG) environment. BMMCs exposed to SMG showed enhanced apoptosis along with the downregulation of Bcl-2, and reduced proliferation compared to Earth's gravity (1G) controls. The reduction in survival and proliferation caused by SMG exposure was recovered by stem cell factor. In addition, SMG impaired mast cell degranulation and cytokine secretion. BMMCs pre-exposed to SMG showed decreased release of β-hexosaminidase, interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) upon stimulation with phorbol 12-myristate-13-acetate (PMA) plus calcium ionophore ionomycin, which correlated with decreased calcium influx. These findings provide new insights into microgravity-mediated alterations of mast cell phenotypes, contributing to the understanding of immune system dysfunction for further space medicine research.

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L. Infection & Immunology [L-17]

TLR inhibitor peptide mitigates ulcerative colitis through the functional modulation of macrophages

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Toll-like receptors (TLRs) play a crucial role not only in triggering innate responses against microbes but in orchestrating an appropriate adaptive immunity. However, deregulated activation of TLR signaling leads to chronic inflammatory conditions such as inflammatory bowel disease (IBD). In this study, we have evaluated the immunomodulatory potential of a TLR inhibitor in a form of a cell-penetrating peptide(cpTLR-i) using an ulcerative colitis animal model. cpTLR-i suppressed induction of pro-inflammatory cytokines such as TNF-α and IL-1β in macrophages. In DSS-induced colitis mice, cpTLR-i treatment ameliorated colitis symptoms, the colonic tissue damage, and mucosal inflammation. Intriguingly, cpTLR-i attenuated induction of TNF-α-expressing proinflammatory macrophages while promoting that of regulatory macrophages expressing arginase-1, along with reduced type 17 helper T cell (Th17) responses in the inflamed colonic lamina propria. *In vitro* study validated that differentiation of monocyte-driven macrophages into mature macrophages with a regulatory phenotype was enhanced by cpTLR-i in a microbial TLR ligand-independent manner. Furthermore, cocultivation of CD4 T cells with macrophages revealed that cpTLR-i suppressed activation of Th17 cells through the functional modulation of macrophages. Taken together, our data demonstrate the immunomodulatory potential of the TLR inhibitor peptide and suggest cpTLR-i as a novel therapeutic candidate for the treatment of IBD.

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L. Infection & Immunology [L-18]

Ubiquitin-specific Protease 4 Inhibits LPS-induced Inflammation by Regulating TAK1 Polyubiquitination in RainbowS Trout

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Deubiquitinase ubiquitin-specific protease 4 (USP4) plays an essential role in the negative regulation of the Toll-like receptor (TLR) signaling-mediated innate immune response. Although USP4 has been identified and actively studied in mammals, its role in TLR signaling pathways in fish remains largely unknown. Here, we investigated the role of USP4 (OmUSP4) in TLR response regulation in rainbow trout, *Oncorhynchus mykiss*. OmUSP4 contained the characteristic domains conserved in other USP4s: domain in USP (DUSP), ubiquitin-like (UBL), and the bi-part catalytic domain known as USP. In RTH-149 cells, the expression of OmUSP4 was increased by stimulation with fish-pathogenic bacteria and bacterial ligands. Gain-of-function and loss-of-function experiments showed that OmUSP4 down-regulates the activation of MAPK and NF-κB and the expression of pro-inflammatory cytokines in LPS-stimulated RTH-149 cells. OmUSP4 interacted with TAK1, a critical mediator in TLR-mediated NF-κB signaling pathways. LPS treatment increased the K63-linked polyubiquitination of TAK1 in RTH-149 cells, which was suppressed when OmUSP4 was forced expression. These findings suggest that OmUSP4, like those of mammals, reduces LPS-induced inflammation in rainbow trout, most likely by regulating the K63-linked ubiquitination of TAK1.

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L. Infection & Immunology [L-19]

Developing engineered Escherichia coli Nissle 1917 to treat Pseudomonas aeruginosa infection

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Broad-spectrum antimicrobials kill without discrimination, which may have adverse clinical effects and increase resistance. An autonomous antibacterial therapy that remains inert until a pathogen is detected and then releases antibacterial compounds on demand to kill the pathogen can be a potential solution. Herein, we aimed to develop genetically engineered *Escherichia coli* Nissle 1917 (EcN) that can detect and eradicate *Pseudomonas aeruginosa* by producing and secreting PA2-GNU7, *P. aeruginosa*-specific antimicrobial peptide, in response to *P. aeruginosa* quorum-sensing molecule N-3-oxododecanoyl homoserine lactone ($3OC_{12}HSL$). Plasmid-based systems were constructed to produce antimicrobial peptides in response to $3OC_{12}HSL$ and secrete them into the extracellular medium via the colicin V secretion system or using YebF as a carrier protein. After transferring these plasmid-based systems to EcN, we demonstrated the ability of the engineered EcNs to express and secrete PA2-GNU7, resulting in the killing of *P. aeruginosa*. This system may serve as a model for producing and delivering antimicrobial peptides to the site of the target pathogen infection.

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L. Infection & Immunology [L-20]

Development of novel antimicrobial peptides for the treatment of acne vulgaris

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Acne vulgaris is a common adolescent skin condition which is mainly caused by *Cutibacterium acnes* overcolonization and subsequent inflammation. Topical and oral antibiotics are commonly used to treat acne, but they predispose to overgrowth of antibiotic-resistant bacteria. Other current acne treatments can be accompanied by undesirable side effects. Thus, it is important to develop alternative agents with fewer adverse effects and high efficacy. In this study, we initially designed a short, 13-meric antimicrobial peptide by comparing amino acid sequences of peptides known to possess potent anti-*C. acnes* activity. Subsequently, we designed several analogs by substituting amino acids from the designed peptide to improve therapeutic potential. Among the designed peptides, AC7 peptide showed potent antimicrobial activity against antibiotics-susceptible and -resistant strains of *C. acnes*, without significant cytotoxicity. AC7 peptide decreased *C. acnes*-induced pro-inflammatory cytokines expression, and it was not digested by exposure to proteases found in the acne lesion for up to 12h. In addition, in a mouse model of acne vulgaris, AC7 exerted both anti-inflammatory and antimicrobial effects against *C. acnes*. These data suggested that AC7 peptide may be a promising candidate drug for the treatment of acne vulgaris.

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L. Infection & Immunology [L-21]

Revisiting the Role of Fibronectin in Dendritic Cells Maturation

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Fibronectin (FN) is a glycoprotein found in the extracellular matrix (ECM) of many tissues and plays an essential role in cell adhesion, migration, and differentiation. Dendritic cells (DCs) are professional antigen-presenting cells that continuously survey the tissue microenvironment for harmful antigens, migrate to lymph nodes, and present them to T cells, thereby initiating an adaptive immune response. Given the availability of ECM proteins like FN *in vivo*, it is likely that ECM influences DC development, migration, and activation status in lymphoid organs such as the spleen. In this study, we investigated the effect of FN on the maturation of bone marrow-derived DCs (BMDCs) by lipopolysaccharide (LPS). FN downregulated the expression of surface maturation markers, like CD86, CD80, CD40, MHC-I, and MHC-II with decreased production of pro-inflammatory cytokines such as TNF-α, IL12p40, and IL-6. FN inhibited LPS-induced maturation of BMDCs by interacting with α4β1 integrin receptor and its dephosphorylation of AKT and IKKα/β but not p38 and ERK1/2 kinases. The inhibitory capability of FN was overturned by using α4β1 inhibitor (BIO5192) or proteases from the supernatant of LPS-activated neutrophils. These findings suggest that FN can modulate DC through the complex interplay between the ECM and immune cells.

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L. Infection & Immunology [L-22]

Assessment of the efficacy/safety of a recombinant Japanese encephalitis virus E-protein vaccine in vivo

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Japanese encephalitis virus (JEV) is a flavivirus that is the causative agent of Japanese encephalitis, a serious neurological infection. The virus is primarily transmitted by mosquitoes. In humans, Japanese encephalitis can cause a wide range of symptoms, from mild flu-like symptoms to severe encephalitis, which can result in permanent brain damage or death. Those who develop symptoms may experience fever, headache, and paralysis. Recently, the number of JEV infections cases has been increasing in Korea, So there is a need for Japanese encephalitis prevention. To this end, we have selected Recombinant JEV E protein for their efficacy evaluation from multiple perspectives. The Envelope (E) protein that constitutes JEV has immunogenic properties as it induces production of protective antibodies. The Baculovirus system produces antigens with folding and modifications that are similar to the actual JEV E protein. So recombinant JEV E proteins have been selected as a final vaccine candidate antigen. Here, we present the results of evaluating the humoral immune response through PRNT and ELISA by administering the selected recombinant JEV-E protein to a mouse model, as well as evaluating the cell-mediated immune response of the vaccine through the ELISpot analysis, which showed limitations in previous diagnostic tests.

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L. Infection & Immunology [L-23]

Pilot-scale production with recombinant foot-and-mouth disease virus and determination tests of antigen amount for manufacturing trial vaccine

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Foot-and-mouth disease (FMD) is a highly contagious disease that is only transmitted to cloven-hoofed animals. FMD is caused by FMD virus (FMDV), which occurs mainly in livestock such as cattle, pigs, and goats. Since the large-scale outbreaks in 2010, Korea has implemented FMD vaccination policy, making it mandatory to vaccinate FMD susceptible livestock nationwide every year. Therefore, Korea has been developing a bivalent vaccine for serotypes O and A with the goal of localizing FMD vaccines. In this study, to determine the optimal amount of antigen for a bivalent vaccine, we produced antigen on a pilot scale (100 L) to produce a bivalent vaccine using two recombinant FMDV. Using the produced antigen, pigs were vaccinated and subjected to a virus challenge test to determine the optimal amount of antigen required for the production of the trial vaccine. The results showed that each O PA-2 and A22 IRQ vaccinated pigs inoculated with more than 10 μg of antigen and more than 5 μg, respectively, were protected against the FMDV challenge. The results of this study are expected to serve as an important basis for the domestication of FMD vaccines in Korea in the future.

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L. Infection & Immunology [L-24]

Imiquimod-induced immunogenic adipose tissue destruction in obese mice exacerbates psoriatic skin inflammation

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Obesity has been shown to be associated with psoriasis, which negatively impacts its prevalence, severity, and response to treatment. However, the systemic inflammatory microenvironment in obesity-associated psoriasis has not been extensively investigated. To explore this relationship further, we used a mouse model of psoriasis induced by the topical application of imiquimod. Our findings show that in obese mice, imiquimod-induced skin responses result in increased expression of inflammatory cytokines in the skin and higher levels of inflammatory mediators implicated in psoriasis pathogenesis in the serum. Interestingly, imiquimod treatment also significantly decreased body weight and fat, which was more pronounced in obese mice. Additionally, the subcutaneous fat layer was thinner in imiquimod-treated obese mice than in lean mice. Further analysis revealed higher macrophage infiltration, upregulation of genes related to the inflammatory response, and increased expression of necrosis-associated molecules in the perigonadal fat of imiquimod-treated obese mice than in lean mice. Based on these findings, we propose that the destruction of adipose tissue inflammation and accompanying systemic increase in inflammatory mediators account for the aggravation of psoriasis in obese individuals.

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L. Infection & Immunology [L-25]

Naringenin alleviates DSS-induced intestinal inflammation and fibrosis in mice with colitis

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The mechanism of naringenin in dextran sulfate sodium (DSS)-induced intestinal inflammation and fibrosis in mice with ulcerative colitis (UC) was investigated. Normal group, model group, 5-aminosalicylic acid group (5-ASA, 50 mg/kg) and naringenin group (30 mg/kg) were set up to induce UC model in mice by drinking water with 2% DSS freely for 7 days while administering drugs. The body weight, the ratio of colon weight to length, DAI scores, histopathological changes of the colon by H&E staining, mucous layer changes of the colon by Alcian Blue staining and glycogen PAS staining were compared among the four groups. Serum cytokine levels and antioxidant levels were detected by ELISA and colonic fibrosis-associated proteins expression were detected by Western blot. After naringenin intervention, the body weight of UC mice was significantly increased, the ratio of colon weight to length, DAI score and inflammatory response were significantly decreased (*PPP*

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L. Infection & Immunology [L-26]

A Novel resveratrol derivative inhibits IL-1β secretion in macrophages by targeting the NLRP3 inflammasome.

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NLRP3 inflammasome is a protein complex that plays a crucial role in triggering the immune response to harmful stimuli. However, when activated excessively, it causes acute and chronic inflammation, which can lead to various inflammatory diseases such as arthritis, sepsis, and inflammatory bowel diseases. Resveratrol is a natural polyphenolic compound found in plants and has been shown to possess anti-inflammatory and antioxidant properties. Thus, this study aims to investigate the therapeutic function of new resveratrol derivatives in macrophages. Among the diverse newly synthesized and purified resveratrol derivatives, a new therapeutic candidate was selected based on its NLRP3 inflammasome targeting feature. The resveratrol derivative, which was named RSV-B, significantly reduced IL-1β secretion and ASC speck production when treated to NLRP3 inflammasome-activated macrophages. Furthermore, the inflammasome inhibiting effect of the RSV-B was better than that of conventional resveratrol. From these findings, we believe that the novel resveratrol derivatives can offer a new class of drugs for treating inflammatory diseases by targeting NLRP3 inflammasome.

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L. Infection & Immunology [L-27]

A preliminary study on the effect of cornetin-3,5-O-biglucoside in ameliorating pentafluorouracil-induced chemotherapeutic intestinal mucositis in BALB/c mice

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To investigate the mechanism of the effect of centaureidin-3,5-O-biglucoside (C35G) on ameliorating pentafluorouracil (5-FU)-induced chemotherapeutic intestinal mucositis in mice.Forty-eight BALB/c male mice were randomly divided into 6 groups: CON group, 5-FU group, C35G prophylactic low and high dose group, and C35G therapeutic low and high dose group. The levels of TNF-α, IL-1β, IL-10 and IL-6 in the serum of mice in each group were measured by ELISA. H&E, PAS, and Alcian Blue staining were used to observe the colonic tissues of each group of mice to observe the pathological changes, goblet cell distribution and secretion of mucin 2 (MUC2). Western blot was used to detect the expression levels of WNT/β-catenin pathway related proteins in the colon. The results showed that C35G treatment significantly reduced the intestinal mucosal damage caused by 5-FU, significantly decreased the levels of TNF-α, IL-1β, IL-6 and increased the level of IL-10 in the serum of mice compared with the 5FU group. C35G significantly increased the expression of WNT/β-catenin pathway-related proteins in colon tissues and promoted the proliferation and differentiation of colon tissue cells and intestinal barrier restoration.C35G has a significant ameliorative effect on 5-FU-induced chemotherapeutic intestinal mucositis in mice.

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L. Infection & Immunology [L-28]

Cyanidin-3,5-O-diglucoside inhibits intestinal fibrosis in mice with experimental colitis by regulating Nrf2 signaling pathway

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To investigate the protective effect of cyanidin-3,5-O-diglucoside(C35G) on intestinal fibrosis in mice with Dextran sulfate sodium (DSS)-induced experimental colitis and its effect on Nrf2 signaling pathway. DSS was added to drinking water to induce colitis in C57BL/6 mice. The physiological indexes of mice were recorded. The levels of inflammatory factors in serum were determined by ELISA kit. HE and Masson were used to observe the histological changes. Western-blot was used to detect the protein expression of factors related to colonic fibrosis and NRF2 signaling pathway. qRT-PCR was used to explore the mRNA expression of oxidative stress factors. C35G can improve the weight loss and colon shortening, reduce the ratio of colon weight to length, and increase the DAI index score. Histological observation found that C35G could reduce the infiltration of inflammatory cells and alleviate the degree of fibrosis. C35G reduced the inflammatory factors IL-1β, IL-6, IL-17A, IL-18 and TNF-α. qRT-PCR results showed that C35G could increase the expression of oxidative stress factors Cat, Sod2, Mgst1, and Sod1. Western-blot results showed that C35G could inhibit the protein expression of α-SMA and Collagen-I, and increase the expression of p-Nrf2. C35G can exert anti-inflammatory and antioxidant properties by activating the Nrf2 pathway.

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L. Infection & Immunology [L-29]

Mitofusin 2 is a key coordinator of innate immune responses against intracellular bacterial infections in mouse macrophages

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Mitochondrial shaping protein mitofusin-2 (MFN2) plays a crucial role in innate immune responses during mycobacterial infection. This study aimed to investigate the mechanistic role of MFN2 in macrophages for innate host defense against intracellular bacterial infections. The study found that MFN2 promoted macrophage inflammatory signaling through optimal induction of aerobic glycolysis via hypoxia-inducible factor (HIF)-1α, triggered by bacterial infection. MFN2 was also required for the activation of xenophagy against Mycobacterium tuberculosis (Mtb) infection through HIF-1α. The study further demonstrated that MFN2 interaction with the late-endosomal protein Rab7 contributed to the activation of xenophagy during mycobacterial infection. We also showed that macrophages from Mfn2 CKO mice had a higher capacity to replicate intracellular bacterial colonies compared to macrophages from Mfn2 WT mice. Therefore, we suggested that MFN2 is a key coordinator of innate immune responses against intracellular bacterial infections through the maintenance of aerobic glycolysis and activation of xenophagy via HIF-1α. These findings provide new insights into the complex role of mitochondria in innate host defense against bacterial infections.

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L. Infection & Immunology [L-30]

Adipose tissue dendritic cells play an indispensable role in regulatory T cell features in obesity-induced inflammation

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Regulatory T cells (Tregs) residing in the visceral adipose tissue (VAT) play an important role in controlling tissue inflammation and alleviating metabolic diseases. However, obesity-specific phenotypic and functional characteristics of VAT Tregs and the mechanisms that shape VAT Tregs in obesity have not been clearly elucidated. Here, we found that obesity severely diminished VAT Tregs accompanied by lower ST2 and restrained proliferation. High expression of migratory molecules is exclusively observed in VAT Tregs but neither in subcutaneous adipose tissue (SAT) Tregs nor splenic Tregs. The VAT from obese mice is strongly enriched with adipose tissue dendritic cells (ATDCs) which express higher MHC II and CD86 costimulatory molecules and are inversely correlated to the VAT Tregs. While ATDCs from SAT and splenic DCs did not show any alteration of Treg differentiation between lean and obese condition, ATDCs isolated from VAT of obese mice reduce Treg differentiation with lower ST2 and IL-10 expression compared with ATMs from obese mice or ATDCs from lean mice. These data uncover ATDCs as the critical contributor to direct VAT Treg phenotype and function during obesity.

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L. Infection & Immunology [L-31]

A cell-based high throughput drug screening of putative compounds for inhibitors of SARS-CoV-2

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The COVID-19 pandemic caused by SARS-CoV-2 resulted in a public health emergency of international concern. Currently, the risk for severity of COVID-19 illness has been greatly reduced, but there is still a possibility of re-infection and immense socioeconomic impact. The numerous variants of SARS-CoV-2 carry key mutations that help it escape antibodies generated by prior infection and/or vaccines. The complex interplay of these COVID variants, vaccination and natural immunity makes epidemic trends far more difficult and less predictable. Consequently, there is still an emerging need to rapidly and accurately develop antiviral drugs for treatment. We implemented a robust screening strategy in both original wildtype and predominant omicron strain to quickly identify new compounds for therapeutic development. To improve hit selection and reduce false positives, we first used molecular fingerprints obtained from previous pilot screening against wildtype and omicron strain and then performed a scaffold similarity search in our diverse chemical space of over 100,000 compounds. We selected putative 6,393 compounds from our 11 libraries that have predicted virial inhibitory effect for rapid assessment in our anti-viral assays. As a proof of concept, we implemented a high-throughput screen to identify SARS-CoV-2 antiviral compounds for omicron strain using immunofluorescence-based cell imaging assay.

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L. Infection & Immunology [L-32]

Ginseng leaf extract attenuates allergic inflammatory responses by inhibiting pro-inflammatory cytokine expression but activating NF-κB/MAPK signaling

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Ginseng leaves contain highly concentrated bioactive ingredients, such as ginsenosides, and have been reported to be effective in the treatment of fungal infections, cancer, obesity, oxidative injury, and age-related diseases. Nevertheless, not much research has been conducted on the extent to which ginseng leaves have the effect of controlling allergic inflammation. For that reason, in this study, we aimed to determine the therapeutic effect of ginseng leaf extract (GLE) on allergic inflammation. To that end, we evaluated the anti-allergic and anti-inflammatory effects of GLE in RBL-2H3 by measuring ß-hexosaminidase and histamine levels, detecting intracellular ROS and NO, and conducting several molecular biological experiments using RT-PCR, western blotting, and ELISA. Our results show that GLE treatment significantly inhibited the transcriptional expression of COX-2, iNOS, and the pro-inflammatory cytokines including interleukin (IL)-4, IL-6, IL-1ß, and tumor necrosis factor (TNF)-α in RBL-2H3 cells. GLE also inhibited the production of IL-4, IL-6, TNF-α, IL-1β, LTC4, PGE2, and intracellular ROS and NO levels in activated mast cells. Our experiments also show that blockage of the NF-κB and MAPK pathways is required for these inhibitory effects to occur. Our findings suggest that GLE is a potential treatment for mast cell-mediated allergic inflammation such as AD.

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L. Infection & Immunology [L-33]

Anti- tuberculosis effect of Bacillus probiotic strain in mice

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Tuberculosis is one of the highly contagious diseases caused by Mycobacterium tuberculosis. In addition, the XDR (extensive drug-resistant) strain of Mycobacterium tuberculosis is gradually increasing, reaching the limit of existing antibiotic-based anti-tuberculosis drugs. Therefore, it is necessary to develop a new medical concept. In this context, probiotics have recently emerged as an alternative to antibiotics to treat antibiotic-resistant infectious diseases. Probiotics have been studied for the treatment of Clostridium difficile infections. It has recently been applied to the field of antibiotic-resistant super bacteria. However, more research on tuberculosis is needed. Based on this background, we are researching the development of anti-tuberculosis drugs using probiotics. In this study, the survival rate of a mouse model of extensive drug resistance (XDR) pulmonary tuberculosis was improved when Bacillus was inhaled. In addition, a 2-week repeated oral toxicity test using guinea pigs judged it to be non-toxic as there were no notable clinical symptoms or changes in body weight in guinea pigs. It showed similar activity to rifampicin, a first-group treatment for tuberculosis. Based on these experiments, the antitubercular activity of the Bacillus species was confirmed.

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L. Infection & Immunology [L-34]

Immune-cell derived extracellular vesicle in plasma as a potential biomarker for diagnosis of active tuberculosis

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Tuberculosis (TB), a respiratory disease caused by Mycobacterium tuberculosis (MTB), is the leading cause of death worldwide due to a single infectious agent. Current representative diagnostic assays for Active tuberculosis (ATB) take a considerable amount of time as well as low sensitivity and specificity. Therefore, a rapid and precise diagnostic method for TB is required. Extracellular vesicles (EVs), secreted by most cell types, are derived from the surrounding cell membrane and involved in cell-to-cell communication by carrying various functional biomolecules, including proteins, nucleic acids, and lipids. Herein, EVs isolated from immune cells in whole blood were proposed as potential biomarkers for TB diagnosis. Based on mRNA analysis and bioinformatic analysis of ATB patients and a healthy control (HC) group, the top four biomarkers were selected and expressed on the immune cell membrane of ATB patients. In addition, clinical analysis of immunoprecipitated EVs between ATB patients and healthy controls showed significant differences in expression and high diagnostic utility. Taken together, these TB-specific extracellular vesicle biomarkers were considered potential biomarkers for the novel ATB diagnostic method.

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L. Infection & Immunology [L-35]

Study on Inactivation of Foot-and-Mouth Disease Virus for Vaccine Production in South Korea

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Foot-and-mouth disease (FMD) is a highly contagious vesicular disease that affects cloven-hoofed animals, such as cattle and pigs, and often causes extensive economic damage to the livestock industry. FMD outbreaks have occurred several times in Korea since 2000, and the FMD vaccination has been administered to ungulates nationwide since December 2010. Because there is no domestic FMD vaccine, all FMD vaccines are imported. To localize the FMD vaccine in the near future, we have developed vaccine candidate strains using FMDV obtained in South Korea and internationally. FMD vaccines must be produced in a biosafety level 3 facility, so the FMDV must be completely inactivated after amplification. This study deals with four FMD vaccine candidate strains for validation of BEI treatment at different concentrations and temperatures to determine the optimal inactivation condition of each virus. Two domestic isolates, O/SKR/Boeun/2017 (O BE) and A/SKR/Yeoncheon/2017 (A YC), and two recombinant viruses, PAK/44/2008 (O PA-2) and A22/Iraq/24/64 (A22 IRQ), were investigated. The O BE and A22 IRQ required 2 mM BEI at 26°C and 0.5 mM BEI at 37°C for complete inactivation. The O PA-2 and A YC required 2 mM BEI at 26°C and 1 mM BEI at 37°C.

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L. Infection & Immunology [L-36]

Development of monoclonal antibody to specifically recognize VP0 not VP4 and VP2 of foot-and-mouth disease virus

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Foot-and-mouth disease (FMD) is a highly contagious disease, caused by FMD virus (FMDV). The capsid of FMDV consists of four structural proteins. Initially, one copy each of the proteins VP0, VP3, and VP1 are folded together into a protomer, and five copies of the protomer compose a pentamer. Finally, 12 pentamers are assembled into an icosahedral capsid. At the maturation stage during RNA encapsidation, VP0 is cleaved into VP4 and VP2. The mechanism underlying VP0 maturation remains unclear. While monoclonal antibodies (mAbs) against VP2 have been developed in previous studies, a mAb specific to VP0 has not yet been reported. In this study, we generated VP0-specific mAbs by immunizing mice with peptides spanning the C-terminal amino acids of VP4 and N-terminal amino acids of VP2. We verified that these mAbs displayed specificity to VP0 with no reactivity to VP4 or VP2. Therefore, these mAbs could prove useful in identifying the role of VP0 in FMDV replication and elucidating the mechanism underlying VP0 cleavage into VP4 and VP2.

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L. Infection & Immunology [L-37]

Establishment of an automatic quantification method for foot-and-mouth disease vaccine antigens using high performance liquid chromatography

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Foot-and-mouth disease (FMD), caused by the FMD virus (FMDV), is controlled by vaccine policy in many countries. For vaccine potency, the content of intact virus particles (146S antigens) is critical, and sucrose density gradient (SDG) centrifugation is the gold standard for the quantification of FMD vaccine antigens (146S). However, this method has several drawbacks. Recently, size-exclusion high performance liquid chromatography (SE-HPLC) was introduced to replace the SDG. The purpose of this study is to adjust the difference between the two methods and establish conditions that can remove the interfering signals in order to accurately measure the FMDV. The bovine enterovirus (BEV) was used as a standard material because the BEV has similarities in density and size to FMDV, while it displayed far better stability than FMDV. Crude cell infection supernatant (CCIS) and semi-purified samples with PEG precipitation (PEG-P) were evaluated. The non-specific signals were distinctively removed by applying chloroform and benzonase as a sample pretreatment method. This result could be used to quantify FMD vaccine antigens in domestic FMD vaccine manufacturing facilities in the future.

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L. Infection & Immunology [L-38]

Improvement of recombinant protein expression by modifying amino acid composition of foot-and-mouth disease virus VP4 protein

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Foot-and-mouth disease (FMD) is a highly contagious vesicular disease that affects cloven-hoofed animals, causing substantial economic losses to the livestock industry. The causative FMD virus (FMDV) comprises four structural proteins (VP1, VP2, VP3, VP4) and several non-structural proteins. Among these, VP4 is considered an ideal candidate with respect to its utility as a diagnostic protein or vaccine antigen for FMD, regardless of viral serotype. However, there is a difficulty in that VP4 is poorly expressed in *Escherichia coli.* Therefore, in this study, we attempted to express the N-terminus glutathione *S*-transferase (GST)-fused VP4 protein in *E. coli*. To investigate the effect of VP4 C-terminal amino acid residues on protein expression, we constructed three VP4 mutants fused to GST, among which the mutant in which the C-terminal 15 amino acid residues had been deleted showed the highest level of protein expression. Furthermore, protein expression was observed even in the mutant in which three amino acid residues (DKK) had been fused to the C terminus. However, unlike the other two mutants, the wild-type VP4 mutant was poorly expressed, thereby indicating that the C-terminal amino acid residues could play a pivotal role in determining expression of the VP4 protein in *E. coli.*

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L. Infection & Immunology [L-39]

Immunostimulatory Activity of Cordyceps militaris Fermented with Pediococcus pentosaceus SC11 Isolated from a Salted Small Octopus in Cyclophosphamide-Induced Immunocompromised Mice and Its Inhibitory Activity against SARS-CoV 3CL Protease

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In this study, we investigated the immune-enhancing and anti-viral effects of germinated *Rhynchosia nulubilis* (GRC) fermented with *Pediococcus pentosaceus* SC11 (GRC-SC11) isolated from a salted small octopus. The cordycepin, β-glucan, and total flavonoid contents increased in GRC after SC11 fermentation. GRC-SC11 inhibits 3CL protease activity in severe acute respiratory syndrome-associated coronavirus (SARS-CoV). GRC-SC11 significantly increased thymus and spleen indices in immunocompromised mice. The rate of splenocyte proliferation was higher in GRC-SC11-treated immunocompromised mice than that in GRC-treated immunocompromised mice in the presence or absence of concanavalin A. In addition, GRC-SC11 increased the phagocytic activity and nitric oxide production in immunocompromised mice. The mRNA expression of interferon-gamma (IFN-γ), interferon-alpha (IFN-α), and interferon-stimulated gene 15 (ISG15) was up-regulated in GRC-SC11 treated RAW 264.7 macrophages, compared to GRC. Our study indicates that GRC-SC11 might be a potential therapeutic agent for immunocompromised patients who are vulnerable to SARS-CoV infection.

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L. Infection & Immunology [L-40]

Generation of 3D Culture Platforms as an Alternative System to Animal Models for SARS-CoV-2 Infection

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Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a worldwide pandemic since its infection was first reported in China in December 2019. To rapidly respond to a newly emerging respiratory virus, there is a need to develop *in vitro* human infection models which mimic *in vivo* conditions. In this study, we demonstrated the establishment of Palatine tonsil (hereinafter referred to as "tonsil") organoid and 3-dimensional bioprinted airway models and the evaluated their antiviral activity using those models. Through optimization of culture media supplements, we achieved three-dimensional tonsil organoid culture from human tissues. The organoids successfully recapitulated the characteristics of the tonsil epithelium, such as its cellular composition, histologic properties and biomarker distribution. Notably, we verified that the basal layer cells of tonsil organoids express key molecules for virus entry, angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2), and are susceptible to the viral infection, resulting in robust viral RNA replication and secretion of progeny viral particles. Here, we suggest that tonsil epithelial organoids could provide a preclinical and translational research platform for investigating SARS-CoV-2 infection-mediated pathology or for evaluating antiviral candidates or immune regulatory molecules.

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L. Infection & Immunology [L-41]

HDAC6 and CXCL13 mediate Atopic Dermatitis by regulating Cellular Interactions and Expression levels of miR-9 and SIRT1

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Histone deacetylase 6 (HDAC6) has been known to regulate inflammatory diseases. We studied the role of HDAC6 in atopic dermatitis (AD) and the mechanisms associated with it. The decreased expression or chemical inhibition of HDAC6 suppressed AD by decreasing autophagic flux and cellular features of AD. AD increased expression levels of the Th1 and Th2 cytokines, but decreased expression levels of forkhead box P3 (FoxP3) and interleukin-10 (IL-10) in an HDAC6-dependent manner. CXC chemokine ligand 13 (CXCL13), which was increased in an HDAC6-depenednt manner, mediated AD. MiR-9, negatively regulated by HDAC6, suppressed AD by directly regulating the expression of sirtuin 1 (SIRT1). The downregulation of SIRT1 suppressed AD. Experiments employing culture medium and transwell suggested that cellular interactions involving mast cells, keratinocytes, and dermal fibroblast cells could promote AD; HDAC6 and CXCL13 were found to be necessary for these cellular interactions. Mouse recombinant CXCL13 protein increased HDAC6 expression in skin mast cells and dermal fibroblast cells. CXCL13 protein was found to be present in the exosomes of DNCB-treated skin mast cells and these exosomes enhanced invasion potentials of keratinocytes. These results indicate that HDAC6 and CXCL13 may serve as targets for the developing anti-atopic drugs.

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L. Infection & Immunology [L-42]

Engineering influenza NS1 and M2e Based broad spectrum Influenza Vaccine Candidate

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Influenza viruses are one of the major viruses that cause public health issues every winter around the world. Because of the rapid mutation on major antigen sites of the Influenza A virus(IAV), a new flu vaccine is produced every year. This vaccine system presents the inconvenience of producing and administering a new vaccine every year. Moreover, there is a possibility that vaccine strains may not match the circulating viruses, and an epidemic may occur due to the emergence of a novel influenza virus. Then, it is necessary to develop a universal vaccine. The IAV NS1 protein is known to promote viral replication by inhibiting host innate immune responses. Recently, a truncated NS1 gene was found in an equine influenza virus and we are utilizing this NS1 gene to develop a universal vaccine platform. In addition, the M2e of the IAV is known as a highly conserved domain, so many researchers target this region as a universal flu vaccine. We engineer the truncated NS1 gene to include the foreign antigen and insert the M2e gene as the universal antigen, it can be utilized as an effective universal flu vaccine.
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L. Infection & Immunology [L-43]

Synergistic anti-inflammatory effects of ethanol extracts from Chrysanthemum zawadskii flower and Cudrania tricuspidata fruit occur via inhibition of the NF-κB signaling pathway

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Chrysanthemum zawadskii (CZ) and *Cudrania tricuspidata* (CT) are both traditional Korea herbal medicines, which is widely used to treat diseases linked to inflammation. Our data revealed that ethanol extracts of CZ flower reduced LPS-induced intracellular reactive oxygen species, and decreased the LPS-induced up-regulations of the mRNAs iNOS, COX-2, and IL6 in RAW264.7 cells at a concentration of 100 μg/ml. An ethanol extract from CT fruit exhibited free radical scavenging capacity *in vitro*, and 5.5 μg/ml of the extract significantly suppressed LPS-induced the mRNA expression levels of iNOS and IL6. Furthermore, as little as 1 μg/ml of the combined ethanol extracts of CZ flower and CT fruit reduced the LPS-induced up-regulations of iNOS and IL6, and decreasing the nuclear localization of NF-κB p65. These results suggest that the observed synergistic anti-inflammatory effects mediated via inhibition of NF-κB signaling. Taken together, these data suggest that ethanol extracts from CZ flowers and CT fruits have synergistic anti-inflammatory effects, and that a combination of the two extracts could prove useful for the treatment of inflammation-related diseases.

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L. Infection & Immunology [L-44]

Antioxidant and Immune Enhancement Effects of Fermented Artemisia argyi H.

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Artemisia argyi H. (*A. argyi* H.) contains various flavonoids, exerting the antioxidant, anti-inflammation, and immunological activities. The aim of this study was to demonstrate antioxidant and immune enhancement effects of *A. argyi* H. fermented by Lactic acid bacteria. An *in vitro* study was conducted to evaluate antioxidant effects of fermented *A. argyi* H. (FAA) including scavenging activity of hydroxyl radical (·OH) and superoxide radical (O_2^-). Moreover, to determine immunostimulatory effects, we evaluated lymphocytes proliferation and T helper (Th) cell cytokines *in vivo*. The mice were divided into 4 groups: Normal (fed a control diet), FAA1, 2, and 5 group (fed a diet consisted of freeze-dried powder of FAA 1%, 2%, and 5%, respectively). In the results, FAA showed high scavenging activity against ·OH and O_2^-. Furthermore, in the FAA5 group, lymphocytes proliferation and the production of IFN-γ, IL-10, and TNF-α were significantly increased compared with the normal group. Particularly, the release of IFN-γ, which is a Th type 1 cytokine, was prominently stimulated in FAA5 group. It meant to differentiate of Th cell into Th type 1 cell. These findings suggest that FAA can be a dietary antioxidant and immunostimulatory agent.

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L. Infection & Immunology [L-45]

Discovery of biomarkers for the diagnosis and treatment of atopic dermatitis using non-invasive methods.

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Atopic dermatitis(AD), which is caused by allergic inflammation and abnormalities in the skin's stratum corneum, often recurs despite treatment with antihistamines, steroid medications, immunosuppressants, and moisturizers, making it difficult to predict the course of the disease. Therefore, we aimed to overcome the limitations of conventional research methods by using a non-invasive method called tape stripping to collect samples and identify the biomarkers necessary for diagnosing atopic dermatitis. Lesional and nonlesional tape-stripped skin was sampled from AD patients before and after treatments and from healthy subjects and analyzed in the expression of mRNA and proteins. Protein or mRNA extraction was performed using the stratum corneum layer obtained from each sample, and Filaggrin, Involucrin, Loricrin, which are markers for keratinocyte differentiation, as well as Claudin, which are tight junction proteins of epithelial cells, were compared using western blot or Real-time PCR. The experimental results showed differences in the expression each marker. Thus tape-strips can be used to evaluate molecular changes with the treatment in AD. Therefore, Tape-strips are an useful non invasive approach to characterize skin biomarkers in atopic dermatitis reflecting therapeutic response in AD patinets before and after treatment including Interleukin inhibitors

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L. Infection & Immunology [L-46]

Chemical mimetics activating p62/SQSTM1 inhibits inflammatory responses and induces mitophagy in macrophages

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The Arg/N-degron pathway is known to be involved in the degradation of proteins containing an N-terminal signal peptide, and it is connected to p62/SQSTM1-mediated autophagy. However, the relationship between this pathway and systemic inflammation is poorly understood. This study aimed to investigate the potential of ATB1021, a p62 ligand that mimics the N-degron Nt-Arg pathway, in decreasing mortality and attenuating pathological inflammation by regulating mitochondrial homeostasis *in vitro* and *in vivo*. Our results demonstrate that ATB1021 alleviates systemic inflammation of lipopolysaccharide-induced septic shock, sepsis induced by cecal ligation and puncture, and acute lung injury in the mouse model. Additionally, ATB1021 can attenuate proinflammatory cytokines and chemokines in macrophages against various innate immune stimuli by activating mitophagy and inhibiting mitochondrial reactive oxygen species production in p62 dependent manner. Together, our findings suggest that ATB1021 plays a crucial role in regulating inflammatory responses by orchestrating mitochondrial homeostasis through activating mitophagy and scavenging mitochondrial reactive oxygen species.

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L. Infection & Immunology [L-47]

Aerial parts of Adenophora triphylla exert Immunostimulatory Activity in Mouse Macrophages, RAW264.7 Cells

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In this study, we evaluated the functionlity of extracts form the Aerial parts of *Adenophora triphylla* (AAT). The AAT fo the Adenophora family and is distributed in China, Russia and Korea. However, only a few studies have investigated on the mechanism of action of immune-enhancing activity of ATT. we evaluated whether ATT immune activation and elucidate its potential mechanism in mouse macrophages ATT. ATT increased the production of immunomodulators such as nitric oxide (NO), inducible nitric oxide (iNOS), cyclooxygenase-2 (COX-2), tumornecrosis factor-α (TNF-α) and interleukin-1β (IL-1β) activated in RAW264.7 cells. The inhibition of toll-like receptor 4 (TLR4) blocked ATT mediated production of immunomodulators in RAW264.7 cells. Taken together, demonstrated that TLR4-MAPKs/NF-κB signaling pathways participated in ATT induced macrophage activation and ATT could be developedas apotential immunomodulating functional food.

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L. Infection & Immunology [L-48]

Evaluation of Anti-mycobacterial efficacy of Bacillus strain and characterizing probiotics-derived Extracellular Vesicles

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Tuberculosis is still a severe disease. About 25 percent of people are infected with *M. tuberculosis* worldwide. The development of new substance characteristics and screening of new drugs are needed. We describe an assay applicable to the screening and the anti-mycobacterial efficacy of probiotics libraries against *M. tuberculosis*. *Bacillus* strain reduced intracellular *Mycobacterium* and drug-resistant strains. It was confirmed that *Bacillus* reduced intracellular *Bacilli* due to increased autophagy through increased lysosomes in cells. Extracellular vesicles (EVs) secreted from probiotics, defined as live microorganisms with beneficial effects on the host, are expected to be new nanomaterials for EV-based therapy. To clarify the usability of EV-based treatment, consider the possibility of developing new microbiome-based drugs. Thus, the Food Grade Medium (FGM) consists of safe ingestible ingredients for human and animal consumption, and a fermentor system was used for scale-up before isolating EVs ultracentrifugation. *Bacillus*-derived EVs secreted tiny amounts of particles, whereas all the EVs showed comparable particle sizes, ranging from 20 to 50 nm. This study has the advantage of being differentiated from existing studies, such as researching probiotics-derived EVs. We are considering the possibility of developing new microbiome-based drugs in the future.

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L. Infection & Immunology [L-49]

Immunostimulatory Activity of Chrysosplenium flagelliferum extract in Mouse Macrophages, RAW264.7 Cells

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Under the COVID-19 pandemic, interest in immune enhancement is increasing. In this study, we investigated whether *Chrysosplenium flagelliferum* extract (CFE) exhibits immunostimulatory activity in RAW264.7 cells. The CFE is distributed throughout Korea except for Jeju Island, and is distributed in Amur, Ussuri, Manchuria, and Japan in Russia. Because it is a biological resource that can be used for ornamental, edible and medicinal purposees, it has research value. but the potential mechanism for the immune activation of the CFE still insufficient. CFE increased the production of immunomodulators such as nitric oxide (NO), inducible nitric oxide (iNOS), cyclooxygenase-2 (COX-2), tumornecrosis factor-α (TNF-α) and interleukin-1β (IL-1β) activated in RAW264.7 cells. TLR2 and TLR4 blocked CFE-mediated production of immunostimulatory factors in RAW264.7 cells. In addition, the inhibition of MAPK signaling pathway reduced CFE-mediated production of immunostimulatory factors. From these results, CFE is considered to have immunostimulatory activity through TLR2/4-mediated activation of MAPKs signaling pathway. Based on these results, CFE is expected to be used as a potential functional agent for immune enhancement.

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L. Infection & Immunology [L-50]

Development of anti SARS-CoV-2 virus Neutralizing Antibodies

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SARS-CoV-2 receptor binding domain (RBD) is a viral protein that plays a major role in SARS-CoV-2 viral infection and is widely known as a target for neutralizing antibodies. Many researchers have done and still do research that blocks RBD.

In early 2020, when the SARS-CoV-2 virus began to spread, YNTOAB Co., Ltd. made an immune library using PBMC of wild type SARS-CoV-2 virus patients and developed neutralizing antibodies using it. The antibodies developed in this way showed neutralization ability not only of the wild type but also of the omicron variant.

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L. Infection & Immunology [L-51]

Development of anti Canine Parvovirus2(CPV2) Neutralizing Antibodies

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CPV type 2 (CPV 2) emerged during the 1970 s and has rapidly spread worldwide CPV 2 causes diarrhea and often death, especially in puppies Vaccination is currently the most effective and important approach, and efficacious antiviral therapies are required in the field therefore, antibody therapy may represent an alternative strategy for the prevention of CPV 2.

An Antibody based approach is promising in CPV 2 associated diagnosis, therapy and prevention. The relevant hyper immunoglobulin G and full length monoclonal antibody mAb have been routinely applied in veterinary practices. We constructed a canine single chain variable region fragment scFv phage display library using cDNA from the splenocytes of the dog previously immunized against CPV 2 (vaccine strain). The scFv was cloned into the mammalian cell line and purified.

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L. Infection & Immunology [L-52]

Development of anti Severe Fever with Thombocytopenia Syndrome (SFTS) virus antibodies

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SFTS was identified in China in 2009 and is a tick-borne viral infection disease that is widely distributed not only in Korea but also in other East Asian countries such as China and Japan, Australia and New Zealand. Since it is a new infection disease, it is difficult to respond effectively in the clinical field due to a lack of academic evidence and clinical experience so far.

YNTOAB Co., Ltd. produced an SFTS immune antibody library using PBMC of patients with severe febrile thrombocytopenia syndrome to make antibodies to SFTS N protein that can be used for diagnosis and to Gn that can be used for treatment.

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L. Infection & Immunology [L-53]

Resistance of hypervirulent Klebsiella pneumoniae to caspase-1-mediated pyroptosis in murine macrophages

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Hypervirulent *Klebsiella pneumoniae* (hvKp) has emerged as a concerning global pathogen in the last decade. However, the host immune responses of the macrophages during hvKp infection are largely unknown. The main aim of the present study was to observe the cytotoxic effect of hvKp compared with the classic *Klebsiella pneumoniae* (cKp) strain within murine macrophages. During hyKp and cKp strains infection, cytotoxicity was higher in cKp strains as a result of measuring sub-G1 population and lactate dehydrogenase (LDH) release in bone marrow-derived macrophages (BMDMs) and RAW264.7 cells. In addition, we found that cKp-infected cells showed higher mRNA expression levels of interleukin (*Il)1b* and *Il18* compared with hvKp-infected cells. Next, we found that the activation of caspase-1/gasdermin D-dependent pyroptosis was higher in cKp-infected macrophages compared with that in hvKp-infected macrophages. These data suggest that hvKp controls the host immune responses differently compared with cKp to maintain survival within the macrophages. Based on these findings, caspase 1-mediated pyroptosis is a potential new target for regulating host immune responses during hvKp infection.

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L. Infection & Immunology [L-54]

Periodontitis Increases Risk of Diabetes in Systemic Manner through Immunological Abnormalities

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Periodontitis (PD) is a chronic inflammatory disease persistently affecting the whole body with low-grade stimuli and increasing the risk of type 2 diabetes mellitus (DM). Although there is extensive knowledge that DM predisposes individuals to PD, little is known about connections from PD to DM at the cellular level. We investigated the immunological response of PD and comorbid PDDM status in a cell-specific manner through single-cell RNA sequencing and found distinctive and common immune coordinating systems. First, monocyte showed elevated antigen-presenting and higher secretion of cytokines, prompting inflammation in both PD and PDDM. Second, PD exhibited terminal differentiation stage similar to PDDM in NK cells and CD8+ effector T cells, which lost the cytotoxic attributes and became exhausted. However, PD status struggled to protect the body against pathogens than PDDM. In the cell-to-cell signals, there were similar networks between PD and PDDM, particularly, the RESISTIN pathway, which increases insulin resistance. The pathway generated an identical cellular network in both PD and PDDM, separating from the healthy group. In conclusion, a comparison of periodontitis and comorbid conditions explain the mechanisms by which periodontitis causes susceptibility to diabetes, along with immunological abnormalities by comorbid conditions.

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L. Infection & Immunology [L-55]

Elucidation of microglia-specific correlation between type 1 interferon signaling pathway and the inflammasome response

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Elucidation of microglia-specific correlation between type 1 interferon signaling pathway and the inflammasome response

Microglia are the brain's resident population that function in immune responses. Several studies report upregulation of the interferon (IFN) pathway in microglia in neuropathological conditions. However, its direct contributory roles to CNS disease pathogenesis remain to be elucidated. We incorporated methods of single-cell RNA-seq, in conditions of systemic inflammation induced by LPS injection unto mice with wild-type and *NLRP3* knockout genotypes, to delineate the microglial response to an inflammatory insult. Along with the upregulation of interferon-stimulated genes (ISGs) in inflamed conditions, the downregulation of the enlisted ISGs in knockout samples was noticeable. Although crosstalk between the two pathways has been previously reported, no reports of molecular mechanisms regarding this interaction exist. Therefore, the objective of this study lies, first in the elucidation of the correlation between the two pathways in microglia. Second, to ascertain the specific factors that lead to this phenotype, and whether this correlation is elicited due to the innate mechanisms of microglia or rather a consequential outcome influenced by CNS-specific environments. Therefore, this study aims to unravel the explicit cellular state of microglia regarding IFN signaling in neuroinflammatory conditions.

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L. Infection & Immunology [L-56]

NLRP3 intercellular communication via extracellular vesicles exacerbates acute infections

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The NLRP3 inflammasome is an important protein in acute and chronic inflammatory diseases, and extracellularly released NLRP3 particles are detected in patients with various diseases. However, whether NLRP3 is released via extracellular vesicles and its accurate biological function have not been clearly demonstrated. We determined that the NLRP3 inflammasome signaling pathway was activated in sepsis patients and animal models and released through extracellular vesicles. Based on a peptide system mimicking extracellular vesicle-mediated NLRP3 transmission, we confirmed that IL-1β maturation and pyroptosis accelerated and amplified via enhancing MAVS aggregation. In acute infectious diseases such as sepsis, the extracellular transmission of NLRP3 severely deteriorated survival rate by amplifying the cytokine storm and promoting the release of intracellular components including damage-associated molecular patterns. Our results provide new insights that the transmission of NLRP3 via extracellular vesicles can exacerbate acute infectious diseases.

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L. Infection & Immunology [L-57]

IRG1/itaconate reinforce IgE isotype switching through STAT6 phosphorylation in B cells

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The mitochondrial enzyme Immune-responsive gene 1 (IRG1), also known as aconitate decarboxylase 1, produces itaconate and has been identified as a new regulator of immune metabolism in inflammation and infection. However, the specific role of IRG1/itaconate in B cells has yet to be fully understood. In this study, we examined the expression of IRG1 in B cells and investigated the effects of IRG1/itaconate on Ig isotype switching and Ab production in mouse B cell cultures *in vitro*. Our findings demonstrate that itaconate selectively enhances IL-4-induced IgE/IgG1 isotype switching and production, as well as the expression of the IL-4-induced circle transcript ε-γ1, which is a molecular marker for sequential switching to IgE via IgG1. Moreover, we observed that IRG1 deficiency specifically reduces IL-4-induced IgE/IgG1 isotype switching and production in B cells. In addition, itaconate enhanced IL-4-induced STAT6 phosphorylation, a critical transcription factor for IgE isotype switching, while IRG1 deficiency inhibited STAT6 phosphorylation in B cells. These findings suggest that IRG1/itaconate selectively enhance IL-4-induced IgE isotype switching through STAT6 phosphorylation, resulting in enhanced IgE production by B cells.

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L. Infection & Immunology [L-58]

Validation of AgNPs for the treatment of myelitis_in vitro

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Background: Myelitis is a disease in which bacteria invade the bone marrow, mainly consisting of fecal resection and antibiotic treatment, but problems such as antibiotic resistance occur. Therefore, in this study, the AgNPs effect was analyzed through in vitro experiments for use in the treatment of myelitis. **Method:** The antibacterial effect of AgNPS is confirmed through an anti-bacterial test using Methicillin-resistant Staphylococcus aureus. After that, calvaria bone of SD rat is collected and osteoblasts are cultured. After securing osteoblasts, the therapeutic effect is confirmed through the expression analysis of biomarkers related to infectious inflammatory diseases through cell characterization and cell molecular biological tests. **Results:** Antibacterial test confirmed that AgNPs have antibacterial properties. When AgNPs were treated in osteoblasts by concentration, the living cell survival rate tended to increase at 50 ppm and 100 ppm than 0 ppm, and the living cell survival rate tended to decrease at concentrations of 1000 ppm or more. As a result of RT-PCR, COX-2 tended to be high at 100 ppm, and TNF-a tended to be low at a concentration of less than 1000 ppm. **Conclusion**: This study suggests that AgNPs can be used to treat myelitis.

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L. Infection & Immunology [L-59]

Appropriate methods and circumstances for quantifying itching in an imiquimod-induced psoriasis mouse model.

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Psoriasis is an inflammatory skin disease that causes itching, redness, and scaling. Psoriasis entails not only various complications but also psychological and economic burdens. The severity of psoriasis has also been linked to high suicide rates. Therefore, early diagnosis and treatment are crucial in managing the condition effectively. Itching is a critical indicator of psoriasis severity and is generally induced by IgE. However, measuring the concentration of IgE in the blood is not enough to assess the frequency and intensity of itching. Therefore, observing the frequency of scratching behavior is the most effective way to assess itching in psoriasis patients. To accurately identify and quantify scratching behavior, we used DEEPLABCUT, a deep learning program, to track mouse behavior in a skin inflammation experiment. We created a shooting cage with different materials and found that transparent and reflective cages were not suitable for filming. The minimum frame rate required to capture the movement of the mouse's feet was 120 fps. Through this study, it will be possible to clarify the criteria for evaluating itching in the mouse model.

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L. Infection & Immunology [L-60]

Ring Finger Protein 178 (RNF178) Negatively Regulates the Type I Interferon Signaling by Targeting TBK1

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RNF178 is an E3 ubiquitin ligase which consist of an N-terminal cytoplasmic tail (CT) domain containing a C4HC3 RING finger (RING-CH finger) motif, two transmembrane (TM) domains, and a C-terminal CT domain. RNF178 is a well-known E3 ligase in the regulation of host immune response by different mechanisms. In this study we present a novel mechanism of RNF178 mediated regulation of host immune response. First we found that knockdown of RNF178 abolish the DNA and RNA virus replication and enhance the cytokine secretion. Furthermore, RNF178 deficient mice were less susceptible than their wild-type counterparts to SeV infection. Mass-spectrometry analysis shows that RNF178 interact with TBK1 upon late time after virus infection. Moreover, domain study found that 192-218 aa region of RNF178 interacts with the 100-200 aa region of TBK1 and this interaction disrupts the Ser172 phosphorylation of TBK1 which inhibit the activation of IFN signaling pathway. Finally, we showed that RNF178 192-218 aa deletion mutant was unable to inhibit the host immune response and enhance DNA and RNA virus replication compared to RNF178. In conclusion, our study presents a novel mechanism of RNF178 mediated inhibition of host immune response by targeting TBK1. [The National Research Foundation of Korea (2021R1A6A1A03045495, RS-2023-00209214)]

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L. Infection & Immunology [L-61]

MyD118 promotes antiviral innate immunity by facilitating G3BP-mediated stress granule condensation

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Stress granules (SGs) are dynamic cytoplasmic foci that assemble in response to different types of stresses, including viral infection, and serve as a signaling hub, that plays a critical role in the type I interferon response to virus infection. G3BP is a major nucleator and organizer of SG assembly and regulates SG formation. Here, we report that myeloid differentiation primary response protein, MyD118 is a novel positive regulator of SGs-mediated interferon signaling by targeting G3BP upon viral infection. MyD118 interacts directly with the RNA binding domain of G3BP and leads to the conformational expansion of G3BP by the dissolution of its autoinhibitory electrostatic intramolecular interaction. In addition, the acidic loop 1 and RNA binding properties of MyD118 markedly increase the conformational expansion and RNA-binding affinity of G3BP-MyD118 complex, respectively. Importantly, MyD118 deficiency significantly impairs the SG formation and SG-mediated activation of RLR signaling *in vitro*. MyD118 knockout mice are highly susceptible to RNA virus infection and a severely impaired potential to induce interferon and cytokine production. These findings suggest a novel role for MyD118 as a critical regulator of G3BP-mediated SG formation, which facilitates RLR-mediated interferon signaling. [NRF of Korea (Grant no. RS-2023-00209214 and 2021R1A6A1A03045495) and KRIBB Program (KGM9942011)]

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L. Infection & Immunology [L-62]

Foot-and-Mouth Disease Virus Protein 2B Targets RIG-I And MDA5 for the Suppression of RLR Signaling Pathway

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Foot-and-mouth disease virus (FMDV) is an RNA virus containing 13 proteins. Many of these proteins show immune modulation capabilities. As a non-structural protein of the FMDV, 2B is involved in the rearrangement of the host cell membranes and the disruption of the host secretory pathway as a viroporin. Previous studies have also shown that FMDV 2B plays a role in the modulation of host type-I interferon (IFN) responses through the inhibition of expression of RIG-I and MDA5. However, the exact molecular mechanism is poorly understood. Here, we demonstrated that FMDV 2B modulates host IFN signal pathway by the degradation of RIG-I and MDA5. FMDV 2B targeted the RIG-I for ubiquitination and proteasomal degradation by recruiting E3 ubiquitin ligase RNF125 and also targeted MDA5 for apoptosis-induced caspase-3- and caspase-8-dependent degradation. Ultimately, FMDV 2B significantly inhibited RNA virus-induced IFN-b production. Importantly, we identified that the C-terminal amino acids 126-154 of FMDV 2B are essential for 2B-mediated degradation of the RIG-I and MDA5. Collectively, these results provide a clearer understanding of the specific molecular mechanisms used by FMDV 2B to inhibit the IFN responses and a rational approach to virus attenuation for future vaccine development. [National Research Foundation of Korea (2018M3A9H4079660, 2021R1A6A1A03045495)]

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L. Infection & Immunology [L-63]

Foot-and-Mouth Disease Virus 3Cpro Mediated Degradation of RIG-I and MDA5 Suppress the Host Type-I Interferon Pathway

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3C protease ($3C^{pro}$), a chymotrypsin-like cysteine protease encoded by the foot-and-mouth disease virus (FMDV), plays an essential role in processing the FMDV P1 polyprotein. Here, we demonstrated that the protease activity of $3C^{pro}$ contributed to the degradation of RIG-I and MDA5, key cytosolic sensors of the type-I IFN signaling cascade in proteasome, lysosome and caspase-independent manner. And also, we examined the degradation ability on RIG-I and MDA5 of wild-type FMDV $3C^{pro}$ and FMDV $3C^{pro}$ C142T mutant which is known to significantly alter the enzymatic activity of $3C^{pro}$. The results showed that the FMDV $3C^{pro}$ C142T mutant dramatically reduce the degradation of RIG-I and MDA5 due to weakened protease activity. Thus, the protease activity of FMDV $3C^{pro}$ governs its RIG-I and MDA5 degradation ability and subsequent negative regulation of the type-I IFN signaling. Importantly, FMD viruses harboring $3C^{pro}$ C142T mutant showed moderate attenuation of FMDV in a pig model. In conclusion, our results indicate that a novel mechanism evolved by FMDV $3C^{pro}$ to counteract host type-I IFN responses and a rational approach to virus attenuation could be utilized for future vaccine development.
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L. Infection & Immunology [L-64]

NQO1 Suppress Host Innate Immune Response by Inhibiting TBK1 Self-Association and Phosphorylation

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TANK-binding kinase 1 (TBK1) is an essential kinase that phosphorylates transcriptional factors, which induce antiviral interferons and inflammatory cytokines against viral infection. We report that NAD(P)H:quinone-oxidoreductase-1 (NQO1) forms a negative feedback loop with TBK1 to regulate innate antiviral immunity. Depletion or reduced expression of NQO1 increased secretion of antiviral cytokines and resulted in less viral replication, while overexpressed NQO1 increased viral replication with less activation of innate immune signaling molecules. NQO1 knockout mice showed more secretion of antiviral cytokines and resistance to virus infection than wild-type. In addition, we showed that NQO1 binds to TBK1 at late time after infection and interferes with the self-association of TBK1, resulting in a reduced activation signal. Furthermore, we also found that TBK1 phosphorylates the 82nd serine of NQO1. Cells genetically edited to express a phosphorylation-mimicking or phosphorylation-resistant mutant of NQO1 showed that the phosphorylation turns on the immune regulatory function of NQO1. Finally, we showed that the phosphorylated NQO1 binds to TBK1 and decreases self-association. In conclusion, our study presents a new regulatory loop of TBK1-NQO1 in antiviral immunity. [National Research Foundation of Korea (Grant no. 2021R1A6A1A03045495, RS-2023-00209214)]

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L. Infection & Immunology [L-65]

E3 Ligase Seven In Absentia Homolog-1 Mediated K27-Linked Ubiquitination and Degradation of USP19 Enhance the Host Immune Response to Virus Infections

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SIAH (Seven in Absentia Homolog), an E3 ubiquitin ligase is mammalian homolog of SINA (Seven in Absentia), a *Drosophila* protein which first identified as involved in the development of the *Drosophila* eye. With a highly conserved N-terminal RING domain, SIAH1 consists of two zinc finger domains and a substrate-binding domain. It is mainly involved in cellular stress responses, but SIAH1 is associated with a broader range of cellular processes including neural functions, hypoxia, DNA damage response, and cell cycle regulation. In this study, we report that E3 ligase SIAH1 is a novel positive regulator of host innate immune signaling upon viral infection. SIAH1 interacted directly with USP19, which is known to be involved in deubiquitinating Beclin1, TRAF3, and TRIF for downregulation of the interferon (IFN) signaling pathway. During the early phase of infection SIAH1 catalyzed K27-linked ubiquitination of 3 Lysine residues on USP19, which resulted in its degradation. Ultimately, knockdown of SIAH1 inhibited type I interferon signaling and enhanced viral replication. Collectively, these data provide a clear understanding of the molecular mechanism of SIAH1 mediated positive regulation of host immune response to virus infections by targeting USP19. [NRF of Korea (2021R1A6A1A03045495, RS-2023-00209214), KRIBB Program (KGM9942011)]

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L. Infection & Immunology [L-66]

Regulation mechanism of E3 ligase RNF172 and its critical role in inflammatory bowel disease

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Dysregulated immune responses and impaired function in intestinal epithelial cells contribute to the pathogenesis of inflammatory bowel disease (IBD). Here, we discovered the critical role of E3 ligase ring finger protein 172 (RNF172) in IBD through regulating the NF-κB signaling. RNF172$^{-/-}$ mice were hyper-susceptible to the 3% DSS-induced colitis, which coincided with the massive production of inflammatory cytokines and chemokines. Tumor necrosis factor (TNF-α) treatment in RNF172-deleted or knockdown macrophages or colon epithelial cells (HT-29) resulted in marked incensement of the immune response. Consequently, RNF172 interacted directly with NEMO in HT-29 cells to catalyze NEMO degradation during the late phase of treatment with TNF-α. Moreover, in resting cells direct interaction of RNF178 with RNF172 inhibits RNF172 activation. TNF-α treatment results in RNF172 dimerization and subsequent catalyzing K63-linked self-ubiquitination on its Lys 127/238 which ultimately activates RNF172 to promote its substrate (NEMO) degradation. Taken together, our study revealed an elegant mechanism that evolved to manipulate the enzymatic activity of E3 ubiquitin ligase RNF172 and expand the existing knowledge of the function of RNF172 and its therapeutic potential in IBD. [The National Research Foundation of Korea (RS-2023-00209214, 2021R1A6A1A03045495)]

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L. Infection & Immunology [L-67]

The expression of NeuN and GFAP gene of jugular vein for nerve regeneration in rat model

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Background: The number of patients with neurological deficits is increasing these days. Methods for treating neuro deficient patients include auto vascular transplantation and nerve graft. Among them, the method of transplanting autologous blood vessels is typical. In this study, in order to confirm the degree of nerve regeneration, a nerve defect was caused in rat, and then jugular vein was transplanted to confirm gene expression. The genes used are GFAP and NeuN.

Materials and Methods: Nerve defect was caused in the rat, and jugular vein were implanted.

After 12 weeks of nerve transplantation, nerve tissue from rats was collected and molecular biology and histological experiments confirmed the expression of genes involved in nerve regeneration.

Results: It was confirmed that nerves were regenerated in the nerve defect area by autologous nerves, and molecular biological analysis showed that the expression of NeuN and GFAP tended to increase.

Conclusions: This study suggests the potential to promote neural regeneration if auto vascular is implanted in nerve-deficient sites.

Keywords: nerve regeneration, nerve defect, jugular nerve.

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M. Metabolism and Metabolic Diseases [M-1]

Transcriptome analysis of pulmonary fibrosis related genes in the lung of mice fed a high-fat diet

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Obesity is increasing all over the world and is associated with the incidence of diverse metabolic diseases including diabetes, cardiovascular disease and cancer etc. Recently, many studies have focused on the progression of lung fibrosis and lung cancer in obesity patients. In addition, high-fat fed diet animal studies demonstrated the small airways, reduced nasopharyngeal volume, and pulmonary inflammation, leading to pulmonary dysfunction. Therefore, we screened and compared genetic patterns in lung tissue of mouse fed a high fat diet (common obesity mimic mouse model) and those fed a normal diet to investigate the relationship between obesity and lung fibrosis. Through the RNA sequencing analysis, we found the differences in the expression of lung cancer related genes, and lymphocyte activation related genes. In GO and KEGG pathway data, we found upregulations in the nitrogen compound metabolic process, VEGF signaling, Notch signaling, non-small cell lung cancer and NK-cell mediated cytotoxicity. In summary, the increased inflammation and lung fibrosis were observed in lung tissue of high fat diet mouse model. Our results may provide the basic information to find critical genetic factors associated with pulmonary dysfunction in obesity.

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M. Metabolism and Metabolic Diseases [M-2]

Metformin ameliorates antipsychotics-induced metabolic side effects

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Olanzapine, one of the antipsychotics that treat schizophrenia, is reported to be frequently associated with hyperphagia-induced obesity and metabolic disorders. Metformin is widely prescribed together to address the metabolic problem caused by olanzapine. This study investigated the effects of olanzapine and metformin on the POMC neurons, which are major regulators of metabolic and energy consumption in the hypothalamus in mice. Female mice were divided into control groups, olanzapine (Oral, 5mg/kg) groups and olanzapine + metformin (Oral, 5mg/kg, 300mg/kg) groups. Drugs were injected for 5 days. Beta-Endorphin expression in the brain was examined through immunofluorescence staining to confirm the distribution of POMC neurons and axonal projection. Olanzapine administration significantly decreased POMC mRNA expression, POMC neuron numbers, POMC projections, and induced leptin resistance before the onset of obesity. Coadministration of metformin with olanzapine not only increased POMC neuron numbers and projections but also improved the leptin response of POMC neurons in the olanzapine-treated female mice. These findings suggest that olanzapine-induced hypothalamic POMC neuron abnormality and leptin resistance, which can be ameliorated by metformin administration, are the possible causes of subsequent hyperphagia.

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M. Metabolism and Metabolic Diseases [M-3]

Statistical Correlation Analysis of Propofol Infusion Rate in Relation to Sex and Body Weight of Patients Who Undergoing Deep Sedated Tumescent Liposuction Using Propofol-Ketamine

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Propofol has a fast onset and short duration of action, allowing for rapid recovery of cognitive and psychomotor functions. However, propofol has disadvantages such as a high incidence of apnea, reduced blood pressure and heart rate, as well as insufficient analgesic effect. These adverse effects of propofol can be reduced by the concomitant use of ketamine. Accordingly, the use of propofol combined with ketamine has become a popular protocol applied for deep sedation under tumescent liposuction. However, there is limited information on the use of propofol-ketamine for deep sedated tumescent liposuction. In this study, we retrospectively investigated the medical records of patients who underwent tumescent liposuction under deep sedation using propofol-ketamine to determine if there is a relationship between sex and body weight using sample *t*-test and ANOVA statistical methods. Because of large differences in the total number and distribution of male and female populations, the propensity score matching method was used to minimize the bias. For the evaluation of the body weight effect on the propofol infusion rate, lean body weight, total body weight, and body mass index were used. The results of our analyses revealed statistically significant differences in propofol administration rate on sex and body weight.

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M. Metabolism and Metabolic Diseases [M-4]

Musclin attenuates lipid deposition in hepatocytes through SIRT7/autophagy-mediated suppression of ER stress

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Musclin, an exercise-responsive myokine, has the ability to attenuate inflammation, oxidative stress, and apoptosis in cardiomyocytes under pathogenic conditions. While the potential benefits of musclin in the cardiovascular system have been well documented, its effects on hepatic endoplasmic reticulum (ER) stress and lipid metabolism are not fully understood. The present study showed that musclin treatment reduced lipid accumulation and lipogenic protein expression in primary hepatocytes exposed to palmitate. Palmitate treatment led to an increase in markers of ER stress, which was reversed by musclin treatment. Musclin treatment increased SIRT7 expression and markers of autophagy in a dose-dependent manner. Small interfering (si) RNA of SIRT7 or 3-methyladenine (3MA) reduced the effects of musclin on lipogenic lipid deposition in hepatocytes under hyperlipidemic conditions. These findings suggest that musclin can suppress palmitate-induced ER stress by upregulating SIRT7 and autophagy signaling, thereby alleviating lipid accumulation in primary hepatocytes. The current study provides a potential therapeutic strategy for the treatment of liver diseases characterized by lipid accumulation and ER stress, such as nonalcoholic fatty liver disease.

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M. Metabolism and Metabolic Diseases [M-5]

Protaetia brevitarsis protein hydrolysate reduces obese-related colitis through anti-inflammatory pathways on high fat diet mice

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Ulcerative colitis is an inflammatory bowel disease that reveals inflammation in the mucosal and submucosal layer of the colon. Currently, obesity is reported to be closely related to the development and progression of colitis, and inhibition of inflammatory responses may be the most effective approach for the treatment of obesity-related colitis.

We focused on the anti-inflammatory effects of polyphenols in *Protaectia brevitas* larvae. The *P. brevitas* prepared as a low molecular protein hydrolysate (PHPB) to increase concentration of anti-inflammatory molecules. In current study, we investigate the anti-inflammatory effect of PHPB on an obesity-induced colitis mouse model. Male C57BL/6J mice were fed with standard diet or high-fat diet (HFD). HFD mice were treated with PHPB (16 mg/100 g of body weight/daily).

When compared to the HFD group, PHPB treatment showed that reduced body/organ/fat weight, appetite/food intake inhibition, hypolipidemic effect on ectopic fat, anti-adipogenic mechanism through *AMPK* signaling pathway attenuates PPARγ and C/EBPα expression, pro-inflammatory molecules inhibition, anti-inflammatory molecules stimulation, probiotic-like effect against obesogenic gut microbiota, inhibition of macrophage polarization into M1, anti-oxidative stress and ER stress, which induces collection of Muc2 misfolding. These several anti-inflammatory responses result goblet cells recovery histologically and functionally, which eventually appears as an improved colitis.

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M. Metabolism and Metabolic Diseases [M-6]

Deacetylation of hypothalamic TTF-1 by Sirt1 regulates energy homeostasis

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Agouti-related peptide (AgRP) and pro-opiomelanocortin (POMC) neurons are located in the hypothalamus and act in response to energy state of the entire body. Previous studies have reported that these neurons were regulated by thyroid transcription factor-1 (TTF-1). However, the mechanisms by which TTF-1 responds to change in the body's energy state are not fully understood. Here, we report that NAD^+-dependent deacetylase Sirtuin 1 (Sirt1) is a key regulator of energy-dependent TTF-1 activation. We confirmed that Sirt1 and TTF-1 genes were highly expressed during energy-deficiency, and that Sirt1-induced overeating was inhibited by blocking TTF-1. Moreover, TTF-1 interacted with Sirt1 and was deacetylated in response to energy-deficiency and Sirt1 induction. Intriguingly, nuclear translocation of TTF-1 was induced by energy-deficiency and Sirt1 induction, while Sirt1 inhibition attenuated energy-deficiency-induced nuclear translocation of TTF-1. Notably, we observed that the transactivity of TTF-1 on AgRP and POMC expression was altered by regulated TTF-1 acetylation using acetyl-lysine point mutation of TTF-1. Consistent with these findings, TTF-1 acetylation was decreased in energy-deficient mice and attenuated by Sirt1 inhibition. Taken together, these observations suggest that energy-deficiency induces the interaction between TTF-1 and Sirt1, reduces TTF-1 acetylation, and regulates the expression of AgRP and POMC genes.

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M. Metabolism and Metabolic Diseases [M-7]

RNA binding protein HuD mediates the crosstalk between β cells and islet endothelial cells by the regulation of Endostatin and Serpin E1 expression

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RNA binding protein HuD plays essential roles in gene expression by regulating RNA metabolism, and its dysregulation is involved in the pathogenesis of several diseases, including tumors, neurodegenerative diseases, and diabetes. Here, we explored HuD-mediated differential expression of secretory proteins in mouse insulinoma βTC6 cells using a cytokine array. Endostatin and Serpin E1 that play anti-angiogenic roles were identified as differentially expressed proteins by HuD. HuD knockdown increased the expression of α chain of collagen XVIII (Col18a1), a precursor form of endostatin, and Serpin E1 by associating with the 3′-untranslated regions (UTRs) of their mRNAs. Reporter analysis revealed that HuD knockdown increased the translation of EGFP reporters containing 3′UTRs of their mRNAs. Co-cultures of βTC6 cells and pancreatic islet endothelial MS1 cells showed that HuD downregulation in βTC6 cells inhibited the growth and migration of MS1 cells. Ectopic expression of HuD decreased Col18a1 and Serpin E1 expression, while increasing the markers of islet vascular cells in the pancreas of db/db mice. Taken together, these results suggest that HuD has the potential to regulate the crosstalk between β cells and islet endothelial cells by regulating Endostatin and Serpin E1 expression, thereby contributing to the maintenance of homeostasis in the islet microenvironment.

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M. Metabolism and Metabolic Diseases [M-8]

Regulation of beige adipocyte thermogenesis by the cold-repressed ER protein NNAT

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Cold stimuli trigger the conversion of white adipose tissue into beige adipose tissue, which is capable of non-shivering thermogenesis. However, what process drives this activation of thermogenesis in beige fat is not well understood. Here, we examine the ER protein NNAT as a regulator of thermogenesis in adipose tissue. Cold exposure or treatment with a β3-adrenergic agonist reduces the expression of adipose tissue NNAT in mice. Genetic disruption of Nnat in mice enhances inguinal adipose tissue thermogenesis. Nnat null mice exhibit improved cold tolerance both in the presence and absence of UCP1. Gain-of-function studies indicate that ectopic expression of Nnat abolishes adrenergic receptor-mediated respiration in beige adipocytes. NNAT physically interacts with the ER Ca2+-ATPase (SERCA) in adipocytes and inhibits its activity, impairing Ca2+ transport and heat dissipation. We further demonstrate that NHLRC1, an E3 ubiquitin protein ligase implicated in proteasomal degradation of NNAT, is induced by cold exposure or β3-adrenergic stimulation, thus providing regulatory control at the protein level. This serves to link cold stimuli to NNAT degradation in adipose tissue, which in turn leads to enhanced SERCA activity. Our study implicates NNAT in the regulation of adipocyte thermogenesis.

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M. Metabolism and Metabolic Diseases [M-9]

The role of adipose tissue NK cells in the development of obesity-induced inflammation and insulin resistance

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Although it has been shown that epididymal adipose NK cells play an important role in the development of obesity-induced inflammation and insulin resistance, it is not fully understood how obesity activates NK cells. Thus, we conducted single-cell RNA sequencing of epididymal (eWAT) and subcutaneous (sWAT) adipose tissues and spleen. We found that NK cells from all three tissues showed 10-13 clusters, suggesting that NK cells are very heterogeneous. Furthermore, all three tissue NK cells showed unique tissue profiles, suggesting different tissue NK cells play different roles in the regulation of immune homeostasis. Moreover, we found an yet-identified new cluster, namely ILC2-specific RORa^{+} ILC1 in those tissues. Finally, obesity changed the frequencies and gene regulations mainly in eWAT NK cells, while the genes for cytotoxicity were not changed by obesity. However, we found a self-proliferating Ki67^{+} cluster and a lipid-metabolism-enriched cluster, both of which were increased by obesity. Preliminary results with NK cell-specific lipid metabolism gene KO mice further showed that deletions of lipid metabolism genes in NK cells protected the development of obesity-induced insulin resistance. These data suggest strongly that lipid metabolism is a key regulator for the activation of NK cells in obesity.

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M. Metabolism and Metabolic Diseases [M-10]

Identification and analysis of potential organokines and their effects on target organs in a mouse model of obesity.

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Organokines regulate metabolic processes and play important roles in metabolic disorders like obesity, diabetes, and cardiovascular disease. Metabolic dysfunction and disorders can contribute to dysregulation of organokines. Understanding their functions and interactions is essential for developing new therapeutic strategies. However, identifying and studying organokines is challenging due to their low abundance and complex properties.

In this study, we identified organ-specific secreted genes based on transcriptome expression and cellular location in obese mice. Using PPI databases, we predicted receptors that interact with these genes and analyzed downstream gene expression patterns to predict potential organokine functions. Through downstream gene expression analysis, we identified potential functions of the liver-derived organokines, including the inhibition of differentiation in brown adipose tissue (BAT), decreased thermogenesis in epididymal white adipose tissue (eWAT), and glucose metabolic disorders in muscle. Additionally, Adipokines, Batokines, and Myokines all exhibited a common inhibitory effect on liver catabolic processes.

In conclusion, the sub-signaling changes of target organ receptors interacting with the potentially predicted organokines in this study were consistent with the roles of previously known organokines. This suggests that these predicted targets could be potential therapeutic targets as well as important biomarkers for development.

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M. Metabolism and Metabolic Diseases [M-11]

Rabbit meat extract promotes the browning of 3T3-L1 adipocytes

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Rabbit meat offers excellent nutritive and dietetic properties. Among its beneficial characteristics, rabbit meat has less fat and cholesterol content than beef, pork, or chicken. However, its role in the improvement of obesity-related metabolic disease is limited. Brown adipocytes (BAT) have many mitochondria and express high levels of uncoupling protein (UCP1), which are specialized for energy expenditure and emerged as an attractive therapeutic target for the treatment of obesity and the metabolic syndrome. The aim of this study was to assess the browning effect of rabbit meat extract in 3T3-L1 adipocytes. 3T3-L1 pre-adipocytes were differentiated and exposed to rabbit meat extract. Rabbit meat extract increased expression of BAT-specific markers. Rabbit meat extract also promoted mitochondrial biogenesis by enhancing the expression of representative subunits of oxidative phosphorylation complexes in a dose dependent manner. In addition, rabbit meat extract increased glucose uptake in adipocytes and myocytes, indicating that rabbit meat extract improved insulin action. Thus, rabbit meat extract may have a potential therapeutic implication of obesity.

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M. Metabolism and Metabolic Diseases [M-12]

Microbiota-derived indole compounds attenuate non-alcoholic fatty liver disease by improving fat metabolism and inflammation.

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Nonalcoholic liver disease (NAFLD) is the most common chronic liver disease, and its prevalence has increased worldwide in recent years. Additionally, there is a close relationship between NAFLD and the gut microbiota and metabolites. However, the mechanisms of NAFLD and its metabolites are still unclear. We found decreased indole-3-propionic acid (IPA) and indole-3-acetic acid (IAA) in the feces of patients with hepatic steatosis compared to healthy controls. In a Western diet-induced NAFLD mice model, IPA and IAA supplementation effectively improved NAFLD activity scores, biochemical markers (AST, ALT, total cholesterol, and TG) cytokines (TNF-α), and chemokines (Cxcl10, Ccl2, Ccl5). Here, we show that IPA and IAA administration ameliorates hepatic steatosis and inflammation in an animal model of WD-induced NAFLD by suppressing the NF-κB signaling pathway through the reduction in endotoxin levels and inactivation of macrophages. We found that Bifidobacterium bifidum metabolizes tryptophan to produce IAA. Our results showed that B. bifidum could prevent hepatic steatosis and inflammation through the production of IAA. Our study demonstrates that IPA and IAA derived from the gut microbiota have novel preventive or therapeutic potential for NAFLD.

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M. Metabolism and Metabolic Diseases [M-13]

Lactobacillus Casei-Fermented herb A (blind, LAX) Mitigates Non-alcoholic Steatohepatitis in a High-fat Diet Mice Model

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The present study aimed to investigate whether L. casei-fermented herb A (LAX, blind) alleviates high-fat diet (HFD)-induced non-alcoholic fatty liver disease. Ten-week HFD-fed C57BL/6J mice were administrated with LAX or AX (200 mg/kg) by gavage once a day from the 4th week for 6 weeks. Simultaneously, metformin was administrated as a positive control. LAX significantly declined liver weight, especially the fat mass in the liver surpassing AX ac-cording to the result of the Dual-Energy X-ray Absorptiometry (DXA) scan. Besides, LAX more sharply ameliorated intrahepatic lipid accumulation, oxidative stress than AX, as evidenced by the positive results of serum aminotransferases, histopathological staining, and hepatic TG, TC levels. The lipid and energy metabolism-related proteins, including GPAM, SREBP-1, PPAR-α, and p-AMPK-α, were also more extensively regulated by LAX than AX. In addition, LAX markedly reduced the serum proinflammatory cytokines, like TNF-α, IL-1β, and IL-6, and normalized the serum TG, TC and LDL-cholesterol. Taken together, LAX mitigates excessive fat intake-caused fatty liver potentially by the activation of PPAR-α and AMPK-α and inhibition of the IRE1α signaling pathway. Consequently, we suggested that the fermentation of AX can be deemed a promising strategy for enhancing therapeutic potential, especially for NAFLD.

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M. Metabolism and Metabolic Diseases [M-14]

Tat-CIAPIN1 prevents pancreatic β-cell loss in hIAPP-induced RINm5F cells and T2DM animal model

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It is well known that cytokine-induced apoptosis inhibitor 1 (CIAPIN1) protein plays an important role in biological progresses as an anti-apoptotic protein. Human islet amyloid peptide (hIAPP), known as amylin, is caused to pancreatic β-cell death in type 2 diabetes mellitus (T2DM). However, the function of CIAPIN1 on T2DM is not studied yet well. Therefore, we investigated the effects of CIAPIN1 protein on hIAPP-induced RINm5F cell and T2DM animal model induced by high-fat diet (HFD) and streptozotocin (STZ). Cell permeable Tat-CIAPIN1 fusion protein was prepared and this protein inhibited cell viability and cytotoxicity levels in hIAPP-induced RINm5F cells. Tat-CIAPIN1 reduced the activation of mitogen-activated protein kinase (MAPK) and regulated the apoptosis-related protein expression levels including COX-2, iNOS, Bcl-2, Bax, and Caspase-3 in the cells. In a T2DM mice model, Tat-CIAPIN1 ameliorates the pathological changes of pancreatic β-cells and reduced the fasting blood glucose, body weight and hemoglobin Alc (HbAlc) levels. In conclusion, Tat-CIAPIN1 showed the protective effects against T2DM by protection of β-cells *via* inhibition of hIAPP toxicity and by regulation of MAPK signal pathway, suggesting CIAPIN1 can be therapeutic protein drug candidate by beneficial regulation of T2DM.

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M. Metabolism and Metabolic Diseases [M-15]

Verapamil-loaded supramolecular hydrogel patch attenuates MAFLD via restoration of autophagic clearance of aggregated proteins and inhibition of NLRP3

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Obesity is linked to chronic metabolic complications including insulin resistance, type-2 diabetes, and metabolic dysfunction-associated fatty liver disease (MAFLD). Current obesity medications are challenged by poor effectiveness, poor patient compliance, and potential side effects. This study aims to develop a verapamil transdermal patch and to evaluate its anti-obesity effects. Verapamil is loaded in biomimetic vascular bundle-like carboxymethyl pullulan-based supramolecular hydrogel patches cross-linked with citric acid and glycerol linkages (CLCMP). Verapamil-loaded CLCMP (Vera@CLCMP) hydrogel patches with hierarchically organized and anisotropic pore structures not only improved verapamil bioavailability without modifying its chemical structure but also enhanced verapamil release through the stratum corneum barrier. Vera@CLCMP patches exhibit low toxicity and high effectiveness at delivering verapamil into the systemic circulation through the dermis in a sustained manner. Specifically, transdermal administration of this patch into diet-induced obese mice drastically improved glucose tolerance and insulin sensitivity and alleviated metabolic derangements associated with MAFLD. Furthermore, we uncovered a distinct molecular mechanism underlying the anti-obesity effects associated with the hepatic NLRP3 inflammasome and autophagic clearance by the vera@CLCMP hydrogel patches. The current study provides promising drug delivery platforms for long-term family treatment of chronic diseases, including obesity and metabolic dysfunctions.

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M. Metabolism and Metabolic Diseases [M-16]

Procyanidin B2-rich extract of Unripe apple alleviates colitis via attenuating colonic mucosa injury and regulating pro-inflammatory cytokines production

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Unripe apples (UA) contain fairly high amount of polyphenols, which has been reported to have various physiological effects including anti-inflammation, anti-cancer activity, anti-obesity, anti-hyperglycemia, anti-allergic and anti-arteriosclerosis. It contains the most procyanidin among polyphenols. Procyanidin has been reported to inhibit inflammation in dextran sulfate sodium (DSS)-induced mouse model. We conducted an analysis of the procyanidin content of apple (Gamhong) and UA, and as a result, we found that UA contain higher procyanidin content. So, this study aimed to investigate the anti-inflammatory effects of UA ethanol extract (UAE) against DSS-induced colitis in mice. The results demonstrated that UAE significantly relieved the loss of body weight, shortening of colon length. The serum cytokine profile demonstrated that tumor necrosis factor-α, interleukin (IL)-1β and IL-6 was significantly lower in the UAE group than in the DSS-induced group. Moreover, UAE ameliorated colonic edema, mucosal damage, and neutrophil infiltration into colonic intestinal tissue in response to DSS challenge. These results demonstrate for the first time that UA has an ameliorative effect on DSS-induced colonic inflammation in mice. In the future, the therapeutic potentials of UA as an effective complementary modality for the treatment of ulcerative colitis.

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M. Metabolism and Metabolic Diseases [M-17]

TMEM135 depletion prevents HFD-induced NAFLD in mice

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Background: Non-alcoholic fatty liver disease (NAFLD) is a condition in which fat is accumulated in the liver without alcoholic consumption. It is associated with obesity, diabetes and insulin resistance. In NAFLD, the function of mitochondria is compromised due to fat accumulation. TMEM135 is a transmembrane protein predicted to be localized in ER, mitochondria, peroxisome, nucleus and lipid droplet. It functions in the regulation of mitochondria dynamics, lipid metabolism and longevity. However, the exact role of TMEM135 remains unclear.

Objectives: To determine the role of TMEM135 in regulating lipid import and accumulation.

Methods: *In vivo* study was conducted in the wild type (WT) and TMEM135 knock-out (TMEM135KO) mice fed with 22 weeks of HFD to induce fatty liver. For the *in vitro* study, sh-Scramble and sh-TMEM135 AML12 cells were treated with free fatty acid (FFA) to mimic the high-fat diet condition.

Results: TMEM135KO mice were resistant to fatty liver in HFD feeding. The protein expression of lipid import and accumulation were downregulated compared to WT mice. Similarly, *in vitro* treatment with FFA shows reduced lipid accumulation in TMEM135-depleted AML12 cells. An increased expression of SIRT1, NAD-dependent histone deacetylase, in TMEM135 depleted conditions indicates its role in preventing lipid accumulation. We need further study to explain the underlying mechanism.

Conclusions: TMEM135 depletion prevents hepatosteatosis by suppression of lipid import and accumulation through SIRT1 activation.

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M. Metabolism and Metabolic Diseases [M-18]

Saturated Fatty Acid-inducible MiR-183-5p Impairs Insulin Signaling by Suppressing IRS-1 in HepG2 hepatocytes

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Excessive saturated fatty acids (SFA) uptake is known to be a primary cause of obesity, a widely acknowledged risk factor of insulin resistance and type 2 diabetes. Although specific microRNAs (miRNAs) targeting insulin signaling intermediates are dysregulated by SFA, their effects on insulin signaling are largely unknown. Here, we investigated the role of SFA-induced miR-183-5p in the regulation of insulin signaling molecules and the development of hepatic insulin resistance. HepG2 hepatocytes treated with palmitate and the livers of high-fat diet mice exhibited impaired insulin signaling resulting from dramatic reductions in the protein expressions of insulin receptor (INSR) and insulin receptor substrate-1 (IRS-1). Differential expression analysis exhibited that miR-183-5p, which tentatively targets the 3′UTR of *IRS-1*, was significantly elevated in palmitate-treated HepG2 and the livers of HFD-fed mice. Dual-luciferase analysis showed miR-183-5p bound directly to the 3′UTR of IRS-1 and reduced IRS-1 expression at the post-transcriptional stage. Moreover, transfection of HepG2 with miR-183-5p mimic inhibited IRS-1 expression and hindered insulin signaling, consequently inhibiting insulin-stimulated glycogen synthesis. Collectively, this study reveals a novel mechanism whereby miR-183-5p induction by SFA impairs insulin signaling and suggests miR-183-5p plays a crucial role in the pathogenesis of hepatic insulin resistance in the background of obesity.

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M. Metabolism and Metabolic Diseases [M-19]

The Role of ATF4 in Cold-induced Adipose Tissue Remodeling

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Adipose tissue (AT) is a heterogenous tissue consisting of various type of cells. Cold exposure has been reported to cause inguinal white adipose tissue (iWAT) remodeling as reflected by higher mitochondria activity and the alteration of resident cell composition. Here we investigated the function of ATF4, a mediator of the mitochondrial stress response, in regulating cold-induced AT remodeling. We generated adipocyte-specific ATF4 knockout mice ($Atf4^{AKO}$) by crossing $Atf4^{flox/flox}$ ($Atf4^{WT}$) mice with the adiponectin-cre mice. We performed cold exposure or CL316,243 injection to activate adrenergic signaling and induce AT thermogenic function. We found that cold exposure induced the expression of ATF4 and mitochondria integrated stress response (ISRmt) since the early period. However, mitochondria complex and mitochondria quantity were induced after chronic cold acclimation. $Atf4^{AKO}$ mice had lower energy expenditure upon CL316,243 injection and less cold-induced iWAT browning. Further transcriptomics analysis revealed higher expression of cytokines related to immune cell recruitment in $Atf4^{AKO}$ mice. Moreover, $Atf4^{AKO}$ mice maintained a higher M1/M2 ratio in iWAT upon cold exposure. Our results demonstrate that cold exposure induced the activation of ATF4 which might be important to maintain immune cell homeostasis during cold-induced iWAT remodeling.

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M. Metabolism and Metabolic Diseases [M-20]

NK Cell Activating Receptor NKp46 Does Not Play a Role in the Development of Obesity-Induced Inflammation and Insulin Resistance

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It has been shown that obesity-induced inflammation plays important roles in the development of insulin resistance and natural killer (NK) cells in epididymal adipose tissue (eWAT) contribute to these processes. Recent study also suggests that NK cell activating receptor NKp46 may regulate NK cell activations in obesity (Nat. Immunol. 16(15), 376). Thus, by using the same NKp46 knockout mice (*Ncr1*$^{gfp/gfp}$) mice tested in this study, we wanted to further investigate the molecular mechanisms how NKp46 controls NK cell functions, eventually to determine how obesity activates NK cells. We examined the metabolic and inflammatory phenotypes of *Ncr1*$^{gfp/gfp}$ in obesity. Obesity increases insulin resistance with increased NK cell numbers only in eWAT. Moreover, NK cell deletion or expansion improves or worsens insulin resistance, respectively, accompanying with corresponding changes in inflammation only in eWAT. However, we found that *Ncr1*$^{gfp/gfp}$ did not show any changes in fasting blood glucose and insulin levels, GTT and ITT, suggesting that NKp46 does not play any role in the development of obesity-induced insulin reisistance. Furthermore, inflammation was not changed in *Ncr1*$^{gfp/gfp}$ either. Thus, contrary to the previous study, our data strongly suggest that NKp46 does not regulate NK cell functions in obesity.

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M. Metabolism and Metabolic Diseases [M-21]

The correlation between growth characteristics and the contents of active compounds in Cudrania tricuspidata fruits

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Cudrania tricuspidata is a traditional medicinal herb in East Asia. However, fewer or no studies have been done on the correlation between growth characteristics and compounds in *C. tricuspidata* fruit. So, we aimed to investigate their relationship. Samples of *C. tricuspidata* fruit and cultivation soil were collected in 28 cultivation sites in October 2021. Six growth characteristics and three active compounds were investigated. We developed and validated an optimized method for quantifying active compounds using ultra-performance liquid chromatography (UPLC), and performed correlation analysis of growth characteristics and contents of active compounds. The UPLC-UV method for determining active compounds was validated by measuring the linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy using UPLC. The LOD and LOQ were 0.01-0.03 μg/mL and 0.04-0.09 μg/mL, respectively. The precision was acceptable with % RSD values less than 2%. The recoveries ranged from 97.25-104.98% with RSD values < 2%, within the acceptable limits. The active compounds were negatively correlated with the size of the fruit (length, width of fruit, and fresh weight of fruit). The results of this study can be used as basic data for the standard cultural practices and quality-control of *C. tricuspidata* fruits.

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M. Metabolism and Metabolic Diseases [M-22]

Empagliflozin attenuates hepatic steatosis, fibrosis and inflammation in db/db mice

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Type 2 diabetes mellitus (T2DM) is considered one of the major risk factors for the faster progression of nonalcoholic fatty liver disease to nonalcoholic steatohepatitis (NASH), advanced fibrosis or cirrhosis. Studies have suggested that empagliflozin (EMPA), a sodium-glucose cotransporter 2 inhibitor and a new type of oral antidiabetic drug, may also have a beneficial effect on NASH in patients with T2DM. However, the mechanisms underlying this effect remain unclear. We aimed to investigate how the EMPA prevent NASH in T2DM mouse model. In this report, we provide preliminary results on the effects of EMPA on diabetic liver disease in db/db mice. Results showed that treated db/db mice with EMPA (10 mg/kg/day for 10 weeks of treatment) significantly reduced hepatic lipid accumulation (decreased triglyceride level, CD36 protein expression), In addition, EMPA attenuated hepatic fibrotis molecules (collagen 1 and 3 and Smad 2 phosphorylation). Moreover, EMPA inhibited NF-κB/IL-1β, IL-18 and enhanced IL-10 expression attenuating liver inflammation compared to the control group. The present data confirmed the hepatoprotective effects of EMPA in diabetic mice and serve as the basis for further molecular mechanism studies.

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M. Metabolism and Metabolic Diseases [M-23]

Weight Cycling Accelerates Nonalcoholic Fatty Liver Disease (NAFLD) Progression through Activation of IGFBP7

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Weight loss intervention is the current standard for NAFLD treatment. However, combatting obesity can lead to large fluctuations in body weight, referred to as weight cycling or yo-yo dieting. Recent studies implicated weight cycling as an independent factor to hasten NAFLD, but the underlying mechanisms of NAFLD development are not well understood. Utilizing diet-switch model, we created obesity, weight loss (WL), and weight regain cycle (WC) in mice. Metabolic parameters were measured by biochemical and histological analysis and liver macrophages were quantified with flow cytometry. While body weights were comparable between high-fat diet (HFD) challenged group (ST-HFD) and HFD re-challenged group (RCHFD), RCHFD displayed aggravated metabolic and liver profile including higher blood glucose, glucose intolerance, increased hepatic TG accumulation, and higher collagen deposition. RCHFD had higher numbers of proinflammatory liver macrophages, which were induced and persisted in HFD and WL periods. Furthermore, liver macrophages from formerly obese mice produced higher amount of IGFBP7. Moreover, IGFBP7 treatment to hepatic stellate cells (HSC) demonstrated the upregulation of smooth muscle actin which is a marker of liver fibrosis. Therefore, worsened NAFLD progression during WC could be attributed in part to the sustained liver macrophage activation and their product IGFBP7 which induced HSCs activation.

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M. Metabolism and Metabolic Diseases [M-24]

Ubiquitin Ligase RNF20 Coordinates Sequential Adipose Thermogenesis with Brown and Beige Fat-Specific Substrates

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In mammals, brown adipose tissue (BAT) and inguinal white adipose tissue (iWAT) execute sequential thermogenesis to maintain body temperature during cold stimuli. BAT rapidly generates heat through brown adipocyte activation, and further iWAT gradually stimulates beige fat cell differentiation upon prolonged cold challenges. However, fat depot-specific regulatory mechanisms for thermogenic activation of two fat depots are poorly understood. Here, using fat depot-selective genetic modulation, we demonstrated that E3 ubiquitin ligase RNF20 orchestrates adipose thermogenesis with BAT and iWAT-specific substrates. Upon cold stimuli, BAT RNF20 was rapidly downregulated to accumulate GABPa protein by controlling protein stability, which stimulated mitochondrial and thermogenic gene expression. Accordingly, BAT-specific *Rnf20* suppression stimulated BAT thermogenic activity via GABPa regulation. Moreover, upon prolonged cold stimuli, iWAT RNF20 was gradually upregulated to stimulate *de novo* beige adipogenesis. Mechanistically, iWAT RNF20 promoted NCoR1 protein degradation, but not GABPa, to potentiate PPARg activity. Together, our data propose fat depot-specific regulatory mechanisms for temporal activation of adipose thermogenesis.

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M. Metabolism and Metabolic Diseases [M-25]

The inhibition of FOXO1 improves cholestatic liver disease-induced sarcopenia

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Primary sclerosing cholangitis (PSC) is a cholestatic liver disease that affects the hepatic bile ducts, leading to inflammation, fibrosis, and eventual liver damage. PSC can also impact other organs, including skeletal muscle, resulting in sarcopenia, a syndrome characterized by generalized loss of muscle mass and strength. The underlying mechanisms of PSC-induced sarcopenia are not well understood, but one potential factor is the transcription factor FOXO1, which is involved in the ubiquitin proteasome degradation pathway. To investigate the role of FOXO1 in PSC-induced sarcopenia, we used a 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet-induced PSC mice model. Our results showed that this model exhibited sarcopenic features, including decreased skeletal muscle mass and increased levels of atrophy-related markers such as atrogin-1 and MuRF1. We also observed decreased diameter and MyHC protein level in C2C12 myotubes, suggesting impaired muscle growth and differentiation. Furthermore, we found that inhibition of FOXO1 led to decreased levels of atrophy-related proteins and improved MyHC protein expression, suggesting that FOXO1 plays a role in PSC-induced sarcopenia. These findings indicate that targeting FOXO1 may be a potential therapeutic strategy for preventing muscle loss in PSC-induced sarcopenia.

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M. Metabolism and Metabolic Diseases [M-26]

Acyloxyacyl hydrolase (AOAH) is enhanced in the NASH and affects NASH development.

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AOAH is the only host lipase that can inhibit an LPS-induced inflammatory response. LPS is a substance that constitutes the outer membrane of Gram-negative bacteria and is largely composed of lipid A and polysaccharide chins. AOAH selectively cuts only the secondary (acyloxyacyl-linked) fatty acyl chains of lipid A and converts them to less stimulatory LPS. Non-alcoholic steatohepatitis (NASH) is a type of non-alcoholic fatty liver disease that causes excessive accumulation of fat in the liver. We examined AOAH levels in the livers of FPC-, and MCD-fed mice to find out the mechanism by which AOAH alleviates NASH. We found that AOAH levels were high in FPC-, and MCD-induced NASH mice. Also, we found that AOAH expression increased dramatically as a result of treating bacterial substances by isolating Kupffer cells and bone marrow-derived macrophage. These results suggest that the cause of increased mRNA levels of AOAH in NASH liver is macrophages after bacterial stimulation. Additionally, We tested the change in FPC diet using AOAH knockout mice. We found that NASH was alleviated in the AOAH knockout mice compared to the WT mice. We will study the function of AOAH to overcome NASH by employing AOAH knockout mice.

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M. Metabolism and Metabolic Diseases [M-27]

Hepatocyte Kctd17 Inhibition Ameliorates Glucose Intolerance and Hepatic Steatosis Caused by Obesity-induced Chrebp Stabilization

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Obesity predisposes to type 2 diabetes (T2D) and nonalcoholic fatty liver disease (NAFLD), but underlying mechanisms are incompletely understood. Potassium channel tetramerization domain-containing protein 17 (Kctd17) levels are increased in livers from obese mice and humans. In this study, we investigated the mechanism of increased Kctd17 and whether it is causal to obesity-induced metabolic complications. Here, we found that Kctd17 expression was increased in HFD-fed mice due to increased Srebp1c activity. Hepatocyte specific Kctd17 knockout (L-Kctd17) or Kctd17 antisense oligonucleotide–treated mice fed on HFD show improved glucose tolerance and hepatic steatosis, whereas forced Kctd17 expression caused glucose intolerance and hepatic steatosis even in lean mice. Kctd17 induced Oga degradation, resulting in increasing carbohydrate response element-binding protein (Chrebp) protein, so concomitant Oga knockout negated metabolic benefits of hepatocyte Kctd17 deletion. In patients with NAFLD, KCTD17 messenger RNA was positively correlated with expression of Chrebp target and other lipogenic genes. Srebp1c-induced hepatocyte Kctd17 expression in obesity disrupted glucose and lipid metabolism by stabilizing Chrebp, and may represent a novel therapeutic target for obesity-induced T2D and NAFLD.

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M. Metabolism and Metabolic Diseases [M-28]

Immature CX3CR1+ myeloid cells promote hepatocellular carcinoma through arginase-1 expression in interaction with stellate cells

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Although the cellular interaction in the tumor microenvironment has been thoroughly investigated in hepatocellular carcinoma (HCC), the mechanism regulating the migration of pro-tumorigenic myeloid-derived cells and their interaction with hepatic cells remain marginal. Here, we demonstrate how a specific subset of immature myeloid-derived cells interact with activated HSCs in the peritumor area to suppress the proliferation of $CD8^+$ T cells, promoting tumorigenesis *in vivo* and *in vitro*. In both clinical and experimental HCC, human ($CD14^+CD11b^+HLA\text{-}DR^-$) and mouse ($CD11b^+Ly6G^-Ly6C^{high}$) myeloid cells elevated in blood and liver tissues highly express CX_3CR1, a chemokine receptor that regulates monocyte chemotaxis. These CX_3CR1^+ immature myeloid-derived cells migrate and interact with activated HSCs expressing CX_3CL1 in the fibrogenic peritumor region where they express arginase-1 in response to retinoids produced from the activated HSCs. Subsequently, the proliferation of $CD8^+$ T cells is suppressed in the presence of arginase-1-expressing immature myeloid-derived cells as $CD8^+$ T cells are deprived of arginine needed for their proliferation. Taken together, our study reveals a novel metabolic cellular interaction involved in the tumorigenesis in HCC.

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M. Metabolism and Metabolic Diseases [M-29]

Mesenchymal-stem cell-derived exosomes attenuate chronic inflammation and improve insulin resistance in diet-induced obese mice through the action of adiponectin

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Mesenchymal-stem cell-derived exosomes attenuate chronic inflammation and improve insulin resistance in diet-induced obese mice through the action of adiponectin.

Recently, there has been growing acknowledgment that exosomes derived from mesenchymal stem cells (MSC) possess therapeutic effects against several chronic inflammatory diseases and exosome-based therapy could be used as an effective strategy. However, most of the mechanisms underlying their effects remain largely to be elucidated. This study was designed to demonstrate the ameliorative effect of MSC-derived exosomes on metabolic disorders and to clearly illustrate the molecular mechanisms using high-fat diet (HFD)-induced obese mice. We found that exosomes reduced body weight, improved glucose tolerance, and alleviated fat accumulation in the liver in exosome-treated HFD-fed mice. Those mice also showed a significantly lowered serum insulin level compared to non-treated HFD-fed mice, indicating an insulin-sensitizing effect of exosomes. Furthermore, serum levels of pro-inflammatory cytokines including TNF-α, IL-1β, and IFN-γ were reduced and the protein levels of adiponectin in both serum and adipose tissues were increased in exosome-treated HFD-fed mice. Our observations suggest that exosomes have the ability to restore homeostasis in glucose and lipid metabolism in adipose tissues and the liver, which is mediated by the anti-inflammatory and insulin-sensitizing actions of adiponectin.

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M. Metabolism and Metabolic Diseases [M-30]

Golgi condensation causes intestinal lipid accumulation through HIF-1α mediated Golga1 ubiquitination and degradation

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Lipid accumulation in epithelial cells of small intestine has been reported to occur due to depletion of Golgi proteins, leading to impaired lipoprotein maturation. Among several factors of lipid accumulation, hypoxia is one that exacerbates the lipid trafficking and the Golgi in epithelial cells. However, the mechanism of lipid accumulation affected by the Golgi under hypoxia remains unknown. In this study, we find that hypoxia enhances lipid accumulation in epithelial cells due to a high-fat diet. At the same time, the Golgi apparatus undergoes structural modifications in response to hypoxia. We also discover that the Golgi condenses with HIF-1α activation, and the structural protein Golga1 is ubiquitinated and degraded by the proteasome. In contrast, inhibiting HIF-1α can prevent the Golgi condensation, thereby ameliorating promoting HDL secretion. We also observe that the HIF-1α inhibitor, PX-478, prevents lipid accumulation and promotes HDL secretion in epithelial cells of HFD mice. In summary, our study reveals that HIF-1α activation is increased in the epithelial cells of HFD mice, leading to structural modification of the Golgi via degradation of Golga1. This Golgi condensation results in reduced secretion of lipoproteins. However, the treatment with the HIF-1α inhibitor PX-478 is able to rescue phenotypes in HFD mice.

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M. Metabolism and Metabolic Diseases [M-31]

Exploration of sinapic acid (SA) based on AMPK/TGF-β1 signaling pathway ameliorative effect on myocardial fibrosis in diabetic mice

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The study aimed to investigate the improvement of myocardial fibrosis in diabetic mice by sinapic acid through AMPK/TGF-β1 signaling pathway. The type 2 diabetes model was established by high-fat diet (HFD) combined with streptozotocin (STZ) for six weeks. After modeling, there were normal group, model group, metformin group (200 mg/kg BW), and sinapic acid groups (20 and 60 mg/kg BW) with continuous gavage for 4 weeks. After euthanizing the mice, samples were taken and the cardiac index was calculated. H&E staining was performed to observe the myocardial pathological changes. Serum levels of TC, TG, MDA, SOD, IL-1β, IL-6 and TNF-α were measured by kits. The protein expression levels of AMPK, TGF-β1, TGF-βR1, α-SMA and CTGF were detected by protein blotting. Histological observations revealed that sinapic acid improved the disorderly arrangement, swelling, and inflammatory cell infiltration of cardiac myocytes in diabetic mice. Compared with the model group, the cardiac index, and the levels of TC, TG , MDA , IL-1β, IL-6, TGF-β1, TGF-βR1, α-SMA, and CTGF were lower but SOD and AMPK were higher ($P<0.05$) in sinapic acid groups. Sinapic acid ameliorates myocardial fibrosis in diabetic mice, and its effect may be related to regulation of AMPK/TGF-β1 signaling pathway.

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M. Metabolism and Metabolic Diseases [M-32]

The role of the autophagic Arg/N-degron pathway in Nucleophagy

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The nucleus, which serves as the central hub for all cellular information, is susceptible to genotoxic stress and general wear-and-tear, necessitating a means for not only constitutive but stress-induced turnover. Although the autophagic turnover of transmembrane receptor-mediated nuclear components in other eukaryotes, most notably yeast, have been well-characterized, the exact mechanisms and physiological relevance of nuclear autophagy in mammals remain poorly understood. In this study, the autophagic Arg/N-degron pathway regulates the stability of both luminal and transmembrane nuclear substrates in basal and genotoxic-induced stress. These nuclear substrates are degraded by p62-mediated and ATE1-dependent macroautophagy. Notably, the functional inhibition or genetic ablation of this circuit abrogates the autophagic targeting of nuclear substrates. Under genotoxic stress, p62- and Nt-Arg-mediated nucleophagy rescues cells from DNA damage toxicity. We confirmed that pharmacological modulation of this pathway via synthetic mimicry of the p62-Nt-Arg interaction is cytoprotective against DNA damage. Our results elucidate the mechanisms of the Arg/N-degron pathway in mammalian nucleophagy and provide a pharmaceutical means to modulate nuclear homeostasis.

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M. Metabolism and Metabolic Diseases [M-33]

Farnesoid X receptor (FXR) agonist attenuates hepatic endoplasmic reticulum stress and glucose metabolic disturbance in mice with type 2 diabetes

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Background: Farnesoid X receptor (FXR) plays an important role in bile acid, lipid, and glucose metabolism. Activation of FXR improves hyperglycemia by repressing hepatic gluconeogenic genes and increasing hepatic glycoegen synthesis. However, whether FXR is involved in hepatic endoplasmic reticulum (ER) stress in type 2 diabetes remains unknown. Here, we show that FXR activation attenuates hepatic ER stress and glucose metabolic disturbance in diabetes.

Methods: We treated *db/db* mice with the FXR agonist GW4064 (30 mg/kg/day) for 4 weeks, and evaluated glucose and lipid metabolism, and hepatic ER stress.

Results: The FXR agonist suppressed weight gain, and significantly improved glucose intolerance and insulin resistance. The FXR agonist significantly reduced liver expression of genes involved in gluconeogenesis and lipogenesis. In addition, the FXR agonist reduced hepatic ER stress.

Conclusion: The FXR agonist attenuates hepatic ER stress and glucose metabolic disturbance in mice with type 2 diabetes.

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M. Metabolism and Metabolic Diseases [M-34]

Grifola frondosa polysaccharides(GFPS) improve follicular development in PCOS rats by modulating oxidative stress and chronic inflammation

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This study was designed to investigate the effect of GFPS on follicular development in PCOS rats by modulating oxidative stress and chronic inflammation. The female SD rats were classified into 4 groups as follows: the normal group, the PCOS group (ingested letrozole), Metformin group and GFPS group(600mg/kg). All rats weighed every three days, Pap staining was used to determine the success of the PCOS model. H&E staining was performed to observe the pathological changes in the ovaries. mRNA expression of antioxidant enzymes and inflammatory factors in rat ovaries was measured by RT-qPCR. GFPS upregulated the mRNA expression of antioxidant enzymes *Cat*, *Sod2*, *Gpx3*, *Mgst1*, *Gsta*4, *Sod1*, *Gsr* and *Prdx3*, downregulated the mRNA expression of inflammatory factors *Nlrp3*, *Asc*, *Caspase1*, *Il1β*, *Il18*, *Tnfα* and *Il*6, and decreased the mRNA expression of TGF-β1, Smad2, Smad3 and Smad4, decreased the number of vesicles, increased the thickness of the granulosa cell layer and the number of corpus luteum. In conclusion, GFPS could improve follicular development in PCOS rats by ameliorating oxidative stress, chronic inflammation in PCOS ovaries.

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M. Metabolism and Metabolic Diseases [M-35]

Mesenchymal stem cell- derived exosomes relieve colitis through enhancing intestinal barrier function and alleviating systemic inflammation in DSS-induced colitis mice.

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Exosome therapy has been proposed as a promising therapeutic approach for the treatment of inflammatory bowel disease (IBD) since they possess potent immunomodulatory and anti-inflammatory properties. In this study, we aimed to evaluate the therapeutic effect of mesenchymal stem cell (MSC)-derived exosomes on IBD and elucidate the molecular mechanisms underlying the effect using dextran sodium sulfate (DSS)-induced colitis mice. We found that exosomes restoring the reduced body weight, the increased disease activity, and the shortened colon length, caused by DSS, compared to that of an existing therapeutic, 5-aminosalicylate. The mRNA expression level of pro-inflammatory cytokines such as TNF-α, IL-1β, and IFN-γ in adipose tissue and the liver of exosome-treated colitis mice was reduced compared to non-treated colitis mice. The mRNA and protein expressions of tight junction proteins such occludin and ZO-1 in the colon of exosome-treated colitis mice increased. The expression levels of a metabolically beneficial hormone, hepatic FGF21, and an adipokine, adiponectin were also observed to be enhanced by exosome treatment. From the results, it was suggested that exosome could relieve the intestinal permeability and systemic inflammation through its anti-inflammatory action and thereby ameliorate chronic inflammation in metabolic tissues such as the liver and adipose tissues.

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M. Metabolism and Metabolic Diseases [M-36]

Ginsenoside Rb2 stimulates adrenergic receptor signaling and accelerates lipolysis in mouse brown adipose tissue.

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Ginsenoside Rb2 is one of components in Panax ginseng, which has been traditionally used to treat metabolic diseases. However, the function of ginseng in brown adipose tissue (BAT), the primary organ involved in metabolism, is not fully understood. In this study, we investigated the function of Rb2 in BAT by intraperitoneally injecting the Rb2 into mouse for two weeks. To examine the systematic changes by Rb2 in BAT, we performed RNA sequencing, and analyzed differentially expressed genes (DEG) and pathways. Our analysis identified 727 genes significantly altered by Rb2 treatment. These genes were linked with BAT activation pathway such as adrenergic signaling, cAMP signaling, PKA signaling and lipolysis. Interestingly, adrb1 and adrb2 were upregulated by Rb2 treatment, while adrb3 was not, suggesting that Rb2 modulates BAT activation via specific adrenergic receptors. To confirm these findings, we conducted molecular experiment and found that Rb2 activates lipolysis via ß adrenergic receptor – cAMP – PKA signaling. Specifically, Rb2 enhanced the phosphorylation of HSL and PLIN, which are well known target of PKA. Our study provides a better understanding of the molecular mechanisms underlying the effects of Rb2 on BAT and may have implications for the development of novel therapeutics for metabolic disorders.

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M. Metabolism and Metabolic Diseases [M-37]

Phellinus linteus polysaccharide (PLP) alleviated ovarian fibrosis in letrozole-induced polycystic ovary syndrome (PCOS) in rats

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To investigate the effects of PLP on ovarian fibrosis in letrozole-induced PCOS rats. All SD rats were randomly divided into normal, PCOS, metformin group (Met, 265 mg/kg) and PLP group (1 g/kg) and weighed every three days. After 21 days of continuous administration, hormonal (FSH, LH, E2 and T) parameters were analyzed and the LH/FSH ratio, the relative weight of bilateral ovaries was calculated. Western blotting and immunohistochemical analysis was carried out for TGF-β1/Smads signaling pathway protein expression in the ovaries. Histological examinations of ovaries were also conducted by H&E and Masson trichrome staining. Treatment with PLP reduced the theca cell layer, increased granular cell layers and corpus luteum number, improved mature follicles and inhibited ovaries fibrosis in PCOS rats. The administration of PLP reduced the body weight and the level of serum T and LH in PCOS rats. The expression analysis of proteins by Western blotting revealed that PLP significantly decreased the level of TGF-β1, p-Smad3, p-Smad2 and Smad4 and reversed the downregulation of Smad7 in PCOS rats. The immunohistochemical results also corroborated the above statement. PLP improved ovarian fibrosis in PCOS rats by regulating the serum hormone level and suppressing the activation of the TGF-β1/Smads signaling pathway.

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M. Metabolism and Metabolic Diseases [M-38]

T factor promotes non-alcoholic fatty liver disease by suppressing AMPK-mediated autophagy

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Autophagy functions in cellular quality control and metabolic regulation. Dysregulation of autophagy is one of the major pathogenic factors contributing to the progression of non-alcoholic fatty liver disease (NAFLD). Autophagy is involved in the breakdown of intracellular lipids and the maintenance of healthy mitochondria in NAFLD. However, the mechanisms underlying autophagy dysregulation in NAFLD remain unclear. Here, we demonstrate that the hepatic expression of T factor was significantly increased in NAFLD conditions. Liver-specific T factor knockout improved lipid accumulation and metabolic properties in high-fat diet-induced NAFLD model. Further, T factor deficiency enhanced autophagy, PINK1 expression and mitochondrial function. Interestingly, T factor knockout increased the cytosolic translocation of AMPK from the nucleus and enhanced its activation through physical interaction. The translocation of AMPK is regulated by direct binding with AMPK and C terminal domain of T factor. Our results indicate a role for T factor in NAFLD progression and suggest that T factor is a potential target for NAFLD treatment. This research was funded by 1.210034.01, 2016M3A9D5A01952411, 2020R1I1A1A01074940, 2021R1I1A2041463, 2018R1A5A1024340, KGM5392212 and IBS-R022-D1.

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M. Metabolism and Metabolic Diseases [M-39]

Investigations on the intervention effect of Irpex lacteus polysaccharides on rats with PCOS

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Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies among women of childbearing age. This study aimed to investigate the effect of *Irpex lacteus* polysaccharide (ILP, 1000 mg/kg) on the letrozole (1 mg/kg)-induced PCOS model in female rats. Metformin (Met, 265 mg/kg) as the positive control. The study suggested that the ILP restored the estrous cycle of PCOS rats and reduced body weight and the relative weight of ovaries. ILP ameliorated the abnormal ovarian structure and fibrosis in PCOS rats. ILP decreased the testosterone (T), luteinizing hormone (LH), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), fasting blood glucose (FBG), insulin (INS) and homeostasis model assessment-insulin resistance (HOMA-IR) levels and increased the estrogen (E2) and follicle-stimulating hormone (FSH) levels in PCOS rats. In addition, ILP treatment inhibited the activation of the fibrosis associated TGF-β1/Smad pathway and downregulated the expression of connective tissue growth factor (CTGF), α-smooth muscle actin (α-SMA) and Collagen Ⅰ in the ovaries of PCOS rats. These suggest that ILP has potential therapeutic ability in letrozole-induced PCOS rats by ameliorating sex hormone levels, metabolic disturbances and ovarian fibrosis.

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M. Metabolism and Metabolic Diseases [M-40]

Effect of cerium oxide nanoparticles on letrozole-induced ovarian fibrosis and sex hormone and lipid levels in PCOS rats

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The aim of this study was to investigate the effects of cerium oxide nanoparticles (CeNPs), a UV shielding agent for food packaging, on hormone levels and ovarian fibrosis in rats with letrozole (1 mg/kg/d) induced polycystic ovary syndrome (PCOS). Female SD rats were randomly divided into control, PCOS and PCOS+CeNPs groups (30 mg/kg/d, 300 mg/kg/d and 600 mg/kg/d). After 28 days of continuous gavage administration, the rats were executed and blood was collected from the abdominal aorta and ovaries. serum hormones FSH, LH, E2, T, TG, TC, LDL-C and HDL-C were measured by ELISA. compared with the PCOS group, Masson staining in the CeNPs-treated group observed increased ovarian fibrosis, serum T, E2, LH, TC, TG, LDL-C and ovarian TGF-β1 and α-SMA expression levels were significantly increased, and FSH and HDL-C levels were significantly decreased, all in a dose-dependent manner. The results of this study suggest that exposure of PCOS rats to CeNPs alters serum sex hormone levels and affects lipid metabolism, while increasing doses exacerbate ovarian fibrosis.

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M. Metabolism and Metabolic Diseases [M-41]

Effects of black onion vinegar on high fat diet-induced obese C57BL/6 mice model

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Consumption of high fat diet (HFD) induces obesity by accumulating triglycerides and inflammation in the body. In the present study, we investigated the effects of black onion vinegar (BV) on HFD-induced C57BL/6 obese mice model. The HFD-fed obese mice were administered black onion juice (BJ) and BV, respectively, for 6 weeks. The HFD-fed group increased body and organ weights compared with normal control diet-induced group. However, administration of BV significantly reduced body and organ weights compared with HFD-fed group. The BJ- and BV-administered groups improved the serum lipid profiles such as total cholesterol and triglyceride, compared with HFD-fed group. In addition, BV-administered group significantly improved serum high-density lipoprotein cholesterol and low-density lipoprotein cholesterol. The BV-administered mice had increased the number and size of adipose cells in the liver and adipose tissues. The administrations of BJ and BV significantly down-regulated adipogenesis transcription factors and proinflammatory proteins in the liver compared with HFD-fed group. In particular, BV-administered group showed stronger attenuation of adipogenesis-related proteins than the BJ-administered group. Therefore, this study demonstrated that administration of BV attenuated HFD-induced obesity, in particular down-regulation of adipogenesis, and it could be developed as a functional vinegar for anti-obesity.

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M. Metabolism and Metabolic Diseases [M-42]

The change of bone marrow monocytes in the development of obesity-induced inflammation and insulin resistance

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Obesity is one of the factors that cause metabolic diseases and develop type 2 diabetes by exacerbating inflammation and insulin resistance. Bone marrow produces immune cells and new blood cells. To unbiasedly investigate the cells in the bone marrow (BM), wild-type (C57BL/6J) mice were treated with a low-fat diet (LFD) and a high-fat diet (HFD) for 12 weeks. We performed single cell RNA-sequencing by sorting whole live cells in the BM. We identified that obesity altered the cell numbers and populations in the BM. We found that there were Cd209a+Ly6c+ monocyte and one Ly6c- monocyte cluster in the BM. Cd209a+Ly6c+ monocytes are known to egress into circulating blood. In this cluster, the frequency of HFD was significantly decreased by obesity. However, the frequency of HFD increased the most in the Ly6c- monocyte cluster. Through trajectory analysis, we found that non-classical monocytes are the last to differentiate. We expect that Cd209a+Ly6c+ monocyte contributes to systemic inflammation and systemic insulin resistance. Based on these findings, we will target the clusters altered by obesity to investigate the molecular functions of inflammation.

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M. Metabolism and Metabolic Diseases [M-43]

Unique Adipose Invariant Natural Killer T Cell Subpopulations Control Adipocyte Turnover by Orchestrating Cellular Crosstalk

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Adipose invariant natural killer T (iNKT) cells are a crucial cell type for adipose tissue homeostasis in obese animals. However, heterogeneity of adipose iNKT cells and their function in adipocyte turnover are not thoroughly understood. Here, we carefully investigated heterogeneous adipose iNKT cells and their hierarchy in lean and obese mice, and found that distinct subpopulations of adipose iNKT cells modulate adipocyte death and birth for adipose tissue homeostasis. Single-cell RNA sequencing analysis revealed that KLRG1+ iNKT cells are a unique iNKT cell subpopulation in adipose tissue. Adoptive transfer experiments showed that KLRG1+ iNKT cells were selectively generated within adipose tissue microenvironment and differentiated into a CX3CR1+ cytotoxic subpopulation in obesity. Intriguingly, CX3CR1+ iNKT cells specifically killed enlarged and inflamed adipocytes and could recruit macrophages through CCL5. Furthermore, adipose iNKT17 cells stimulated adipose stem cell proliferation via amphiregulin secretion. Collectively, our data suggest that each adipose iNKT cell subpopulation plays key roles to control adipocyte turnover by orchestrating cellular crosstalk in adipose tissue.

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M. Metabolism and Metabolic Diseases [M-44]

Lactobacillus delbrueckii subsp lactis CKDB001 ameliorates hepatic lipid accumulation through gut microbiome restoration

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Many studies reported that gut microbiome eubiosis could prevent/treat several diseases, such as inflammatory, metabolic disease, cancer, and even mental disorder. Therefore, live biotherapeutic product (LBP) has been proposed one of the innovative treatments. We isolated and identified many strains from several origins and selected *Lactobacillus delbrueckii* subsp. *lactis* (*L. lactis*) CKDB001 through *in vitro* screening. In this study, we prepared *L. lactis* CKDB001 following optimized manufacturing process and viable cell concentration was measured. To examine non-alcoholic fatty liver disease (NAFLD)-therapeutic effect, 5-week-old C57BL/6J male mice were fed Gubra amylin NASH (GAN) diet for 16 weeks to induce NAFLD. After disease-induction period, *L. lactis* CKDB001 were treated for 8 weeks with each dose: 1.0×108, 1.0×109 or 1.0×10^{10} CFU/day. Until the end of the experiment, no specific clinical symptoms were observed. *L. lactis* CKDB001-treated groups showed reduction of liver weight and fat mass and improvement of liver enzymes and lipid profiles in serum. In addition, hepatic triglycerides and cholesterol were significantly decreased. In gut microbiome analysis, we found that the restoration of gut microbiota and clustering of *L. lactis* CKDB001-treated groups. Our findings suggest that *L. lactis* CKDB001 has a therapeutic potential for NAFLD through gut microbiome restoration.

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M. Metabolism and Metabolic Diseases [M-45]

The novel roles of hepatic G12 family proteins in energy metabolism

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The impact of liver pathophysiology on whole-body energy metabolism is largely attributed to fine-tuned regulation of both lipid and glucose homeostasis upon metabolic challenge. Heterotrimeric G proteins converge with activated GPCRs to control cell-signaling pathways to maintain metabolic homeostasis. Here, we investigated the regulatory role of G12 family proteins (Gα12 and Gα13) on hepatic and whole-body energy metabolism in mice. Gα12 ablation markedly augmented fasting-induced hepatic steatosis. Defective induction of SIRT1 upon fasting was observed in the liver of *Gna12*-KO mice, which was reversed by Gα12 overexpression in hepatocytes. *Gna12*-KO mice showed higher susceptibility to diet-induced liver steatosis and obesity due to decrease in energy expenditure. We also found that expression of G protein α-13 (Gα13) was decreased in the liver of mice and humans with diabetes. Liver-specific *Gna13* gene deletion in mice resulted in systemic glucose intolerance. Comparative secretome analysis identified ITIH1 as a protein secreted by liver that was responsible for systemic insulin resistance in *Gna13*-KO mice, showing positive correlation with surrogate markers for diabetes in patients with diabetes. Collectively, our results demonstrate that Gα12 and Gα13 play a key role in regulating lipid and glucose metabolism, respectively, suggesting promising therapeutic targets against metabolic disease.

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M. Metabolism and Metabolic Diseases [M-46]

MLK3 kinase inhibitor, GSS3, can ameliorate NASH development by blocking CXCL10 expression

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Non-alcoholic steatohepatitis (NASH) is a type of non-alcoholic fatty liver disease (NAFLD) and caused by lipotoxicity-induced hepatic damage and inflammation. Even though many researchers and pharmaceutical companies have tried to develop a drug for NASH, no drug has been approved for NASH treatment. Recently, it has reported that CXCL10 plays a crucial role in steatosis and hepatitis during NASH development. The previous reports suggested that MLK3-p38-STAT1 signaling pathway induces CXCL10 expression. In Genesen Co. Ltd., we have developed MLK3 inhibitor, which is a small molecule drug called GSS3, and examined its anti-inflammation and anti-steatosis effect in *in vitro* and *in vivo* model. According to in vitro experiment results, the expression of cytokines (CXCL10, IL-1β), lipogenesis-related genes (SREBP1, PPARγ), and fibrosis-inducing gene (col1a1) were significantly reduced by GSS3 treatment, indicating that GSS3 can inhibit hepatitis as well as steatosis and fibrosis. As expected, *in vivo* experiments showed the resolution of disease activity in acute liver injury, steatohepatitis, and liver fibrosis by treatment of GSS3. Taking all together, GSS3 has a therapeutic potential to cure NASH by blocking MLK3 activity, which leads to inhibition of hepatitis and steatosis.

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M. Metabolism and Metabolic Diseases [M-47]

Unveiling the Contributing Factors of NAFLD Progression: A Systematic Review using High-fat/High-fructose Diet Rodent Model

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Background: The aims of this study are to unveil the contributing factors of NAFLD progression and provide a detailed preclinical study design focusing on high-fat, high-fructose diet rodent model.

Methods: The PubMed database was searched between March and September 2022, focused on NAFLD and high-fat, high-fructose rodent model. We extracted the characteristics of the rodent models, diet type, and NAFLD progression. To establish the contributing factors of NAFLD, moderation analysis and hierarchical multiple regression analysis were applied.

Results: 7 variants (inducing period, calories from carbohydrates, cholesterol and sucrose in pellet, fructose and glucose in drinking water, and interaction between sex and fructose in water) were found to contribute to NASH Activity Score and 5 variants (age, inducing period, calories from pellet, cholesterol and sucrose in pellet) contribute to Fibrosis Score. Using these contributing factors, NAFL and NASH progression could be explained 53.4% and 75.2% of fibrosis progression by regression equations.

Conclusions: This systematic analysis establishes the contributing factors of NAFLD spectrum and provides a detailed preclinical study design using NAFLD rodent model. Also, the progression of NAFLD can be predicted by regression model. Therefore, this review is meant to suggest the most relevant NAFLD rodent model for clinical development.

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M. Metabolism and Metabolic Diseases [M-48]

Callistemon citrinus leaf extract exerts antoi-obesity effect by regulation of lipid metabolism in high-fat diet fed mice

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Obesity is an epidemic metabolic disease, and its associated metabolic disorders are among the major health challenges of our time. This study examined the anti-obesity effect of *Callistemon citrinus* (CC) by using its leaf extract in high-fat diet fed mice. Male eight-week-old C57BL/6J mice were divided into five groups, which were fed either a normal diet (CON), a high-fat diet (HFD), HFD plus orally administered Orlistat (50 mg/kg), 50 or 200 mg/kg of CC (CC 50 or CC 200) for 12 weeks. The HFD group showed increased body weight and fat mass of various tissues compared to the CON group. In contrast, CC 200 group showed significantly lower body weight than the HFD group. The weights of epididymal, mesenteric, perirenal, and retroperitoneal adipose tissues were significantly reduced in CC 200 compared to the HFD group. In addition, total cholesterol and low-density lipoprotein-cholesterol levels were analysed, and the CC 200 group significantly reduced compared to the HFD group. We are investigating various lipid metabolism factors, including AMP-activated protein kinase and uncoupling protein-1, to clarify the molecular mechanism of anti-obesity effect of CC. Thus, CC leaf extract might be a good candidate for prevention or treatment of obesity and related metabolic diseases.

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M. Metabolism and Metabolic Diseases [M-49]

Ginsenoside CK inhibits the early stage of adipogenesis via AMPK-MAPK-AKT signaling pathways.

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Obesity is emerging as a potential risk factor that can harm health because it is a cause of cardiovascular diseases such as dyslipidemia, hypertension, myocardial infarction, and type 2 diabetes. Compound-K, an active ingredient in ginseng, has been known as a functional food with potential antioxidant, anti-inflammatory, and anti-cancer properties. Although CK was used for various therapeutic applications, its role for improving obesity-related metabolic disease focused on adipocyte differentiation remains unknown. In this study, we found that CK significantly inhibited lipid accumulation and reduced expression of adipogenesis-related genes and proteins. Furthermore, we confirmed that the inhibited expression of CDK2 and cyclin B1 proteins and increased expression of p21 and p27 during clonal expansion of adipocyte differentiation could be a result of cell cycle arrest at the G2M phase. CK treatment significantly inhibited the activation of AKT, ERK, and P38 and increased phosphorylation of AMP-activated protein kinase AMPK and ACC protein expression. Interestingly, in 3T3-L1 cells, co-treatment with Dorsomorphin, an AMPK-selective inhibitor, reduced CK-induced AMPK and ACC phosphorylation, and PPAR-γ expression was also significantly reduced compared to CK alone. These data suggest that CK exerts its anti-adipogenic effects regulating the early stage MCE during adipocyte differentiation through the AMPK-MAPK signalling pathways.

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M. Metabolism and Metabolic Diseases [M-50]

Echinochrome A prevents diabetic nephropathy by inhibiting the PKC-iota signaling pathway in db/db mice

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Echinochrome A (EchA), a natural bioproduct extracted from sea urchins, exerts antioxidant and beneficial effects in a variety of inflammatory disease models. However, its effects on diabetic nephropathy (DN) remain poorly understood. In the present study, seven-week-old diabetic and obese db/db mice were injected with EchA (3 mg/kg/day) intraperitoneally for 12 weeks, while db/db control mice and wild-type (WT) mice received an equal amount of sterile 0.9% saline. EchA improved glucose tolerance and reduced blood urea nitrogen (BUN) and serum creatinine levels but did not affect body weight. In addition, EchA decreased renal malondialdehyde (MDA) and lipid hydroperoxide levels. Histologically, EchA treatment ameliorated renal fibrosis. Mechanistically, EchA suppressed oxidative stress and fibrosis by inhibiting protein kinase C-iota (PKCι)/p38 mitogen-activated protein kinase (MAPK), downregulating p53 and c-Jun phosphorylation, and attenuating NADPH oxidase 4 (NOX4) and transforming growth factor-beta 1 (TGFβ1) signaling. Collectively, these findings demonstrate that EchA prevents DN by attenuating renal fibrosis and oxidative stress via inhibiting PKCι/p38 MAPK pathway.

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M. Metabolism and Metabolic Diseases [M-51]

Curcumin ameliorates alcohol-related fatty liver disease by downregulating ANGPTL4

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Alcoholic fatty liver disease (AFLD) is caused by excessive alcohol consumption, impairing liver function. Curcumin, an active component in *Curcuma longa*, has been proposed as a substance for AFLD therapy due to its biological functions including antioxidative properties. However, the mechanism by which curcumin protects the liver disfunction caused by alcohol has not been fully elucidated. Therefore, we investigated the protective effect of curcumin on lipid metabolism of liver and its mechanisms against AFLD. Curcumin showed modulatory effects on the level of triglyceride and free fatty acid in liver and serum in vivo AFLD model and reduced lipid contents in ethanol-treated hepatocellular carcinoma (HepG2) cells. Curcumin treatment downregulated expressions of genes mediating triglyceride and cholesterol synthesis along with angiopoietin-like 4 (ANGPTL4), which is a key regulator of lipid metabolism. The expressions of peroxisome proliferator-activated receptors (PPARs), major transcription factors of ANGPTL4, were also downregulated by curcumin. Such inhibitory effect of curcumin on the cellular lipid levels contents was alleviated by knockdown of ANGPTL4. Our results suggest that curcumin has a hepatoprotective effect attenuating the lipid accumulation in hepatocytes involved in AFLD through downregulating ANGPTL4.

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M. Metabolism and Metabolic Diseases [M-52]

Plasma lipidomic profiles in acute ischemic stroke patients with atrial fibrillation

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Atrial fibrillation (AF) is one of the most important risk factors for ischemic stroke and is associated with increased stroke severity. AF-related stroke patients had higher mortality, lower discharge rates, and longer hospital stays than those caused by other reasons. This study aimed to identify the plasma lipidomic signatures associated with AF-related stroke. LC-MS-based-lipidomic analysis was performed from plasma samples of ischemic stroke patients with AF (n=92) and without AF (n=391). Among several lipid classes, the levels of lipid classes such as free fatty acids (FFAs), lysophosphatidylcholines (LysoPCs), phosphatidylcholines (PCs), and triacylglycerols (TAGs) were significantly changed in ischemic stroke patients with AF. The levels of FFAs showed the most characteristic differences and the compositions of FFAs were also altered depending on the existence of double bonds in stroke patients with AF. In this study, the associations between the alterations in FFAs and AF-associated stroke were examined and these results suggest that FFAs might be closely related with ischemic stroke with AF.

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M. Metabolism and Metabolic Diseases [M-53]

M14, novel reno-protective agent, ameliorates TGF-beta-induced renal fibrosis.

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Chronic kidney disease (CKD) is increasing in prevalence and can cause social and economic problems. CKD is characterized by tubulointerstitial fibrosis and accumulation of extracellular matrix. Unfortunately, very few anti-renal fibrotic agents exist. M14, discovered through drug screening, is an anti-cholinergic agent; however its effects on renal fibrosis remains unknown. We investigated whether M14 can attenuate TGF-β-induced renal fibrosis in kidney fibroblasts and renal tubular epithelial cells.

In cultured NRK-49F and HK-2, M14 significantly inhibited TGF-β-stimulated expression of collagen type I, plasminogen activator inhibitor-1 (PAI-1) and α-smooth muscle actin (α-SMA). M14 inhibited TGF-β-induced PAI-1 secretion as well as PAI-1 promoter activity in both renal cells. To determine the molecular mechanism by which M14 inhibits renal fibrotic factors, we examined whether M14 inhibits Smad3 activity. M14 inhibited TGF-β-stimulated the phosphorylation of Smad3 in NRK-49F and HK-2. It is necessary to investigate the additional inhibitory effect of renal fibrosis of M14 through in vivo study

Our results suggest that M14 ameliorates renal fibrosis through inhibition of TGF-β/Smad3 signaling pathway and has the potential of novel reno-protective agent.

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M. Metabolism and Metabolic Diseases [M-54]

The evaluation of Mefloquine drug repurposing on kidney disease

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Drug repurposing is a strategy to identify new uses for approved drugs that are outside the scope of the original medical indication, and is emerging as an important tool in the fight against many diseases. We found very interesting results for mefloquine, a medication used to prevent or treat malaria against plasminogen activator inhibitor-1 (PAI-1), one of fibrogenic factors, in renal cells. However, the effect of mefloquine on renal fibrosis remains unknown. We investigated whether mefloquine can inhibit renal fibrotic factors in cultured renal cells.

In renal fibroblasts, NRK-49F and renal tubular epithelial cells, HK-2, mefloquine significantly inhibited TGF-β-stimulated PAI-1 secretion as well as PAI-1 expression. In addition, mefloquine inhibited TGF-β-stimulated PAI-1 promoter activity. To investigate the effects of mefloquine on other renal fibrogenic factors, we identified the expression of collagen type 1 and α-smooth muscle actin (α-SMA) in both renal cells. Mefloquine decreased TGF-β-stimulated the expression of collagen type 1 and α-smooth muscle actin (α-SMA). We investigated the effect of mefloquine on Smad3 activity to elucidate the its mechanism on renal fibrosis. Mefloquine inhibited TGF-β-induced phosphorylation of Smad3 in both renal cells.

These results show that mefloquine attenuates kidney fibrotic factors via inhibition of TGF-β/Smad3 signaling pathway. We propose that mefloquine is a potential therapeutic agent for kidney disease through drug repurposing.

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M. Metabolism and Metabolic Diseases [M-55]

Hepatic fat accumulation exacerbates acetaminophen-induced hepatotoxicity via inflammasome pathway

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Obesity is a risk factor for chronic fatty liver, but its mechanism is largely unknown. It is however, believed that consumption of high fat diet (HFD), which often underlies obesity, plays a role in the mechanism. This study aimed to identify the effect of acetaminophen-induced acute liver injury, the most common cause of acute liver failure, in an obesity model increased via HFD intake. Mice were fed with HFD for eight weeks and then administrated with acetaminophen (APAP) on last day. Serum alanine aminotransferase (ALT)/aspartate aminotransferase (AST) levels, hepatic malondialdehyde formation, and hepatocellular injury in mice fed HFD and APAP (HFD+APAP) were significantly increased in mice fed control diet (CD) and APAP (CD+APAP). However, hepatic glutathione content was more reduced in HFD+APAP mice compared to CD+APAP mice. Furthermore, HFD treatment enhanced APAP-induced expression of NLRP3-target protein by accelerating NF-κB activation. Interestingly, HFD exacerbated acetaminophen-induced acute liver injury in an inflammasome pathway-dependent manner. These results suggested that hepatic fat accumulation exacerbated the pathology of acetaminophen-induced liver injury through activation of the inflammasome pathway.

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M. Metabolism and Metabolic Diseases [M-56]

An optimized herbal medicine containing Scutellaria baicalensis ,Alisma canaliculatum ,and Atractylodes macrocephala Koidz attenuates hyperlipidemia by activating AMPK/SREBP2/PCSK9 signaling pathway

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The prevalence of hyperlipidemia is rising globally, and traditional herbal medicine is gaining the spotlight as a treatment for dyslipidemia. HTB is an optimized complex formula containing three herb medicines, Scutellaria baicalensis, Alisma canaliculatum, and Atractylodes macrocephala Koidz. In this study, we investigated the effect of HTB on hyperlipidemia mouse models and its potential biological mechanisms. Compared to the poloxamer 407-control group, HTB treatment considerably reduced high plasma and hepatic triglyceride (TC) and cholesterol (CHOL) levels. Studies using a high-fat diet showed that HTB treatment significantly reduced weight gain, plasma TC and LDL-CHOL, hepatic lipid contents, tissue damage marker, and inflammatory cytokines, but improved glucose tolerance, muscle weight, and physical activity. Biochemical studies revealed that HTB down-regulated the expression of fatty acid synthase, SREBP2, and HMGCR while simultaneously up-regulated the expression of PGC1α and ABCA1. Additionally, we found that HTB treatment promoted hepatic AMPK activity, then blocked PCSK9 activity, which regulates LDL receptor degradation. Taken together, we demonstrated that HTB ameliorates hyperlipidemia in P407- or/and high fat-induced obese mice via AMPK/SREBP2/PCSK9 signaling pathway. Hence, HTB could be used as a new lipid-lowering drug for the prevention and treatment of hyperlipidemia-associated disorders.

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M. Metabolism and Metabolic Diseases [M-57]

Anti-adipogenic effects of YBS1 in 3T3-L1 pre-adipocyte differentiation by p38 MAPK/PPARγ signaling

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An imbalance between energy intake and energy expenditure causes obesity. Obesity-related disorders such cardiovascular disease, arteriosclerosis, high blood pressure, and cancer also impact society and the economy. The inhibition of adipogenesis, which is the process of white adipocyte differentiation, is still a potential treatment for obesity. Oil Red O staining during 3T3-L1 differentiation was used for screening of 20 Lactobacillus species, and YBS1 (*Lactobacillus acidophilus* DS0079) was finally selected for further investigation. YBS1 treatment during 3T3-L1 development reduced Triglyceride (TG) accumulation and the mRNA expression of major adipogenic marker peroxisome proliferator-activated receptor gamma (PPARγ) and its downstream target genes such adipocyte fatty acid binding protein 4 (aP2) and adiponectin is also nearly abolished. YBS1 suppressed early-stage (day 0-2) adipocyte differentiation, but there was no significant difference between mid-(day 2-4) and late-stage (4-6) differentiation. During early adipogenesis, YBS1 treatment activated p38 MAPK, which was abolished by SB203580 inhibitor. Our findings showed that YBS1 treatment suppressed early adipogenesis through activating p38 MAPK signaling to regulate PPARγ expression. In conclusion, studies reveal that YBS1 may be effective in anti-obesity supplements and needs further investigation.

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M. Metabolism and Metabolic Diseases [M-58]

Characterization of protein-protein interaction of Topoisomerase IIα as a transcriptional coactivator with the nuclear receptor LXRβ

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The Liver X Receptor (LXR) is a transcriptional factor belonging to nuclear receptor superfamily, regulating lipid metabolism. Our previous study showed that DNA Topoisomerase IIα (TOPO2A) specifically regulates the transcriptional activation of LXRβ. To support it, this study aims to characterize a protein-protein interaction (PPI) between TOPO2A and LXRβ. Homology comparison showed that the two isoforms of TOPO2 TOPO2A and TOPO2B were highly homologous. However unlikely to TOPO2A-II, 248~692 A.A of TOPO2A that may be responsible for the coactivator function, TOPO2B-II did not affect transcriptional activities of LXRβ on ABCA1 and ABCG1 promoters. In GST pull down assay, TOPO2B-II showed less interaction with LXRβ than TOPO2A-II. The TOPO2A-II mutant was constructed by swapping the regions of the two isoforms, predicted for coactivator function domain, resulting in disappearance of the effects of TOPO2A. The next study was to identify a subdomain of LXRβ, which is responsible for the specific interaction with TOPO2A. GST pull down assay showed that LXRβ hinge-LBD showed the most robust interaction with TOPO2A, indicating the important role of LXRβ hinge-LBD on the PPI with TOPO2A. Therefore, these results confirmed that TOPO2A is a transcriptional coactivator of LXRβ through the specific PPI between LXRβ hinge-LBD and TOPO2A.

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M. Metabolism and Metabolic Diseases [M-59]

Involvement of a novel cAMP signaling mediator for beige adipogenesis

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Exposure to cold temperature stimulates the sympathetic nervous system that activates β-adrenergic receptor signals in brown and beige adipocytes, leading to the induction of adaptive thermogenesis in mammals. Prominin-1 (PROM1) is a pentaspan transmembrane protein that is widely identified as a marker for stem cells, although the role of this protein as a regulator of many intracellular signaling cascades has been recently delineated. In this study, we found out that Prom1 KO mice displayed an impairment in cold- or β3-adrenergic agonist-induced adaptive thermogenesis in subcutaneous adipose tissues (SAT) but not in brown adipose tissues (BAT). By fluorescence-activated cell sorting (FACS) analysis, we identified that PROM1 positive cells are enriched in AP cells from SAT. Prom1-deficient AP cells from SAT showed reduced potential for beige adipogenesis. Furthermore, AP cell-specific depletion of Prom1, but not adipocyte-specific depletion of Prom1, displayed defects in adaptive thermogenesis as evidenced by resistance to cold-induced browning of SAT and dampened energy expenditure in mice. In molecular level, PROM1 might be involved in the cAMP signaling-mediated induction of beige adipogenesis from AP cells through PROM1-ERM axis. To conclude, we found that PROM1 positive AP cells are essential for the adaptive thermogenesis by ensuing stress-induced beige adipogenesis.

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M. Metabolism and Metabolic Diseases [M-60]

Transcriptional regulation of ABCG5/8 by TRAP80 as a coactivator of LXRα

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ATP-binding cassette (ABC) transporter G5 (ABCG5) and G8 (ABCG8) play a critical role in cholesterol homeostasis by mediating the efflux of dietary sterols from enterocytes back into the intestinal lumen and from the liver to the bile duct. These transporters are transcriptionally regulated by the ligand-dependent activation of Liver X receptor (LXR), which is a transcriptional factor belong to nuclear receptor superfamily. The LXR response element (LXRE) has been previously identified in human ABCG5/8 but not in mouse ABCG5/8. It has been reported that TRAP80 as a transcriptional coactivator of LXRα regulates other ABC transporter ABCA1 and ABCG1 involved in reverse cholesterol transport pathway. Therefore, this study aimed to identify LXRE in mABCG5/8 and to investigate whether transcription of mABCG5/8 is also regulated by TRAP80 as a coactivator of LXRα. Deletion promoter studies revealed a region containing DR4 putative LXREs, which is responsible for the transcriptional activation of mABCG5/8 by TRAP80. The transcriptional regulation by TRAP80 was reduced by mutation of putative LXREs. Taken together, these results suggest that ABCG5/8 are regulated by TRAP80, differently from ABCA1

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M. Metabolism and Metabolic Diseases [M-61]

The protective effects of garlic on LPS-induced acute intestinal injury

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Garlic has long been known as an herb with medicinal properties, including anti-inflammatory effects. The purpose of this study was to investigate the potential benefits of garlic extract in treating lipopolysaccharide (LPS)-induced acute intestinal injury.

ICR mice were orally administered a garlic extract once daily for three days, and then LPS was intraperitoneally injected one hour after the last garlic extract treatment. Mice were sacrificed 24 hours after the LPS injection. Biomarkers and inflammation markers were measured in serum, and changes in protein expressions were analyzed by Western blot.

In the garlic-treated groups, serum levels of aspartate aminotransferase, alanine aminotransferase, and reactive oxygen species were lower compared to the LPS-injected control group. Additionally, the expressions of calprotectin and C-reactive proteins, which were elevated by LPS injection, were reduced by treatment with the garlic extract. Interestingly, the low-dose garlic treatment group showed a greater reduction in these markers than the high-dose group.

In tissue, levels of p-IκBα and NF-κBp65 were significantly increased in the control group, however, the activation of these proteins was suppressed in the garlic-treated group. Similarly, inflammatory factors were decreased in the garlic-treated group compared to the control group. Moreover, garlic treatment reduced expressions of apoptotic factors and increased expressions of anti-apoptotic factors.

These results suggest that garlic may play an important role in regulating inflammation and apoptosis in the LPS-treated intestine, and could potentially be developed as a natural agent for treating acute intestinal damage.

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M. Metabolism and Metabolic Diseases [M-62]

Screening of Korean herb medicines for improving both esophageal mucosa defense and inflammation

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Background: This study evaluates MUC5AC expression with several Korean herb medicines for esophageal mucosa defense and analyzes their associated mechanisms involved in inflammation.
Methods: 40 kinds of Korean herb medicines measured in vitro antioxidant activities and also analyzed the expression of MUC5AC under pH 4.5 conditions in human esophageal epithelial cells (Het-1A) through real-time PCR. Among them, 8 kinds of extracts with increased mucus secretion were treated with 480 μM bile salts and pH 5.5 acid medium for 3 hr and subsequently measured ROS production. Inflammation-associated signaling molecules were also measured by western blotting.
Results: Compared with the Normal group, ROS generation was higher in the Control group. Such increased ROS levels were significantly reduced in 4 groups (Linderae Radix, Citri Unshius Pericarpium, Ginseng Radix, and Aucklandiae Radix). Especially, water extract of Linderae Radix exerted the superior to other groups. Here, surprisingly, ethanol extract of Aucklandiae Radix possessed the cytotoxic effect at 200 μg/mL. Moreover, inflammatory proteins decreased through the inhibition of nuclear factor-kB (NF-κB) activation in two groups (Linderae Radix and Citri Unshius Pericarpium). The protein expression of the extracellular regulatory kinase (ERK) by treatment of Linderae Radix and Citri Unshius Pericarpium is significantly reduced via the suppression of MAPK/ERK kinase (p-MEK). Besides, similar to the above result, mRNA levels of proinflammatory cytokines including interleukin (IL)-6 and IL-8 downregulated in two groups.
Conclusions: Taken together, 2 groups (Linderae Radix and Citri Unshius Pericarpium) showed significant elevation of MUC5AC secretion and the reduction of both ROS and inflammatory proteins without cytotoxicity. These extracts could be used as a potential target for treating esophageal inflammation.

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M. Metabolism and Metabolic Diseases [M-63]

Pinus sylvestris Exerts Antioxidant Effects in U2OS Cells and Anti-inflammatory Effect in Osteoarthritis Rat Model

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In this study, we explored the effect of *Pinus sylvestris* (PS) in U2OS cells and MIA-induced rat model. MitoSox and MitoTraker were used to visualize oxidative stress in U2OS cells through IF. To induce osteoarthritis in SD rats, MIA (50 μL with 80 mg/mL) was treated. PS (200 mg/kg b.w.) was orally treated once daily for 2 weeks from 7 days after MIA injection. The changes in the weight distribution of the hind paws (HWD) were recorded. Moreover, western blotting and serum analysis were used to assess the activation indicators linked to the inflammatory response and cartilage deterioration. In the IF assay, MitoSox dramatically reduced whereas MitoTraker significantly increased. In vivo animal experiments, the decreased HWD in the MIA control group confirmed a meaningful elevation after PS treatment. Also, PS not only inhibited the inflammatory cytokines including TNF-α, IL-6, and IL-1β but also significantly suppressed the increased MMP-2 and MMP-13 levels. These results indicated that PS treatment exhibited markedly antioxidant effects on H_2O_2-induced oxidative stress and also showed the inhibition both of inflammatory cytokines release and collagen degradation.

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M. Metabolism and Metabolic Diseases [M-64]

Effect of Rhei Radix et Rhizoma and Citri Unshius Pericarpium Mixture in Chronic Acid Reflux Esophagitis Rats

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This study was to evaluate the protective effect of Rhei Radix et Rhizoma and Citri Unshius Pericarpium mixture (RC) in rats with chronic acid reflux esophagitis (CARE). CARE was surgically induced and rats with CRAE except for Normal rats were divided into Control, Vitamine E 30 mg/kg, RC 100 mg/kg, and RC 200 mg/kg groups. Drugs were treated orally for 2 weeks. Malondialdehyde levels were measured in serum and esophageal tissue. Moreover, protein expressions related to the pro-inflammatory and tight junction were analyzed by Western blot. RC treatment noticeably reduced malondialdehyde levels of both serum and esophagus tissue. The elevated levels of PPARγ/RXR significantly inhibited NF-κB activation. Subsequently, NF-κB inactivation by RC supplementation effectively led to the reduction of pro-inflammatory mediators and cytokines. However, the reduced tight junction factors were dramatically increased by RC treatment. Moreover, in RC groups, the elevation of MMPs expressions was inhibited, whereas, the reduction of TIMPs expressions increased. RC possesses mucosal protective effects by suppressing inflammation via the inhibition of the PPARγ/NF-κB pathway and by increasing via the elevation of tight junction expressions.

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M. Metabolism and Metabolic Diseases [M-65]

Effects of moderate or high interval intensity Aerobic Exercise in the Fasted State on Fat Browning and Expression of Adipomyokines in eWAT and Blood of Rats

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Fat browning converts white adipose tissue (WAT) to thermogenic brown or beige adipose tissue, which induces weight loss and alleviates metabolic complications of obesity. Aerobic exercise, especially in a fasted state, can speed up fat browning by inducing thermogenic genes and adipomyokines in WAT. To investigate the effects of fasting and exercise on fat browning, male Sprague Dawley rats were divided into four groups: fed control, 24-hour fasted, moderate-intensity aerobic exercise in a fasted state, and high-intensity interval exercise after a 24-hour fast. Regardless of exercise intensity, fasting exercise activates fat browning and thermogenic factors in peripididymal white adipose tissue (eWAT). Aerobic exercise in the fasted state also increases blood levels of adipomyokine associated with fat browning. The increase in β-HB according to exercise intensity has a strong correlation with blood adipomyokine. Further evidence is needed to support a direct relationship between upregulation of β-HB and fat browning by aerobic exercise in the fasted state, but moderate-intensity or higher aerobic exercise may promote fat browning.

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M. Metabolism and Metabolic Diseases [M-66]

TGFBI remodels adipose metabolism by regulating the Notch-1 signaling pathway

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Extracellular matrix proteins are associated with metabolically healthy adipose tissue and regulate inflammation, fibrosis, angiogenesis, and subsequent metabolic deterioration. In this study, we demonstrated that transforming growth factor-beta (TGFBI), an extracellular matrix (ECM) component, plays an important role in adipose metabolism and browning during high-fat diet-induced obesity. TGFBI KO mice were resistant to adipose tissue hypertrophy, liver steatosis, and insulin resistance. Furthermore, adipose tissue from TGFBI KO mice contained a large population of CD11b+ and CD206+ M2 macrophages, which possibly control adipokine secretion through paracrine mechanisms. Mechanistically, we showed that inhibiting TGFBI-stimulated release of adipsin by Notch-1-dependent signaling resulted in adipocyte browning. TGFBI was physiologically bound to Notch-1 and stimulated its activation in adipocytes. Our findings revealed a novel protective effect of TGFBI deficiency in obesity that is realized via the activation of the Notch-1 signaling pathway.

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M. Metabolism and Metabolic Diseases [M-67]

Hepatoprotective role of tumor necrosis factor-inducible gene 6 protein against alcohol-induced liver damage in mice

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Alcohol-associated liver disease (ALD) is globally prevalent chronic liver disease caused by chronic or binge consumption of alcohol. Tumor necrosis factor-inducible gene 6 protein (TSG-6) is one of the anti-inflammatory cytokines released from mesenchymal stem cells. Recent studies show that TSG-6 reduce liver fibrosis and improves regeneration in chronic liver disease. Thus, we investigated the effect of TSG-6 on ALD. 7-week-old WT male mice were fed Lieber-DeCarli alcohol liquid diet for 12 weeks. After 9 weeks, TSG-6 or vehicle was administered every other day along with feeding liquid diet for 3 additional weeks. Chronic alcohol feeding increased the liver weight to body weight (LW/BW) ratio and induced liver damages such as lipid accumulation, hepatocytes ballooning and inflammation. However, TSG-6 treatment significantly relieved LW/BW ratio and liver damages. Also, the expression of proinflammatory, profibrotic markers and accumulation of collagen fibrils were significantly decreased in the livers of alcohol-fed mice treated with TSG-6. These results demonstrate that TSG-6 attenuates alcohol-induced damages in the livers of mice, and suggest therapeutic potential of TSG-6 for ALD.

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M. Metabolism and Metabolic Diseases [M-68]

Role of Tribbles 3 in Brown Adipose Tissue

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Obesity, caused by the imbalance between energy intake and expenditure, is a serious health risk factor associated with diabetes and other metabolic syndromes. The unique expression of UCP1 enables brown adipose tissue (BAT) to serve as an energy-consuming organ by dissipating energy into heat. Therefore, activation of BAT is considered a promising approach for the prevention of obesity.

Tribbles 3 (TRIB3) is a member of pseudokinase family that involved in metabolic cancer, lipid metabolism and etc. Metabolic function of TRIB3 has been explored in done on skeletal muscle, cancer, diabetes, and etc. However, its role in BAT has not been largely studied and remains to be elucidated. In this study, we used TRIB3 whole body knock out (KO) mice to investigate the role of TRIB3 in BAT. Body weight and fat mass was lower in TRIB3 KO mice than control group. Differentiation of BAT was higher and mRNA expression level of mitochondrial markers, such as SDHA, PDH, and UCP1 was higher in TRIB3 KO mice than control group. In conclusion, inhibiting TRIB3 could lead to increase the activity of BAT which leads to high energy consumption and prevent the incidence of obesity.

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M. Metabolism and Metabolic Diseases [M-69]

Chemical Characterization of the Functional Natural Products from the Fruits of Sea Buckthorn (Hippophae rhamnoides) that Control Osteoblast Differentiation

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In the screening assay, we found that the ethanolic extract of the fruits of sea buckthorn (*Hippophae rhamnoides*) showed anti-osteoporosis effects *in vitro* and *in vivo*. A bioassay-guided fractionation and LC/MS-based isolation of the active ethanolic extract resulted to the isolation of potential bioactive compounds (**1-6**), chemical structures of which were identified as four triterpenes, ursolic acid (**1**), uvaol (**2**), oleanolic aldehyde (**3**), and ursolic aldehyde (**4**) by the interpretation of their spectroscopic NMR data and LC-MS analysis as well as HR-ESI-MS data. The isolated compounds were tested for the efficacy of promoting osteoblast differentiation and the expression of mRNA biomarkers related to osteogenesis. Firstly, their effects on osteoblast differentiation of mouse mesenchymal stem cell C3H10T1/2 were tested. As a result of quantification by alkaline phosphate staining, ursolic aldehyde (**4**) at 10 μg/mL concentration showed 11.2 times higher activity than that of negative control, and four kinds of gene expressions were significantly increased in bone formation-related biomarker analysis. These findings provide the experimental evidence that ursolic aldehyde (**4**) derived from the extract of *H. rhamnoides* can be the active constituent for bone health.

N. Microbiology [N-1]

The SpACE-CCM: A Facile and Versatile Cell Culture Medium-based Biosensor for Detection of SARS-CoV-2 Spike-ACE2 Interaction

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The COVID-19 pandemic is an ongoing global public health threat. COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, and binding of the SARS-CoV-2 spike to its receptor, angiotensin-converting enzyme 2 (ACE2), on host cells is critical for viral infection. Here, we developed a luminescent biosensor that readily detects interactions of the spike receptor-binding domain (RBD) and ACE2 in cell culture medium ('SpACE-CCM'), which was based on bimolecular complementation of the split nanoluicferase-fused spike RBD and ectodomain of ACE2 and further engineered to be efficiently secreted from cells by adding a secretory signal peptide. The SpACE-CCM biosensor was validated through molecular and pharmacological experiments. Importantly, the SpACE-CCM biosensor responded well in assay-validating conditions compared with conventional cell lysate-based NanoLuc Binary Technolgy, indicating its advantage. We further demonstrated the biosensor's versatility by quantitatively detecting neutralizing activity in blood samples from COVID-19 patients and vaccinated individuals, discovering a small molecule interfering with the spike RBD-ACE2 interaction through high-throughput screening, and assessing the cross-reactivity of neutralizing

antibodies against SARS-CoV-2 variants. Because the SpACE-CCM is a facile and rapid one-step reaction biosensor that recapitulates the native spike-ACE2 interaction, it would be advantageous in experimental and clinical applications associated with this interaction.

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N. Microbiology [N-2]

Natural Agonist of Insect Olfactory Receptors: Behavior, Physiology and Structure

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The olfactory nervous system recognizes and distinguishes many different chemicals in the general living environment. Insects have evolved a group of odorant-gated ion channels composed of highly-developed olfactory receptors capable of distinguishing and distribution between various chemicals with symbolic or evasive specificities. Recently, aphid genomes related to olfaction, including olfactory receptors and proteins, have been identified and olfactory receptors have been reported that are differentially differentiated from Drosophila. The genome of the olfactory receptor has a very conservative sequence and a systematic signaling system. A representative receptor, odorant-gated ion channels comprised of a highly conserved co-receptor (Orco) has a homotetramer channel structure with four subunits arranged symmetrically around the central hole. It has a very similar structure to the 7-transmembrane receptor present in the human body and has a very similar structural form and gating mechanism to receptors of neurotransmitters.

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N. Microbiology [N-3]

Analysis of Transcriptional Slippage for Production of P3N-PIPO in Potyviruses, Using Plant RNA-seq Data

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Potyviruses have a small open reading frame known as PIPO, which is expressed as a trans-frame protein P3N-PIPO via a transcriptional slippage mechanism. P3N-PIPO is involved in cell-to-cell movement and linked to the spread of the virus throughout the host plant. A total of 1110 distinct virus contigs, which included the P3 region of potyvirus, were identified from 1092 high-throughput plant RNA-seq data. Out of these, nine species of potyvirus with 50 or more contigs were selected: potato virus Y (PVY), 132 contigs; turnip mosaic virus (TuMV), 100; sugarcane mosaic virus (SCMV), 97; soybean mosaic virus (SMV), 67; zucchini yellow mosaic virus (ZYMV), 64; leek yellow stripe virus (LYSV), 59; watermelon mosaic virus (WMV), 55; plum pox virus (PPV), 53; and onion yellow dwarf virus (OYDV), 50. The identified potyviruses exhibited the following average rates of transcriptional slippage: PVY, 3.71%; TuMV, 3.04%; SCMV, 2.74%; SMV, 1.33%; ZYMV, 3.64%; LYSV, 4.37%; WMV, 3.60%; PPV, 2.97%; and OYDV, 2.37%. This study demonstrates that plant RNA-seq data are useful for quantitation of potyvirus genome variants generated by transcriptional slippage and that potyvirus species have distinct transcriptional slippage rates.

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N. Microbiology [N-4]

Suppression of SARS-CoV-2 replication by cell penetrating peptide targeting C-terminal domain of spike protein

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Peptides have several advantages such as specificity, easy synthesis and fine-tuning, therefore they are considered as promising therapeutic agents for the treatment of COVID-19. Previously, we demonstrated that a cell permeable peptide corresponding to the SARS-CoV-2 Spike C-terminal domain inhibits the interaction between the S and N proteins resulting in the SARS-CoV-2 replication. In this study, we designed more potent all-D form short peptide, R-t-Spike CD(D) and evaluated its inhibitory effect against replication of SARS-CoV-2 S clade as well as various SARS-CoV-2 variants. The R-t-Spike CD(D) was successfully internalized into Vero cells and Calu-3 cells and suppressed replication of SARS-CoV-2 S clade and delta variant in the infected cells with higher potency compared to the original peptide. The intranasally administrated R-t-Spike CD(D) mitigated pathological change in lungs and increased survival rate of hemizygous K18-hACE2 mice after infection of SARS-CoV-2 S clade and delta variant. Therefore, our *in vitro* and *in vivo* data suggest that R-t-Spike CD(D) can be applied as potential antiviral therapeutics against SARS-CoV-2 infection.

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N. Microbiology [N-5]

Immune Enhancing Activity Fermented Circium setidens(Dunn) Nakai Extract in Mouse Macrophage RAW264.7

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In this study, we investigated whether fermented *Circium setidens* (CS) extract exhibits immunostimulatory activity in RAW264.7 cells. CS is special plants that grows only in Korea worldwide all. CS increased the production of NO in RAW264.7 cells. The expression of the immune enhancing factor iNOS, IL-1β, IL-6 and TNF-α was increase in RAW264.7 cells. The inhibition of TLR2 and TLR4 blocked CS mediated production of immune stimulation factors in RAW264.7 cells. To measured macrophage activity is neutral red assay was used to evaluate, and CS showed cell activity in a concentration dependent manner. In addition, morphological changes of the cells were also confirmed. Based on these results, CS is expected to be used as a potential functional agent for immune enhancement.

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N. Microbiology [N-6]

Dexamethasone-induced muscle atrophy was inhibited by Compound Pae in C2C12 myotubes.

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Compound Pae was discovered as a drug candidate for sarcopenia. This study was conducted to elucidate the suppressive effect of compound Pae on dexamethasone-induced muscle atrophy in the C2C12 myotubes and its mechanism of action. C2C12 myoblasts were differentiated for 5 days and treated with 100 μM of dexamethasone for 48 hours to induce muscle atrophy. The length and width of myotubes, which were reduced by dexamethasone treatment, were significantly increased by compound Pae. Muscle-specific ubiquitin E3 ligases, MurF1 and MAFbx, which were increased by dexamethasone treatment, were significantly decreased by compound Pae. MuRF1 was known to degrade myosin heavy chain (MyHC), which plays a major role in muscle contraction, and MAFbx degrades myoblast determination protein-1 (MyoD), which induces muscle differentiation. MyHC and MyoD, which were decreased by dexamethasone treatment, were significantly increased by compound Pae. Compound Pae, also reduced the NF-kB signaling and NF-kB nuclear translocation, which is known to play a role in the transcription of MuRF1 and MAFbx. These results suggest that compound Pae inhibits dexamethasone-induced muscle atrophy by reducing the expression of muscle-specific E3 ligase and NF-kB signaling in C2C12 myotubes.

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N. Microbiology [N-7]

Identification of interactions between Doxorubicin-induced haploinsufficency genes through systematic target screening in fission yeast

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Doxorubicin (DOX)-based chemotherapy is one of the most effective methods for the treatment of human cancer. However, clinical use is limited due to serious side effects. Therefore, if the target of a drug is identified, a new drug with fewer side effects can be developed or improved in a form with improved effects. In this study, Doxorubicin-induced haploinsufficency genes were found through systematic target screening in fission yeast and verified in several ways. As a result, surprisingly, it was found that two functionally unrelated genes showed sensitivity to Doxorubicin. In this study, we identified target genes of Doxorubicin using the heterozygous gene deletion library and characterized the action mechanisms of the screened target genes as a proof-of-concept that supports harnessing this genome-wide screening system for the identification and characterization of target genes affected under any condition of interest.

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N. Microbiology [N-8]

Target Screening of miconazole using the fission yeast gene-deletion library and the next generation sequencing (NGS) method

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Miconazole is antifungal widely used to treat mucosal yeast infections, including both oral and vaginal infections. Miconazole is thought to act primarily through the inhibition of fungal CYP450 14α-lanosterol demethylase enzyme, resulting in altered ergosterol production and impaired cell membrane composition and permeability, which in turn leads to cation, phosphate, and low molecular weight protein leakage. In addition, miconazole inhibits fungal peroxidase and catalase while not affecting NADH oxidase activity, leading to increased production of reactive oxygen species (ROS). Increased intracellular ROS leads to downstream pleiotropic effects and eventual apoptosis. In addition, miconazole is an inhibitor of CYP2C9 and CYP3A4, but drug interaction studies have not been conducted separately for this drug. Therefore, the effect of miconazole on concomitant administration with other drugs has not been sufficiently evaluated. To solve this problem, we performed drug target screening using a gene library of 4,843 heterozygous deletion mutants of fission yeast. And the targets analyzed by using Next Generation Sequencing (NGS). The information on the new Miconazole-induced haploinsufficiency targets identified through this method is expected to greatly contribute to improving the efficacy of Miconazole and minimizing drug side effects.

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N. Microbiology [N-9]

Genome-wide screening of fission yeast deletion library to search for putative target genes of alkylating agent carmustine

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Carmustine (BCNU) is a nitrosourea alkylating agent that can cross the blood brain brarrier. Carmustine is non-molecularly charged, lipophilic, and relatively small sized drugs, so it passively diffuses across the cell membrane on its own or actively via a transporter. Carmustine induces DNA damage by adding an alkyl group to guanine, resulting in inhibition of DNA replication and transcription. Carmustine also inhibits the conversion of glutathione disulfide to glutathione by carbamylation of glutathione reductase.

Genome-wide targeted gene deletion is a systematic method to study gene function by replacing target genes with deletion cassettes. Fission yeast currently has 5,064 annotated protein-coding genes, and a genome-wide deletion collection has been constructed with 4,845 genes deleted. We identified carmustine-sensitive genes through screening of the fission yeast heterozygous gene deletion library. The systematic screening of carmustine-sensitive genes was performed as previously described (Han et al., 2013, Kim et al., 2010). And carmustine target of screening with S.pombe gene deletion library was analyzed with Next generation sequencing. By doing this research, the expected targets have been secured and they are being validated.

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N. Microbiology [N-10]

Bacterial factors associated with the clinical prognosis of non-tuberculous mycobacterial lung disease (NTM-PD) patients: Mixed infection and colony morphology

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Non-tuberculous mycobacterial pulmonary disease (NTM-PD) is an infectious disease with a steadily increasing prevalence. It can be transmitted not only from environmental sources such as water supply systems and soil, but recent reports also suggest that person-to-person transmission is possible. Despite this severity, the lack of known markers in NTM-PD poses a major challenge for treatment. In this study, we investigated the microbiological characteristics of 87 patients who started treatment for *Mycobacterium avium* complex (MAC, *M. avium* and *M. intracellulare*) pulmonary disease, among whom 38 patients achieved culture conversion within six months of treatment, and 49 patients failed. We determined the minimum inhibitory concentration of the major drugs for the cultured bacteria isolated from the patients' sputum through drug-susceptibility testing. We also examined the texture and color of the colonies grown on 7H10 medium and visually inspected whether different types of colonies were mixed. In conclusion, patients with *M. intracellulare* infection had a significantly lower culture conversion rate compared to those with *M. avium* infection, and in cases of mixed colony types cultured (mixed infections), the culture conversion was significantly unsuccessful. Additionally, bacteria with a sticky colony texture or a yellow colony color were significantly associated with treatment failure.

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N. Microbiology [N-11]

Systematic genetic analysis with fission yeast deletion library against AZD1208

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AZD1208 is an anticancer compound, developed to treat Acute Myeloid Leukemia(AML). It is a potent and selective pan-Pim kinase inhibitor and ATP-competitive drug. The pan-Pim kinase is overexpressed in AML. These proteins promote cell proliferation and survival downstream of cytokine and growth factor signaling pathways. AZD1208 is an orally available compound that effectively inhibits all isoforms at < 5 nM or

It kills well at low concentrations, despite the lack of Pim kinase in fission yeast. These result strongly suggest that AZD1208 have antifungal effects. For a better understanding antifungal action mechanisms of AZD1208, we systematically identified AZD1208 sensitive genes in fission yeast through the drug-induced haploinsufficiency screening of the fission yeast heterozygous and Haploid gene deletion library. We are currently receiving and analyzing NGS results.

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N. Microbiology [N-12]

Identification of Target Genes for Beta-lapachone by a Genome-wide Screening of the Fission Yeast Deletion Library

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Beta lapachone (β-lap) is a natural compound extracted from the South American Lapacho tree and is a promising agent that shows the effect as an anticancer drug. NAD(P)H: quinone oxidoreductase 1 (NQO1) is known to be the main determinant of β-lap -induced cytotoxicity, but the actual mechanism of action has not yet been identified. For better understanding the cytotoxicity action mechanisms of β-lap, we systematically identified β-lapa-sensitive genes through the microarray screening of the fission yeast heterozygous gene deletion library. Through validation processes by spotting assay, 12 targets were finally yielded.

In a later study, We discovered DNA fragmentation and performed a cell cycle arrest assay.

Natural compounds derived from nature are an important source of new drug development, and β-lap also shows various physiological effects, so it is a drug that shows sufficient potential as an anticancer agent.

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N. Microbiology [N-13]

Mass Spectrometry-Based Proteomics Analysis of Serum Samples for Discovery of MDR-TB Biomarker Signature

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Tuberculosis (TB), caused by the *Mycobacterium tuberculosis* (*M.tb*) complex has further complicated the major health problems in the world (10 million were infected, and 1.5 million died in 2020). Especially multi-drug resistant tuberculosis (MDR-TB) means resistant to the two most potent first-line drugs, isoniazid and rifampicin, making it more challenging to treat and control. Where there are insufficient indicators to confirm treatment progress and failure in TB patients, proteomics-based analysis of the MDR-TB patient's serum is expected to help understand the treatment course. This study collected MDR-TB patients' sequential serums undergoing anti-TB treatment. We analyzed the 14 most abundant depletion serum proteins with high throughput LC-MS/MS based on proteomics. The bioinformatics analysis showed that the functions of differential serum proteins during the MDR-TB treatment were significantly correlated to the complement coagulation cascade, suggesting a coagulation disorder in TB. Here, we identified five potential candidate biomarkers such as C6 (Complement component C6), FGA (Fibrinogen alpha chain), FGG (Fibrinogen gamma chain), FGB (Fibrinogen beta chain), and MBL2 (Mannose-binding protein C). Our study may contribute to elucidating the mechanisms underlying MDR-TB.

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N. Microbiology [N-14]

Quantification of Viable Salmonella enterica by Viability-PCR

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Salmonella enterica is a gram-negative bacterium with various serotypes. It is the most common pathogen causing foodborne illnesses world wide. Determining the colony-forming unit (CFU) is the simplest and most widely used method to quantify viable cells. However, the metho d is time-consuming and labor-intensive. Quantitative polymerase chain reaction (qPCR) and droplet digital PCR (ddPCR) are very accurate and sensitive, but do not distinguish live and dead cells. Therefore, an analytical method termed viability-PCR (v-PCR) was studied. The method uses propidium monoazide (PMA) and Triton X-100. This photoreactive dye inhibits amplification by covalent binding to the DNA of dead cells and This Detergent took the cell wall, making it easier for PMA to penetrate.

S. enterica suspensions were normalized as an optical density at 600 nm of 1.0. The activity of PMA was further increased the concentration of PMA reached 100uM. The qPCR and ddPCR analyses were performed using the extracted genomic DNA using the InvA prim er and probe set, which are the most commonly used primer pairs for the detection of *S. enterica*. Various co nditions for the PMA treatments were tested.

v-PCR is a great method to quantify viable bacteria. It makes an alternative to CFU, q-PCR, ddPCR

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N. Microbiology [N-15]

Isolation of extracellular vesicles from major lactic acid bacteria using ultracentrifugation

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Extracellular vesicles (EVs) are in the limelight as a next-generation drug as an effective treatment for various diseases. This trend of research has recently led to a keen interest in bacterial-derived EVs, but it is still in its infancy. In particular, in the case of lactic acid bacteria, which are well known for their health-promoting effects, little is known about these things. Against this backdrop, we conducted research with the goal of isolating EVs from the major strains of lactic acid bacteria that have traditionally been most widely consumed. Strains of the genera Bacillus, Enterococcus, Lactobacillus and Pediococcus were used in this experiment. They were first cultured at 37℃ for 48 hours under aerobic conditions and subsequently concentrated with a 3 kDa membrane filter. Then, a 38 ml open tube was filled with the concentrated supernatant, installed in a SW-32-TI rotor, and centrifuged under vacuum at 165,000 g and 4℃ for 3 hours. These isolated extracellular vesicles were quantified and characterized by transmission electron microscopy. The results of this study suggest a standard method for isolating EVs from lactic acid bacteria, and more sophisticated optimization is required, but it is expected to be used as basic data in the field.

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N. Microbiology [N-16]

Comparison of microbial diversity and abundance in patients with Sjogren's syndrome and Healthy people

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Sjogren's syndrome is one of the chronic autoimmune diseases characterized by dry mouth and eyes, causing excessive drying of mucous membranes, which interferes with daily life. The cause of Sjogren's syndrome is believed to be genetic and hormonal abnormalities, but the exact cause is not yet known. The disease is treated with steroids and rheumatism, but excessive use causes side effects such as reduced immunity.

While microbiome technology has recently emerged as an alternative to overcome this in the face of the need to develop new treatments, this study conducted skin microbial analysis of patients with Sjogren syndrome and healthy people.

In this analysis, Shannon's, Observed Features, Faith's Phylogenetic, and Evans' methods were used for α-diversity, and Jaccard, Bray-Curtis, and Unweighted UniFrac methods were used for β-diversity.

As a result of the analysis, a significant difference in bacillary community between the two groups was confirmed, and harmful bacteria increased as the beneficial bacteria dominant on the patient's skin decreased, which is believed to be closely related to Sjogren's syndrome disease.

In depth analysis is needed in future experiments, but this study is expected to be used as basic data helpful for the diagnosis, treatment, and prognosis of Sjogren's syndrome.

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N. Microbiology [N-17]

Ohmyungsamycin enhances host defense against Mycobacteroides abscessus infections by promoting inflammatory responses and NO production.

Sang Min Jeon[1,2,3,4], Young Jae Kim[1,2,3,4], Bui Thi Bich Hanh[5], Jinsheng Cui[6], Dong-Chan Oh[6], Jichan Jang[7], Eun-Kyeong Jo[1,2,4*]

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Ohmyungsamycin A (OMS) is a newly identified cyclic peptide that exerts antimicrobial effects against *Mycobacterium tuberculosis*. However, its role in nontuberculous mycobacteria (NTMs) infections has not been clarified. *Mycobacteroides abscessus* (Mabc) is a rapidly growing NTM that has emerged as a human pathogen in both immunocompetent and immunosuppressed individuals. In this study, we demonstrated that OMS treatment of BMDMs at a safe dose (5 μM) significantly increased antimicrobial effects against Mabc in macrophages. Also, OMS treatment increased *in vivo* anti-Mabc effects in both immunocompetent and immunocompromised mice after intranasal Mabc infection. After infection with Mabc, OMS treatment produced significantly higher mRNA levels of tumor necrosis factor (TNF)-α, and IL-12p40 in bone marrow derived macrophages (BMDMs) or peritoneal macrophages compared to untreated control conditions. Consistent with these data, Mabc-induced nuclear translocation of nuclear factor-κB p65 subunit was significantly increased in BMDMs by OMS treatment compared to untreated controls. Additionaly, OMS treatment increases iNOS expression and NO production, but reduced M2 resoponses during Mabc infection. Collectively, these data strongly suggest that OMS upregulates the production of inflammatory cytokine and NO in BMDMs and mice during Mabc infection.

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N. Microbiology [N-18]

Temporal variations in the gut microbial diversity in response to high-fat diet and exercise

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The prevalence of high-fat diet-induced obesity has increased globally due to sedentary lifestyles and Westernized eating habits. Obesity is a leading risk factor for several non-communicable diseases, such as diabetes and cardiovascular diseases. Conversely, physical exercise is linked to improved metabolic health and overall well-being, and may positively impact gut microbial diversity and inflammation. To investigate the gut microbiota variations associated with exercise and high-fat diet over time, we conducted a longitudinal study that examined the effects of these variables on microbial diversity and composition using V4 region sequencing of 16S bacterial rRNA gene. We divided young mice into four groups: Chow-diet (CHD), high-fat diet (HFD), high-fat diet + exercise (HFX), and exercise only (EXE). Following 12 weeks of experimental intervention, we collected fecal samples at 1-week and 12-week intervals for DNA extraction, and we analyzed the resulting 16S libraries using QIIME 2 and R software. The Bacteroidetes to Firmicutes ratio was reduced 5-fold in the HFD and HFX groups compared to the CHD and EXE groups, but increased in the EXE group over time. We found that alpha diversity was significantly increased in the EXE group longitudinally, as measured by several indices (Shannon, Faith's PD, observed features, ACE, Chao1, Inverse Simpson, Fisher, and Pileou's evenness), whereas diversity and richness were significantly reduced in the HFD and HFX groups over time. Additionally, beta diversity, as measured by Jaccard, Bray-Curtis, and unweighted UniFrac distance metrics, was significant among the groups as determined by PERMANOVA. We observed differential abundances of Oscillospira, Ruminococcus gnavus, Helicobacter sp. flexispira taxon, Lactococcus, and Butyricimonas in the high-fat diet-fed groups (HFD, HFX), while Mucispirillum schaedleri was differentially abundant in the HFD group over time. Exercise reduced the proportions of Oscillospira over time in the HFX group. Moreover, Prevotella, Paraprevotella, and Lactobacillus were differentially abundant in the CHD group. Our results suggest that diet, age, and exercise all significantly influence the gut microbiota's community structure and diversity.

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N. Microbiology [N-19]

A resveratrol derivative compound V46 regulates antimicrobial responses against nontuberculous mycobacterial infection

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Over the past years, *Mycobacteroides abscessus,* a rapid growing mycobacteria (RGM), known for a genotypic and phenotypic heterogeneity, have emerged as an important human pathogen. Resveratrol is well-known for its antimicrobial and anti-inflammatory properties in mycobacterial species. Here, we purposed one of the derivatives of resveratrol named as "Compound V46" having more potent antimicrobial activities *in vivo* and *in vitro* as compared to original resveratrol. We found compound V46 was able to alleviate the levels of both mRNA expression and protein production of pro-inflammatory cytokines and chemokines on a dose-dependent manner induced by all *M. abscessus* subsp. *abscessus* infections in murine macrophages. Furthermore, we have found that the inhibited *Sirt1* and *Sirt3* expressions during *M. abscessus* infection were complemented by the treatment with compound V46, but independent for its antimicrobial action. Moreover, the amount of TFEB nuclear translocation was inhibited in *M. abscessus*-infected murine macrophages, and it was overcome by the compound V46 treatment at an early time point. Additionally, compound V46 suppressed activation of the Akt-mammalian target of rapamycin pathway under conditions of Mabc infection. Thus, our findings suggest that the compound V46 promotes antimicrobial activities against *M. abscessus* infection regulated by TFEB-involving mechanisms.

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N. Microbiology [N-20]

Korean Gut Microbiome Bank (KGMB) Infranet

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Over the past decades, the gut microbiome has been spotlighted for its importance in various diseases, encompassing obesity, cancer, and immune disease, as well as aging and development. Previous studies have isolated various bacterial species and sequenced their genome; however, there is still an unmet need for the system to share gut microbiome resources. To this end, we established the Korean Gut Microbiome Bank (KGMB) and the database for related information. The database for KGMB contains clinical information of more than 800 healthy Koreans, metagenomics data, and information on more than 13,000 strains of obligate anaerobes isolated from fecal samples. The KGMB database is accessible to the public at https://www.kobic.kr/kgmb. The database provides a taxonomic profile, sample source, and the number of contigs, CDSs, genes, tRNAs, and rRNAs. In addition, users can search for the strain they need through a user-friendly interface. The isolated real resources (anaerobic bacteria) can be distributed through the distribution website (https://www.kobic.kr/kgmb_dist).

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N. Microbiology [N-21]

Sinonasal microbiome and inflammatory profiles in fungal ball and chronic rhinosinusitis

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Fungal balls (FB) are the main form of non-invasive fungal rhinosinusitis in immunocompetent hosts. Bacterial coinfection affects clinical symptoms. We investigated the sinonasal microbiome and inflammatory profiles in FB and chronic rhinosinusitis (CRS) patients. Thirty-three participants were prospectively recruited. Nasal swab samples and sinonasal tissues were collected from controls, and FB and CRS patients. Microbiome analysis using 16S rRNA sequencing was performed. Inflammatory cytokine levels in the sinonasal tissues, blood eosinophil counts, and serum total IgE were measured. The sinus bacteria composition differed among the groups. At the phylum level, *Firmicutes* in FB were significantly depleted compared with those in CRS, while *Proteobacteria* were more enriched in FB than that in controls and CRS. At the genus level, in FB, *Staphylococcus* and *Corynebacterium* were significantly decreased compared to those in controls. Blood eosinophil counts and IL-5 and periostin levels in FB group were significantly lower than those in CRS. FB patients had different microbiome compositions and fewer type 2 inflammatory profiles than CRS patients did. However, whether these findings cause FB or result from bacterial and/or fungal infection remains unclear. Further studies are needed to reveal how these differences occur and affect the development of FB and clinical symptoms.

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N. Microbiology [N-22]

Inducing skin regeneration through the antibacterial activity of lactic acid bacteria

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Skin regeneration is a physiologically normal process that arises at the cellular level, in other words, is a constant renewal of cells. It is known about proteins have crucial roles in tissue repair and the formation of new tissues. Also, the immune system and microbiome are reported to have roles in repairing and renewing tissue structure [1]. Here, we assessed the function of lactic acid bacteria in wound healing, immune response, and inflammation in the skin. Lactic acid bacteria (LAB) are well-used in the food and pharmaceutical industries for their antibacterial qualities due to the lowering of pH and hydrogen peroxide production. These antibacterial effects are known to be brought about by Lactic acid (LA) and Phenyl-lactic acid (PL). Moreover, a variety of complexes between microorganisms and host cells, such as keratinocytes and immune cells, end up affecting the homeostasis of the skin. The effect of LAB on the immune system and antioxidant activity was measured by western blot. The cytotoxicity of LAB was measured by FACS and MTS assay. Furthermore, we used normal human keratinocytes for verifying the efficacy of LAB. This study confirmed that LAB induced the skin repair system in normal human keratinocytes after skin damage.

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N. Microbiology [N-23]

Characterization of elevated extracellular vesicle-mediated tigecycline resistance in Acinetobacter baumannii

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Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is the most detrimental pathogen that causes hospital-acquired infections. A powerful antibiotic called tigecycline (TIG) is now used to treat CRAB infections, however overuse of this drug causes isolates to become resistant to it. In this investigation, we identified the lipid-bilayered nanoscale structures known as bacterial extracellular vesicles (EVs) as TIG resistance mediators. We showed that TIG-R AB produced more EVs than the control TIG-S AB using laboratory-made TIG-R AB (TIG-S AB). TIG-R EVs proteins are important elements in TIG resistance transfer, according to transfer studies of TIG-R AB-derived EVs treated with proteinase or DNase to TIG-S AB. Further transfer spectrum research showed that *Escherichia coli*, *Salmonella typhimurium*, and *Proteus mirabilis* were the only bacteria that acquire EV-mediated TIG resistance. Nevertheless, *Staphylococcus aureus* and *Klebsiella pneumonia* did not exhibit this behavior. Lastly, we demonstrated that EVs are more likely than antibiotics to cause TIG resistance. According to our research, EVs are powerful cell-derived components that selectively and often cause TIG resistance in nearby bacterial cells.

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N. Microbiology [N-24]

The therapeutic potential of Shihosogan-tang and Yijung-tang in improving gastrointestinal function and modulating intestinal flora in dyspepsia rats

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Over the past few years, there has been growing interest in herbal medicine due to its potential advantages, such as providing fewer side effects and better compliance for treating gastrointestinal disorders. The aim of this investigation was to explore the potential of Yijung-tang (YJT) and Shihosogan-tang (SST) in enhancing dyspepsia by influencing gut microbiota and activating intestinal genes. Sprague-Dawley (SD) rats with loperamide-induced dyspepsia were treated orally with YJT and SST for one week. After treatment, dyspepsia-related factors were evaluated by monitoring phenotypes or detecting mRNA expression of critical markers involved in intercellular connexins, inflammation, water absorption, and contractility in the small intestine. Additionally, the impact of YJT and SST on gut microbiota composition was assessed using 16S rRNA gene sequencing. The administration of YJT and SST to constipated rats improved intestinal motility, suppressed inflammatory responses, protected the gut barrier, and enhanced water metabolism in the intestinal tract. These therapeutic effects were associated with the corrected gut microbiota dysfunction, which conferred the advantage of producing intestinal metabolites. In summary, this study suggests that YJT and SST may improve dyspepsia by having a prebiotic impact on gut microbiota and intestinal metabolites.

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N. Microbiology [N-25]

In silico-based functional enhancement of a novel antimicrobial peptide identified from the spider Pardosa astrigera transcriptome

Min Kyoung Shin[1], Kyung-Bin Bu[1], Bo-Young Jang[1], Seung-Ho Lee[1], Jung-Suk Sung[1*]

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As multidrug-resistant bacteria have become a worldwide threat, there is an urgent need to develop new antibiotic reagents. Antimicrobial peptides (AMPs) are short, cationic peptides expressed universally in living organisms, contributing to host defense by the rapid and efficient killing of microbes. Animal venom is a rich source of functional peptides, serving as a useful platform for identifying AMPs. In this study, a putative AMP LYTX-Pa1a was identified from the transcriptome of the spider *Pardosa astrigera* via homology analysis. A 15-mer region predicted with strong antimicrobial activity was truncated and was introduced with an amino terminal Cu(II)- and Ni(II)-binding (ATCUN) motif. The peptide showed stronger antimicrobial activity than the original template against both gram-negative and positive pathogens. ATCUN-Pa1a exhibited a synergistic effect with conventional antibiotics, which enabled the completed elimination of multidrug-resistant *Pseudomonas aeruginosa*. In addition, ATCUN-Pa1a induced the production of reactive oxygen species and DNA cleavage in bacteria. The results demonstrated the successful application of *in silico* methods for the identification and development of AMP from spider transcriptome. [This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR20233202).]

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N. Microbiology [N-26]

Antibacterial and Antifungal Effects of Novel Toxin-like Peptides Derived from the Spider Argiope bruennichi Venom Gland

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[1] *Life science, Dongguk University-Seoul, Goyang-si 10326, Korea*

Bioactive peptides in spider venom have well-known physiological properties such as cytolytic activity and neurotoxicity. However, functional peptides derived from endemic spiders in Korea are not well characterized yet. In this study, we identified venom-derived peptide from the spider *Argiope bruennichi* (*A. bruennichi*). Cytolytic peptides showing alpha-helical structures with positive net charge often possess antimicrobial properties, which mediate bacterial membrane destruction. We selected toxin-like peptides by homology analysis and identified distinct structural features by secondary structure characterization. Antimicrobial activity of peptides was validated by the growth inhibition assay. Selected peptides significantly inhibited the growth of gram-negative (*E. coli, E. carotovora*) and gram-positive (*S. aureus, B. cereus*) bacteria, and suppressed the viability of fungi (*F. oxysporum*). The mechanistic study revealed the induction of cell membrane permeabilization upon treatment of toxin-like peptides on pathogenic microbes. In conclusion, the toxin-like peptides from *A. bruennichi* were identified based on transcriptome analysis and characterized for antimicrobial activity, providing beneficial information for developing possible antimicrobial agents against multi-drug resistant pathogens. [This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR20233202).]

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N. Microbiology [N-27]

Effects of Baekhogainsam-tang via Modulation of Intestinal Microbiota and Alteration of Gene Expression in High Fat Diet Induced Metabolic Dysfunction Mice

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Obesity is the main cause of type 2 diabetes, a metabolic disease characterized by insulin resistance, hyperglycemia, and abnormalities in insulin secretion. We examined how Baekhogainsam-tang (BHGIST) affected the metabolic dysfunction induced by a high-fat diet (HFD) in mice. C57BL/6 mice were fed HFD along with fructose in their water, and then treated with BHGIST or metformin for 10 weeks. The administration of BHGIST resulted in a significant improvement in HFD-induced metabolic disorders in mice, as evidenced by reduced body weight gain and calorie intake, as well as decreased weight of major adipose tissues and liver tissues. In addition, it improved glucose homeostasis and insulin sensitivity. These effects have also been linked to the inhibition of low-grade intestinal inflammation and an improvement of hepatic function. These results indicated a difference in the distribution pattern of gut microbial communities between groups. Changes in microbiota composition could be partially caused by a SCFA-producing bacterium, *Parabacteroides, Parasutterella* and *Faecalibaculum*, which were decreased in the HFD group and subsequently increased in the BHGIST group. Based on all of these results, treatment with BHGIST appears to be effective in improving signaling pathways associated with metabolic disorders in HFD-induced mice.

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N. Microbiology [N-28]

Beneficial Effects of Newly Isolated Bacteroides dorei (BD) Strains from the Human Gut on Obesity and Reduction of Fat Production

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The identification of new probiotics with anti-obesity properties has attracted considerable interest. In the present study, the anti-obesity activities of *Bacteroides dorei* (BD) strains isolated from human stool samples were evaluated using a high fat-diet (HFD)-fed mice model. The anti-lipid activity of BD was verified by 3T3-L1 cells, and the anti-lipogenic, anti-lipogenic and anti-obesity properties of BD were further evaluated in HFD-induced obese mice. When 3T3-L1 cells were treated with individual strains, BD showed a significant inhibitory effect on lipid accumulation (P

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N. Microbiology [N-29]

FCS-Like MYM Zinc Finger Protein of the African Swine Fever Virus Interacts with MITA and 2’3’-Cyclic GMP-AMP to Suppress Interferon Signaling

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2'3'-Cyclic GMP-AMP is an intracellular second messenger synthesized in response to cytosolic double-stranded DNA (dsDNA) and activates the endoplasmic reticulum (ER)-localized adaptor protein MITA. Upon activation, MITA translocates from the ER to the Golgi, recruiting TANK-binding kinase 1 (TBK1), which phosphorylates interferon regulatory factor 3 (IRF3); the phosphorylated IRF3 dimerizes and translocates to the nucleus to trigger antiviral interferon signaling. However, many viral infections adapt mechanisms to hijack such host immune responses for virulence. African swine fever virus (ASFV) is a highly contagious viral disease of domestic and wild pigs, whose mortality rate can reach 100%, and no commercially available vaccine or drug for the prevention and treatment of this disease. Previous studies have reported that ASFV-encoded proteins could effectively inhibit cell and host defenses, which are essential for establishing immune evasion. Our study found that ASFV B175L with FCS-like MYM Zinc finger motif could bind MITA and 2'-3'-cyclic GMP-AMP. The B175L-MITA interaction occurred at the cyclic dinucleotide (CDN) binding domain (CBD) of MITA that decreased its Golgi trafficking to trigger downstream interferon signaling. In summary, this molecular mechanism of ASFV B175L will provide new strategies to advance vaccines and drugs against ASFV. [National Research Foundation of Korea (2021R1A6A1A03045495)]

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N. Microbiology [N-30]

African Swine Fever MyD116 Homolog Downregulates STING-Mediated Type I Interferon Signaling

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African swine fever virus (ASFV) is a highly contagious viral disease of domestic and wild pigs. Incursions of ASFV can generate substantial economic losses in affected pig sectors, given its high mortality in pig populations and dislocations in the pig market. At present, no commercially available vaccine or drug for the prevention and treatment of this disease. Many ASFV proteins have been found to suppress the antiviral interferon signaling to evade the host's innate immune responses. Therefore, the advancement of an effective vaccine against ASFV is truly challenging. Here, we demonstrate DP71L, the MyD116 homolog of ASFV known to inhibit the eIF2alpha-ATF4-CHOP pathway as a type I interferon (IFN-I) suppressor. DP71L interacted on the C-terminal tail of the Stimulator of interferon genes (STING) to obstruct the STING-TANK-binding kinase 1 (TBK1) interaction that drove the blockade IFN-I production. Altogether, our study of DP71L on the STING-mediated interferon signaling provides new insights into the broad operative immune evasion strategies of ASFV during the ASF infection. [National Research Foundation of Korea (2021R1A6A1A03045495), and Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea (ASFV) 119081-5]

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N. Microbiology [N-31]

African Swine Fever Virus UK gene Targets IRF3 to Downregulate the cGAS-mediated IFN Pathway

Niranjan Dodantenna[1], Buddhika Nuwan Gamage[1], Jong-Soo Lee[1*]

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The highly contagious African swine fever virus (ASFV) is currently giving rise to a pandemic affecting wild boars and domestic swine causing catastrophe to the pig industry. For efficient infection in the host, ASFV has developed multiple strategies to eliminate host innate immune responses. However, the evasion mechanism of ASFV remains elusive. In this study, we demonstrated a novel immune evasion mechanism of the ASFV UK (Uridine Kinase) gene that blocks IRF3-dependent antiviral responses. Ectopic expression of the UK gene enhanced both DNA and RNA virus replication and blocked the production of IFN-β and IL-6 in porcine alveolar macrophages (PAM). Mechanistically, the ASFV UK gene interacted with the IRF3 nuclear localization signal (NLS) and inhibited IRF3 nuclear translocation which in turn suppressed IFN-mediated antiviral responses. Especially, the association between IRF3 and its adapter protein, karyopherin (KPNA), was declined by the ASFV UK gene resulting in impaired subsequent nuclear translocation of IRF3. Taken together we here confirm that the highly conserved UK gene is an excellent candidate for live attenuated vaccine development against ASFV infection. [National Research Foundation of Korea (2021R1A6A1A03045495), and Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea (ASFV) 119081-5]

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N. Microbiology [N-32]

The structural protein VP4 of the foot-and-mouth disease virus inhibits the nuclear translocation of IRF3 to down-regulate host type-I interferon responses

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Foot-and-mouth disease (FMD) causes acute vesicular diseases that affect cloven-footed animals.VP4 is one of the 4 structural proteins which is also involved in evading the type-I interferon (IFN) pathway. Overexpression of FMDV-VP4 downregulates the IFN signaling in porcine (PK15, PAM, and LFBK) and human (HEK293T) cell lines, which is demonstrated by increasing Vesicular Stomatitis Virus (VSV-GFP) and Influenza virus (PR8-GFP) replication and reduced secretion of pro-inflammatory cytokine and negative regulation the transcription of IFN stimulatory genes in the virus replication studies. IFN promoter activity is downregulated by VP4 in the IFN-related luciferase reporter assay until IRF3-5D. Initial mechanism studies revealed that VP4 does not inhibit IRF3 dimerization and phosphorylation. The current study demonstrates the inhibition of IRF3 nuclear translocation from VP4 by competitively interacting with KPNA2 and KPNA4 in vitro. KPNA molecules are the transporters of IRF3 to the nuclear compartment. Both KPNA molecules interacted with VP4 in overexpression and endogenous conditions, and the competition assay shows VP4 has a higher binding affinity to KPNA molecules than IRF3. These results collectively suggest that FMDV-VP4 negatively regulates the type-I IFN pathway and leads to severe FMDV infection. [National Research Foundation of Korea (2018M3A9H4079660, 2021R1A6A1A03045495)]

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N. Microbiology [N-33]

Lactobacillus reuteri BSA218 as a Probiotic Confers Protection against Influenza Virus by Modulating Innate Immunity

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The influenza A virus (IVA) is one of the severe respiratory viral infections in humans that cause seasonal epidemics worldwide. Due to rapidly evolving virus resistance, medical treatments such as vaccines and antiviral drugs often grant limited protective efficacy. Our study found the live cells and culture supernatants of *Lactobacillus reuteri* BSA218 as probiotics against IVA *in vitro* and *in vivo*. The pretreatment of BSA218 into RAW264.7 cells functioned against DNA and RNA viruses and stimulated the phosphorylation of antiviral signaling molecules and transcription of antiviral genes. The oral administration of BSA218 conferred protection against subsequent lethal infection with IVA subtypes, prevented significant weight loss, and lowered lung viral loads in C57BL/6NHsd mouse model. Mice treated with BSA218 showed high levels of cytokines and mRNA expressions of different antiviral molecules in the serum, bronchoalveolar lavage fluids (BALFs), and small intestinal fluids (SIFs), and a low degree of inflammation upon infection with the influenza virus. Finally, we found that bioactive lipids of BSA218 provide prophylactic potential against viruses. Taken together, our findings provide new insights into BSA218 for the inhibition of IVA replication both *in vitro* and *in vivo* and probiotics-based antiviral research. [National Research Foundation of Korea (2021R1A6A1A03045495)]

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N. Microbiology [N-34]

Toll-like receptor-7 agonist GS9620 is a promising vaccine adjuvant for Influenza

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GS-9620 is a TLR7-specific small-molecule agonist that induces IFN-α over proinflammatory cytokines. GS-9620 has been identified to reduce the hepatitis B virus (HBV) in animal models and currently, it is in the development stage as a treatment for chronic HBV. Even though there is much-published knowledge on the use of GS-9620 as a potential immune stimulant, there is no reported evidence for use of GS-9620 as a vaccine adjuvant. In this study, we investigated the efficacy and mode of action of GS-9620 as an immune stimulant for vaccine adjuvant. GS-9620 induced TLR-7 mediated cytokine production and immune response gene expression in human and murine cells. In vivo, influenza recombinant protein sM2HA2 and inactivated A/Puerto Rico/8/34 virus (iPR8) were used to evaluate the effect of GS-9620 as an immune-stimulant. Administration of sM2HA2 and iPR8 with GS-9620 induced antigen-specific humoral and cell-mediated immune responses than TLR7 agonist Imiquimod. Moreover, it elevated clearance of lung virus, protective efficacy of the sM2HA2 and iPR8 against the lethal challenge of different influenza subtypes in a murine model. Altogether, these results evidence that GS-9620 as influenza vaccine adjuvant for provide effective mucosal and systemic immune response. [The National Research Foundation of Korea (2019M3E5D5066834, 2021R1A6A1A03045495)]

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N. Microbiology [N-35]

African Swine Fever virus EP364R and C129R degrade 2'3'cGAMP and Negatively Regulate the cGAS-STING Pathway

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African swine fever virus (ASFV) is a highly pathogenic swine DNA virus with high mortality that causes African swine fever (ASF) in domestic pigs and wild boars. For efficient viral infection, ASFV developed strategies to evade key components of innate immune responses. Upon ASFV infection, cyclic GMP-AMP (2′,3′-cGAMP) synthase (cGAS), a cytosolic DNA sensor, recognizes ASFV DNA and synthesizes the second messenger 2′,3′-cGAMP, which triggers interferon (IFN) production. In this study, we demonstrated a novel immune evasion mechanism of ASFV EP364R and C129R, which blocks cellular cyclic 2′,3′-cGAMP-mediated antiviral responses. ASFV EP364R and C129R with nuclease homology inhibit IFN-mediated responses by specifically interacting with 2′,3′-cGAMP and exerting their phosphodiesterase (PDE) activity to cleave 2′,3′-cGAMP. Particularly notable is that ASFV EP364R had a region of homology with the stimulator of interferon genes (STING) protein containing a 2′,3′-cGAMP-binding motif and point mutations in the Y76S and N78A amino acids of EP364R that impaired interaction with 2′,3′-cGAMP and restored subsequent antiviral responses. These results highlight a critical role for ASFV EP364R and C129R in the inhibition of IFN responses and could be used to develop ASFV live attenuated vaccines. [National Research Foundation of Korea (2021R1A6A1A03045495), and Ministry of Environment (NIWDC-2021-SP-02)], Republic of Korea

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N. Microbiology [N-36]

Foot-and-Mouth Disease Virus VP3 inhibits MAVS aggregation leading to down-regulation of the type-I interferon signaling

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As a structural protein of Foot-and-mouth disease virus (FMDV), VP3 plays an important role in virus assembly and escaping the host's innate immune response to promote FMDV replication. Previous studies demonstrated that FMDV VP3 blocks the type-I IFN response by inhibiting the mRNA expression of the mitochondrial antiviral-signaling protein (MAVS), however, the underline mechanism is poorly understood. Here we identified that the specificity of VP3 interaction with MAVS for the negative regulation of type-I IFN antiviral responses for effective replication of FMDV. Further, we describe that the transmembrane (TM) domain is the specific region of MAVS that interacts with the FMDV VP3. The TM domain of MAVS governs the mitochondria localization of MAVS and it is a key factor in type-I IFN signaling transduction via MAVS aggregation. Thereby, the interaction of FMDV VP3 with the TM domain of MAVS leads to the inhibition of MAVS mitochondria localization, self-association and aggregation, resulting in the suppression of type-I IFN response as shown in the results. Taken together, these data provide a clear understanding of a key molecular mechanism used by the FMDV VP3 for the suppression of IFN responses via targeting MAVS. [National Research Foundation of Korea (2018M3A9H4079660, 2021R1A6A1A03045495)]

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N. Microbiology [N-37]

The novel immunobiotic Clostridium butyricum S45-5 displays broad spectrum of antiviral activity in vitro and in vivo

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Probiotics are likely to have an impact on gut mucosa by inhibiting the growth of pathogenic microorganisms and by enhancing local and systemic immune responses in the host body. Our study revealed the broad antiviral spectrum of *Clostridium butyricum* S45-5 *in vitro* and the prophylactic efficacy against divergent influenza A subtypes in BALB/c mouse model. An effective dose of *C. butyricum* S45-5 significantly reduced the replication of Influenza A virus, Newcastle Disease Virus, and Herpes Simplex Viruses in immune (RAW264.7) cell. Further, oral administration of C. butyricum S45-5 exhibited prophylactic effects in BALB/c mice against lethal doses of highly pathogenic influenza A sub types (H1N1, H3N2 and H9N2) and displayed reduced lung viral titers. Moreover, *C. butyricum* S45-5 in mice caused increased production of IFN-β, IFN-γ, IL-6, and IL-12 in the serum, small intestine, and lung fluid. Furthermore, *C. butyricum* S45-5 treated mice showed lower local and systemic pro-inflammatory cytokine levels and a comparatively higher level of anti-inflammatory cytokine secretion at late time of influenza infection. Taken together, our data reveal a novel immune-regulatory and antiviral role of *C. butyricum* S45-5 through inducing *in vitro* and *in vivo* antiviral states. [The National Research Foundation of Korea (2021R1A6A1A03045495)]

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N. Microbiology [N-38]

African Swine Fever Virus protein L11L targets dual host immune factors IRF3 and PKR to escape the host innate immune responses

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African Swine Fever Virus is a large, complex DNA virus that infects domestic pigs and wild boars, causing a severe and often fatal disease known as African swine fever, which renders 100% mortality. ASFV holds diverse immune evasion mechanisms to increase its pathogenesis by producing different kinds of viral proteins with specific functions. L11L is one of those ASFV proteins produced at late time points, the exact function is unknown. In this study, we investigated the molecular mechanism of L11L in evading the type-I interferon pathway in order to escape the host immune response. L11L downregulated the IFN-β production by interacting with the interferon regulatory factor 3 (IRF3) via inhibiting IRF3 phosphorylation, dimerization, and nuclear translocation. Interestingly, we found that L11L targets Protein Kinase R (PKR) for inhibits its dimerization, thereby preventing the phosphorylation of its substrate eIF2α to promote viral protein synthesis. Altogether these findings conclude that L11L escapes the host immune response by targeting IRF3 and promotes viral protein synthesis by targeting PKR.
[National Research Foundation (2021R1A6A1A03045495), and Ministry of Environment (NIWDC-2021-SP-02)], Republic of Korea

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N. Microbiology [N-39]

Characterization analysis of abalone shell fermented by lactobacillus

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Abalone shell is well known for the protection of oxidative stress, and antioxidant and anti-aging effects on skin cells damaged by ultraviolet ray, and abalone shells, which account for more than half of abalone, are discarded as waste. Bioconversion is widely used in the production of useful substances such as amino acids and vitamins as well as the production of pharmaceutical raw materials including antibiotics and streoids. However, there is still a lack of complete report data on the impact of bioconversion on the compound composition of abalone shells. In this study, the abalone shell was fermented by lactobacillus and then studied the changes in amino acid composition and metabolomics analysis. Analysis of amino acid composition showed that total amino acid content and essential amino acids were higher in fermented abalone shell extract. Among the 18 amino acids, tryptophan content was the ghighes, followed by glycine and glutamic acid. To examine the metabolic analysis, we used HPLC and LC-MS/MS of fermented abalone shell extract and control. Through the study, it was shown that there is a possibility of using abalone shell in the future due to the compounds detected in the abalone shell extract.

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O. Molecular Medicine and Imaging [O-1]

Self-assembled hyaluronic acid nanomedicine: implication as a topical therapeutic agent for the treatment of psoriasis

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Although conventional topical approaches for treating psoriasis have been offered as an alternative, there is still an unmet medical need to overcome limitations such as low skin-penetrating efficacy and off-target adverse effects. Self-assembled hyaluronic acid nanoparticles (HA-NPs) have been studied extensively as a nanocarrier for target-specific and long-acting delivery of various drugs, owing to their excellent physicochemical and biological characteristics. Here, we identify drug-free HA-NPs as topical therapeutics for treating psoriasis using ex vivo and in vivo skin penetration studies and psoriasis animal models. Transcutaneously-administered HA-NP was found to be accumulated and associated with pro-inflammatory macrophages in the inflamed dermis of a psoriasis mouse model. Importantly, HA-NP exerted potent therapeutic efficacy against psoriasis-like skin dermatitis in a size-dependent manner by suppressing innate immune responses and restoring skin barrier function without overt toxicity signs. The therapeutic efficacy of HA-NP on psoriasis-like skin dermatitis was attributed to the presence of a self-assembled hydrophilic HA shell, independent of the molecular weight of HA and hydrophobic moiety, and comparable with that of other conventional psoriasis therapeutics widely used in the clinical settings. Overall, drug-free HA-NPs have the potential as a topical nanomedicine for treating psoriasis effectively and safely.

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O. Molecular Medicine and Imaging [O-2]

Evaluation of Cerenkov luminescence imaging of interscapular brown adipose tissue using a TSPO-targeting PET probe in the UCP1 reporter mouse

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[^{18}F]FDG-PET is used as a standard method for imaging the activity of intersacpular brown adipose tissue (iBAT), but it is difficult to obtain iBAT-specific imaging due to competitive uptake in tumors, skeletal muscle, and inflammatory regions. A reporter mouse that monitors the expression of uncoupling protein 1 (UCP1) as an iBAT biomarker was developed, and translocator protein-18 kDa (TSPO) co-localized in mitochondria has potential for iBAT imaging. Cerenkov luminescence imaging (CLI) using Cerenkov radiation from the PET probe has been proposed as an alternative optical imaging technique for PET as it is cost-effective, more accessibility. In this study, we aim to compare the between [^{18}F]FDG /or [^{18}F]fm-PBR28-d_2 imaging for iBAT using PET and CLI in UCP1 reporter mouse. TSPO-targeting PET probe, [^{18}F]fm-PBR28-d_2, is superior to acquiring iBAT imaging than [^{18}F]FDG. The high molar activity of [^{18}F]fm-PBR28-d_2 is essential factor for CLI as well as PET. Both TSPO-PET and CLI signals of iBAT were clearly increased after cold stimulation than thermoneutral condition. Under prolonged isoflurane anesthesia, TSPO-targeting images showed higher signals from iBAT in the short-term than long-term group.

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O. Molecular Medicine and Imaging [O-3]

Inhibitory effect of parnassin, a novel peptide on atopic dermatitis-like skin lesions via suppression of JAK2 and STAT1 activation

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Atopic dermatitis (AD) is a chronic inflammatory skin disease which requires continuous treatment. The current treatment includes steroids and nonsteroidal agents targeting inflammation but long-term administration causes various side effects such as skin atrophy, hirsutism, and hypertension. Thus, a safe therapeutic agent with fewer side effects is required for the treatment of AD. Peptides are small biomolecule drug which are highly potent and remarkably has lesser side effects. Parnassin is a tetrapeptide with anti-microbial activity that was selected through an in silico therapeutic peptide screening platform in our previous study. In this study, the effect of parnassin on AD was confirmed in vivo and in vitro using a DNCB-induced AD mouse model and HaCaT cells. Parnassin significantly alleviated AD-like skin lesions in the animal model without side effects. In TNF-α/IFN-γ-stimulated HaCaT cells, parnassin inhibited JAK2 and p38 MAPK signaling kinases through inhibition of STAT1, thereby suppressing the expression of the Th2-type chemokine CCL17 and CCL22 genes. Parnassin also significantly reduced the gene expression of TSLP and IL-31, which are pruritus-inducing cytokines. Collectively, parnassin shown to alleviate AD like skin lesion via its immune modulating effect and thus it could be used as a candidate for prevention and treatment of AD.

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O. Molecular Medicine and Imaging [O-4]

Contribution of overlooked KCNQ4 variants to hearing impairment

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Voltage-gated channel subfamily Q member 4 (KCNQ4) plays a crucial role in the potassium recycling in the cochlea. Mutations in KCNQ4 result in progressive, high-frequency-affected hearing loss that begins in adulthood and eventually affects all frequencies. To comprehend the effect of *KCNQ4* variants on hearing impairment, whole-exome and genome sequencing data were analyzed on individuals with hearing loss and those with unknown hearing phenotypes. It was discovered that nine hearing loss patients had seven missense and one deletion KCNQ4 mutations, whereas the Korean population with an unclear hearing loss phenotype had 14 missense variants. In both groups, the p.R420W and p.R447W variants were identified. Except for p.G435Afs*61, all KCNQ4 variants displayed normal expression patterns. p.R331Q, p.R331W, p.G435Afs*61, and p.S691G variants, which were identified in patients with hearing loss, exhibited lower potassium current density than the wild-type. KCNQ activators retigabine or zinc pyrithione restored the channel activity of p.S185W, p.R216H, p.V672M, and p.S691G, while p.G435Afs*61 was partially rescued by sodium butyrate, a chemical chaperone. Moreover, the AlphaFold2-predicted structures of the variants demonstrated impaired pore configurations. The study suggests that *KCNQ4* variants contribute to adult-onset hearing loss and, because some of these variants are medically treatable, genetic screening for KCNQ4 is essential.

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O. Molecular Medicine and Imaging [O-5]

Excessive noise exposure causes sustained PERK activation and is attenuated by chemical chaperones.

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Exposure to external noise can lead to temporary (TTS) or permanent (PTS) hearing loss. However, the underlying mechanisms and therapeutic targets for noise-induced hearing loss remain poorly understood. In this study, a longitudinal transcriptome investigation was performed on adult mice for up to 2 weeks after exposure to TTS and PTS-inducing noise. The results showed that exposure to noise induced endoplasmic reticulum (ER) stress and unfolded protein response (UPR) in the cochlea. Western blot reveals that the IRE1α and PERK branches of UPR activated after TTS- and PTS-inducing noise exposure differed between noise conditions after two weeks of exposure. The PERK branch was continually activated during PTS, and the expression of CHOP, a proapoptotic factor, was elevated in inner hair cells and outer hair cells. To further clarify the involvement of PERK branch in hearing recovery, we examined the effects PERK inhibitor, GSK2656157 on TTS. Intriguingly, GSK2656157 hindered hearing recovery in TTS, indicating that the activation of PERK is necessary for hearing recovery in TTS. We demonstrated that chemical chaperones prevented hearing loss despite PTS-inducing noise exposure. In conclusion, this study identified the role of UPR in response to noise and suggested the therapeutic target for noise-induced hearing loss.

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O. Molecular Medicine and Imaging [O-6]

Investigation of artificial intelligence integrated fluorescence endoscopy image analysis with indocyanine green for interpretation of precancerous lesions in colon cancer

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Indocyanine green (ICG) has been used in clinical practice for more than 40 years and its safety and preferential accumulation in tumors has been reported for various tumor types, including colon cancer. However, reports on clinical assessments of ICG-based molecular endoscopy imaging for precancerous lesions are scarce. We determined visualization ability of ICG fluorescence endoscopy in colitis-associated colon cancer using 30 lesions from an azoxymethane/dextran sulfate sodium (AOM/DSS) mouse model and 16 colon cancer patient tissue-samples. With a total of 60 images (optical, fluorescence) obtained during endoscopy observation of mouse colon cancer, we used deep learning network to predict four classes (Normal, Dysplasia, Adenoma, and Carcinoma) of colorectal cancer development. ICG could detect 100% of carcinoma, 90% of adenoma, and 57% of dysplasia, with little background signal at 30 min after injection via real-time fluorescence endoscopy. Correlation analysis with immunohistochemistry revealed a positive correlation of ICG with inducible nitric oxide synthase (iNOS; $r > 0.5$). With artificial intelligence training, the accuracy of image classification into four classes using data sets, such as fluorescence, optical, and fluorescence/optical images was assessed. Fluorescence images obtained the highest accuracy (AUC of 0.8125) than optical and fluorescence/optical images (AUC of 0.75 and 0.6667, respectively).

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O. Molecular Medicine and Imaging [O-7]

Two-photon intravital imaging of the interplay of lipid droplets and lysosomes in a diet-induced mouse model of non-alcoholic fatty liver disease

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Intravital microscopy (IVM) is a powerful technique for real-time visualization, quantification, and monitoring of various biological processes. Using multiple fluorescent probes, this technique can improve our knowledge of physiological processes and in vivo disease progression. Non-alcoholic fatty liver disease (NAFLD) is a condition in which excess fat accumulates in the liver and is a rapidly increasing chronic liver disease accompanied by hepatic steatosis, inflammation, fibrosis, and severe liver failure. In this study, we established a method of intravital imaging to detect dynamic alteration of hepatic lipid droplets and lysosomes in the liver of a live mouse using two novel two-photon fluorescent dyes, LD1 to label lipid droplets and BLT to label lysosomes. We visualized and quantitatively analyzed the accumulation of lipid droplets and dynamic changes of lysosomes during the progression of NAFLD. The hepatic lipid droplets dynamically interacted for a long time and quickly fused. The number and size of lipid droplets continuously increased as the disease progressed. The lysosomes enclosed the tiny particles of lipid droplets but not large ones. Also, vacuoles surrounded by lysosomes increased as the disease progressed. Intravital liver imaging will be valuable in elucidating the poorly understood mechanisms surrounding liver inflammation and NAFLD progression.

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O. Molecular Medicine and Imaging [O-8]

Optical transparency of rodent bones by using novel bone clearing technique

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Recent developments in hydrogel-based tissue-clearing methods such as the CLARITY and PACT have allowed three-dimensional analysis of biological-structures in whole, intact tissues, thereby providing greater understanding of spatial relationships and biological-circuits. Nonetheless, issues remain in maintaining structural-integrity and preventing tissue expansion/shrinkage with clearing and prevent the wide application of these techniques to hard-bone tissues, such as femur and tibia. Here, we present a PACT-based novel bone-clearing method, Bone-mPACT+, that incorporates four decalcify processes to improve bone-tissue clearing efficiency without sacrificing optical-transparency. We also present a further modified Bone-mPACT+ Advance protocol specifically optimized for processing the largest and hardest rat bones for easy clearing and imaging using established tissue-clearing methods. We provide proof-of-concept support for our optimized protocol by investigating the endogenous fluorescence and the expression of osteogenesis marker, and the relatively understudied proto-oncogene in rodent bones.

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O. Molecular Medicine and Imaging [O-9]

Optimization of the PACT-based embryo clearing method for high resolution analysis in small mouse embryos

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Recent developments in various tissue clearing protocols have significantly advanced the 3-D analysis of biological structures in whole, intact tissue, providing greater understanding of spatial relationships and biological circuits. Nonetheless, studies have reported issues with maintaining structural integrity and preventing tissue disintegration, limiting the wide application of these techniques to fragile tissues such as developing embryos in experimental animal. Here, we present an optimized passive tissue clearing technique (PACT)-based embryo clearing method, IMPACT-Basic, that improves tissue rigidity without compromising optical transparency. We also present IMPACT-Advance, which is specifically optimized for thin slices of mouse embryos past E13.5. We demonstrate proof-of-concept by investigating the expression of two relatively understudied proto-oncogene PRDM family proteins such as PRDM10 and PRDM13, in intact cleared mouse embryos at various stages of development. We observed strong PRDM10 and PRDM13 expression in the developing nervous system and skeletal cartilage, suggesting a functional role for these proteins in these tissues throughout embryo-genesis.

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O. Molecular Medicine and Imaging [O-10]

Development of functional cell-based assay that probes the specific interaction of MERS-CoV N-protein with beta-coronavirus packaging signal RNA sequence

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The beta-coronavirus nucleocapsid (N) protein serves multiple functions in viral replication, transcription, and assembly of the viral genome complex. Beta-coronaviruses specifically package genomic RNA into assembled virions, and in MERS-CoV, SARS-CoV and SARS-CoV-2, it is reported that this process is driven by an interaction between the N-protein and a packaging signal (PS) encoded within the viral RNA. While recent studies have uncovered the sequence of this packaging signals, little is known about the specific interaction between the N-protein and the packaging signal sequence, and the mechanisms by which this interaction drives viral genome packaging. In this study, we examined the interaction between the MERS-CoV N-protein and MERS-CoV and SARS-CoV PS RNAs, as well as other viruses within the Coronaviridae family by using optimized in vivo cell-based assay platform. Our results demonstrate that the MERS-CoV N-protein specifically recognizes the SARS-CoV PS with greater affinity compared to signals from MERS-CoV or other coronaviruses species. These results describe, for the first time, in vivo evidence for an interaction between the MERS-CoV N-protein and SARS-CoV PS RNA, and demonstrate the feasibility of using this cell-based assay to further probe viral RNA-protein interactions in future studies.

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Category : **O. Molecular Medicine and Imaging** [O-11]

High-resolution monitoring of tumor xenograft mouse model via 9.4T DW-MRI.

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Peritumoral edema is an important phenomenon that determines the drug efficacy and prognosis of cancer patients. In particular, non-invasive identification of peritumoral edema from xenograft model is meaningful as an imaging biomarker. Thus, we have performed quantitative pattern analysis using high-resolution DWI and T2w MRI to compare the characteristics between the tumor tissue and the peritumoral edematous tissue. The established cancer-cell-implanted xenograft mice model were weekly monitored by 9.4T MRI (Bruker). MR imaging-based tumor volume was compared to standard calipers-measured volume. DWI-based ADC maps were generated using the MIPAV software (National Institute of Health). ROIs were placed on the area covering tumor and peritumoral edema or by limiting the region of tumor region. For 5-weeks monitoring via MRI, we found a critical problem for the calipers-measured tumor volume due to peritumoral edema. In addition, significant differences in the ADC values were confirmed between the tumor and the peritumoral edema. In the case of the tumor region except for the edematous region, especially, the ADC intensity was deceased as the tumor growth. These results demonstrate that the correlation analysis between imaging-based tumor growth information and microscopic histology of excised tissues is thought to be important information for evaluating tumor prognosis.

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O. Molecular Medicine and Imaging [O-12]

Effect of Rosmarinic Acid on Osseointegration between MC3T3-E1 Preosteoblasts and Titanium

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For successful Titanium (Ti) based dental implants, pre-osteoblasts in contact with Ti must survive and maintain cell viability, and have cell adhesion through the activation of extracellular matrix protein complexes, integrins, cytoskeletal proteins and focal adhesion kinase. Focal adhesions (FAs) and proliferation of pre-osteoblasts are the first stage for a successful dental implant. Improving FAs and cell proliferation of pre-osteoblasts on Ti surface leads to successful Ti based dental implant through increasing osseointegration. Rosmarinic acid (RA) is a natural phenolic substance that have therapeutic potential with anti-oxidative, anti-bacterial, anti-viral, anti-inflammatory and anti-mutagen activity. We used MC3T3-E1 preosteoblasts, on Ti discs incubated in medium with or without 14 ㎍/㎖ RA to reveal the effects of RA on cell adhesion including FAs and cell proliferation and signal pathways for them. As a result, RA not only enhances FAs between MC3T3-E1 preosteoblasts and Ti surfaces through the FAK/Paxillin signal pathway, but also increases cell proliferation through the FAK/Grab2/Ras/ERK1/2 signaling pathway. From the above results, RA can be applied as an effective functional and therapeutic substrate to improve FAs and cell proliferation of MC3T3-E1 preosteoblats on Ti surface, which are essential in the first stage of osseointegration between implanted Ti and bone tissue.

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O. Molecular Medicine and Imaging [O-13]

Anti-inflammatory effects of ethanol extracted Cordyceps militaris in rat brain microglial cells via Nrf2/HO-1 pathway

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Microglia play the prime effectors in immune and inflammatory responses of the central nervous system (CNS). Under pathological conditions, the activation of these cells helps restore CNS homeostasis. Cordyceps militaris, a traditional medicinal mushroom has been shown to have many pharmacological activities in traditional oriental medicine. The specific aim of this study was to examine whether ethanol extracted Cordyceps militaris (ECM) represses microglial activation in rat brain microglial cells mediated by Heme oxygenase-1 (HO-1) up-regulation. These cells were prepared from cerebral cortices of one-day-old rat pups. And then confirm the isolation of microglia through the expression of an anti-OX-42 antibody, which are markers of primary microglia, to confirm the effect of ECM on the LPS-induced inflammatory response after culture. As a result, nitric oxide (NO) increased due to LPS treatment, IL-1β, IL-6, and most of TNF-α were significantly reduced by ECM pretreatment. ECM induced HO-1 transcription and translation through NFE2-related factor (Nrf-2)/antioxidant respons e element (ARE) signaling. Taken together, our results indicate that exposure to ECM exhibits anti-inflammatory activity in microgilal cells through suppressed pro-inflammatory cytokine production and induced Nrf2/HO-1 pathway. In conclusion, our findings indicate that Cordyceps militaris could be used as a natural anti-neuroinflammatory and neuroprotective agent.

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O. Molecular Medicine and Imaging [O-14]

The influence of the effects of Ultrafine particles (UFPs) on diabetic wounds.

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Ultrafine particles (UFPs) are substances that can increase the risk of respiratory diseases, hypertension, cardiovascular diseases, and other negative health effects in humans. But, However, the effects of UFPs on diabetic wounds are not yet known. In this studies pro- inflammatory enzymes, cyclooxygenase-2 (COX-2) and pro-inflammatory cytokines, tumor necrosis factor (TNF)-α, Interleukin-1 beta (IL-1β) and interleukin (IL)-6 evaluation of the influence of Diesel exhaust particles on wounds. **Materials and Methods:** Fibroblast cells were derived from streptozotocin-induced diabetic rats. Cell Counting Kit-8 assays were used to determine cell viability. The expression of pro-inflammatory cytokines including tumor necrosis factor-α (TNF-α), cyclooxygen- ase-2 (COX-2), Interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) was evaluated by reverse transcription polymerase chain reaction and western blot analysis. **Results:** The cell proliferation of fibroblasts treated with UFPs decreased at a concentration of 160 μg/mL. The messenger ribonucleic acid expression of TNF-α COX- 2, IL-1β and IL-6 in UFPs -treated fibroblasts increased in diabetic fibroblasts. The protein expression of TNF-α COX- 2, IL-1β and IL-6 in UFPs-treated fibroblasts increased compared to samples not exposed to UFPs**. Conclusions.** UFPs regulate the expression of pro-inflammatory cytokines such as IL-6, TNF-α, and COX-2, IL-1β which may impede on diabetic wounds.

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O. Molecular Medicine and Imaging [O-15]

Enhancing Skeletal-Muscle Regeneration with Injectable Hydrogel Loaded with Myogenesis Activating Nanoparticles.

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In skeletal-muscle regeneration, it is critical to promote efferocytosis of immune cells and differentiation of satellite cells/postnatal muscle stem cells at the damaged sites. With the optimized poloxamer 407 composition gelled at body temperature, the drugs can be delivered locally. The purpose of this study is to develop a topical injection therapeutic agent for muscle regeneration, sarcopenia, and cachexia. Herein, we construct an injectable, in situ hydrogel system consisting of CD146, IGF-1, collagen I/III, and poloxamer 407, termed CIC gel. The secreted CD146 then binds to VEGFR2 on the muscle surface and effectively induces efferocytosis of neutrophils and macrophages. IGF-1 promotes satellite cell differentiation, and biocompatible collagen evades immune responses of the CIC gel. Consequently, these combined molecules activate muscle regeneration via autophagy and suppress muscle inflammation and apoptosis. Conclusively, we provide an applicable concept of the myogenesis-activating protein formulation, broadening the thermoreversible hydrogel to protein therapeutics for damaged muscle recovery.

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P. Neuroscience [P-1]

Long-term oral administration of Rosa multiflora and Zizyphus jujuba ameliorates sleep impairment in mice

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Sleep is an essential component of quality of life. The majority of people experience sleep problems that impact their quality of life. Melatonin is currently a representative sleep aid. However, it is classified as a prescription drug in most countries, and consumers cannot purchase it to improve their sleep. This sleep induction experiment in mice aimed to identify a natural combination product (NCP) that can create synergistic sleep-promoting effects. Based on the mechanism of action of sleep, we investigated whether phenomenological indicators of sleep quality change according to the intake of NCP.

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P. Neuroscience [P-2]

Green tea polyphenol (-)-epigallocatechin gallate attenuates oxidative stress and inflammation in CoCl2-stimulated microglia cells

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Hypoxia-induced neuroinflammation in stroke, neonatal hypoxic encephalopathy, and other diseases, subsequently contributes to neurological damage and neuronal diseases. Microglia are the primary neuroimmune cells that play a crucial role in cerebral inflammation. Epigallocatechin gallate (EGCG) has a protective antioxidant and anti-inflammatory effect against neuroinflammation. However, the effect of EGCG on hypoxia-induced inflammation in microglia and the underlying mechanism is not clear. In this study, we investigated whether EGCG has a protective effect against hypoxia injury in microglia by treatment with $CoCl_2$ to establish a hypoxic model of BV2 microglia cells following EGCG pre-treatment. Exposure to $CoCl_2$ increased IL-6, iNOS and COX-2, were ameliorated by EGCG via inhibition of NF-κB pathway. In addition, EGCG attenuated the expression of HIF-1α and the generation of ROS in hypoxic BV2 cells. Further, suppression of hypoxia-induced IL-6 production by EGCG was mediated via inhibiting HIF-1α expression and suppressing ROS generation in BV2 cells. Notably, EGCG increased the Nrf-2 levels and HO-1 levels in the presence of $CoCl_2$. Additionally, EGCG suppressed hypoxia-induced apoptosis in BV2 microglia, via PARP and caspase-3. In summary, EGCG protects microglia from hypoxia-induced inflammation and oxidative stress via abrogating NF-κB pathway as well as activating Nrf-2/HO-1 pathway.

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P. Neuroscience [P-3]

A FMRFamide-like neuropeptide FLP-12 signaling regulates head locomotion of C. elegans

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Neuropeptides regulate a multitude of behaviors in many organisms. However, the mechanisms by which neuropeptides orchestrate complex behavioral programs are not fully understood. Here, we show that the FMRFamide-like neuropeptide FLP-12 modulates head locomotion, including foraging-like behavior in *C. elegans*. Previous studies showed that *flp-12* is expressed in a set of neurons, including the SMB motor neurons, and mediates male turning behavior(Liu et al., 2007). To determine the function of *flp-12,* we quantified the head locomotion of *flp-12* mutants and found that mutants exhibited increased head lifting and foraging-like behavior, which were rescued by expressing FLP-12 in SMB. We performed RNAi screening to identify neuropeptide receptors for FLP-12 and found a G-protein coupled receptor FRPR-8 expressed in the AVD interneuron of which mutants also exhibited increased foraging-like behavior. In addition, we found that a G-protein α subunit *gpa-7* was co-expressed in AVD with *frpr-8,* and *gpa-7* mutants showed increased foraging-like behavior, indicating that the GPA-7 is coupled to the FRPR-8. Moreover, heterologous expression of FRPR-8 using HEK cells conferred a response to FLP-12. Taken together, these results indicate that *C. elegans* FMRFamide neuropeptide FLP-12 acts as a modulator for foraging-like behavior via the FRPR-8 GPCR and the GPA-7 G-protein.

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P. Neuroscience [P-4]

Exposure to polystyrene particles leads to developmental disruption in the mouse brain

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The organisms are consistently exposed to polystyrene particles (PS-Ps) owing to the increasing production of plastic waste. It was reported that PS-Ps accumulated in an organism induce a negative impact on various organs. However, the effects of PS-Ps on brain development were not studied clearly. We investigated the PS-Ps impacts on brain development using primary cortical neurons and mice brains exposed to PS-Ps in the developmental stage. First, we demonstrated that PS-Ps induced neurite growth inhibition, apoptosis, and disruption of proliferation on primary cortical neurons. PS-Ps downregulated brain development-associated genes in embryonic brains and reduced Gabra2 expression in both embryonic and adult brains. Finally, PS-Ps induced anxiety-, depressive-like behavior, and social deficit. Taken together, we concluded that PS-Ps exposure leads to brain development disruption in mice.

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P. Neuroscience [P-5]

Maternal exposure to 4-tert-Octylphenol induces long-lasting disruption of microglial homeostasis in offspring mice

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4-tert-Octylphenol (OP), one of the endocrine-disrupting chemicals (EDCs), is widely used to manufacture industrial products such as rubbers and epoxy resins. Through its estrogenic activity, prenatal exposure to OP has adverse effects on various organs. However, the effects of OP on neuroinflammation in developing neural networks are still unknown. Therefore, here we investigated whether exposure to OP affects the activation of microglia using primary microglial culture and mice exposed to OP during the developmental period. We found that exposure to OP increased the number of microglia and morphological transition to amoeboid form in a dose-dependent manner on primary microglial culture. In addition, adult offspring exposed to OP showed an increased number of Iba-1 positive cells, a microglial-specific marker, in their cortex compared to OP-treated neonates. Taken together, our study represents that perinatal OP exposure affects microglial activities and disruption of microglial homeostasis may last in adulthood.

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P. Neuroscience [P-6]

Regulatory role of Cullin-RING E3 ubiquitin ligase 4 in axonal morphogenesis

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Neuritogenesis is a critical event for the neuronal migration and connection during brain development. Its failure or deregulation leads to neurodevelopmental disorders including intellectual disability, mental disorder and epilepsy. In this study, we reveal a novel role of Cullin-RING E3 ubiquitin-ligase 4 (CRL4) complex in neuritogenesis during neurodevelopment. Cul4a and Cul4b, core scaffold proteins of CRL4 complex, are highly activated in the cytosolic compartment of developing neuron, and they are down-regulated by N-methyl D-aspartate receptor signaling as neurons mature. Notably, CRL4 interacts with cytoskeleton-regulating proteins including Doublecortin (Dcx). Genetic perturbation of CRL4 modifies axonal outgrowth and branching in developing neuron. Furthermore, CRL4 restricts the stability of Dcx recruited by Cereblon, which is an adapter molecule for CRL4. Conclusively, we suggest that CRL4 controls axonal morphogenesis in developing neurons by regulating cytoskeleton-regulating proteins.

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P. Neuroscience [P-7]

Effects of maternal exposure to Bisphenol S and Bisphenol F on behavior in mouse offspring

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Bisphenol A (BPA) is a representative endocrine-disrupting chemical that exhibits hormonal disturbance reactions. Various alternatives, such as Bisphenol S (BPS) and Bisphenol F (BPF), are being developed. BPS and BPF are used in consumer products such as polycarbonate plastics and epoxy resins. They have structures similar to those of BPA and have also been proven to be exogenous endocrine disruptors. However, there are few studies on the neurobiological effects of BPS and BPF. In particular, considering that BPS and BPF can pass through the placenta, studies on the effects of exposure to BPS and BPF on offspring during brain development are insufficient. Therefore, in this study, we analyzed neurobehavioral changes in offspring mice exposed to BPS and BPF during brain development by administering BPS and BPF to pregnant mice. We found that maternal exposure to BPS and BPF did not affect anxiety-and depression-like behaviors, locomotion, sociability, memory, or cognition functions in offspring mice. However, exposure to BPS and BPF decreased the preference for social novelty in the offspring mice. Taken together, these findings suggest that maternal exposure to BPS and BPF does not result in significant behavioral changes in offspring mice.

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P. Neuroscience [P-8]

PEP-1-Prohibitin1 protects hippocampal cell damage and brain injury in cerebral ischemia animal model.

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Prohibitin1 (PHB1) has emerged as a key regulator of neuronal cell survival by stabilizing the mitochondria function and suppressing oxidative stress. However, it is not well studied whether PHB expression involves in protection of neuronal cells against brain ischemic injury. Therefore, we generated cell permeable PEP-1-PHB1 fusion protein to transduce into cells, and examined the effects of PEP-1-PHB1 protein against oxidative stress-induced HT-22 cell damage and ischemic injury animal model. In the present study, we showed that transduced PEP-1-PHB1 protein increased cell survivals by reduction of cytotoxicities such as intracellular ROS, DNA fragmentation and mitochondria damage levels under oxidative stress condition. PEP-1-PHB1 protein also regulated the activation of MAPK and apoptosis signaling pathway. Furthermore, PEP-1-PHB1 protein transduced into the brain *via* through the blood-brain barrier (BBB) and reduced brain damage in ischemic injury animal model. Those results indicate that PEP-1-PHB1 protein significantly inhibits hippocampal neuronal cell damage *in vitro* and *in vivo*, suggesting that PHB1 protein may be useful to ameliorate brain damages of oxidative stress-related neuronal disorders including ischemic injury.

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P. Neuroscience [P-9]

Tat-PIM2 alleviates dopaminergic neuronal cell death by regulating the apoptosis signaling

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The pathogenesis of Parkinson's disease (PD) is related with oxidative stress, inflammation, and apoptosis and Proviral integration moloney 2 (PIM2) plays an important role in the cell cycle, anti-inflammation and apoptosis. However, the relationship between PIM2 and PD is not studied yet. In this study, the role of PIM2 in PD was investigated by using the cell permeable Tat-PIM2 whether this fusion protein can protect against dopaminergic neuronal cell death. In the SH-SY5Y cells pretreated with 1-methyl-4-phenylpyridinium (MPP^+), we found that Tat-PIM2 significantly reduced ROS production and protected cell death and this protein regulated expression levels of apoptotic related proteins. Tat-PIM2 transduced into brain and inhibited dopaminergic neuronal cell death in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD animal model and this fusion protein increased the expression of antioxidant proteins and regulated the expression levels of apoptotic related proteins. Those results indicate that Tat-PIM2 protects dopaminergic neuronal cell death by suppressing the apoptotic signaling pathway and cellular toxicity, suggesting Tat-PIM2 act as a therapeutic target for PD.

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P. Neuroscience [P-10]

GAP-43 closely interacts with BDNF in hippocampal neurons and is associated with Alzheimer's disease progression

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GAP-43 has been termed a neuronal plasticity protein because it is widely expressed at high levels in neuronal growth cones during axonal regeneration. GAP-43 protein expressed in mature adult neurons is functionally important for the neuronal communication of synapses as required for learning and memory. Brain-derived neurotrophic factor is closely related to the neurodegeneration and synaptic plasticity during aging process. Moreover, GAP-43 and BDNF are highly expressed in the healthy adult hippocampus brain region and inversely correlate with the Amyloid beta which is related the Alzheimer's disease. We show that GAP-43 and BDNF are inversely associated with AD pathogens in Aβ PFFs treated sample. In addition, we obtained a GAP-43 and BDNF protein three-dimensional structure modeling analysis. We studied the GAP-43 knock-down or BDNF knock-down cellular and animal model with 7.8-dihydroxyflavone compounds that specifically activate TrkB. We demonstrate that both deprivated of GAP-43 and BDNF trigger hippocampal neuronal cell death and memory dysfunction in Aβ PFFs induced AD mouse model. These results highlight the GAP-43 and BDNF as direct binding partners in hippocampal neurons to prevent memory loss and the potential of targeting GAP-43/BDNF molecular signaling in AD drug development.

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P. Neuroscience [P-11]

Akkermansia muciniphila is associated with BDNF/serotonin secretion via gut-liver-brain axis

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Recently, a protective role against metabolic and inflammatory diseases has been suggested for the next generation of beneficial microbes, akkermansia muciniphila. In this study, we sought to evaluate the effects of akkermansia muciniphila on inflammatory markers involved in liver fibrosis in a mouse model of liver injury induced by 3,5-diethoxycarboncyl-1,4-dihydrocollidine (DDC) diet. To understand the phenomenon of cognitive impairment commonly observed in patients with cirrhosis, we have now validated the existence of cognitive impairment in DDC diet animal models. Interestingly, in animal models of cirrhosis, gut-liver-brain damage was confirmed by tissue staining techniques, and BDNF/serotonin expression was drastically reduced in the gut and brain. In addition, administration of akkermansia muciniphila and pasteurized akkermansia muciniphila to this animal model significantly reduced the extent of tissue damage in the intestine and brain, and increased the expression of BDNF/serotonin. Through the vagus nerve isolation and vagus nerve immunohistostaining, we confirmed that the expression of serotonin increased with akkermansia muciniphila feeding, demonstrating the correlation of the gut-liver-brain axis. Thus, our findings provide a molecular understanding of brain dysfunction commonly observed in patients with cirrhosis and suggest akkermansia muciniphila as a therapeutic candidate to alleviate the symptoms of cirrhosis and cognitive impairment.

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P. Neuroscience [P-12]

XperCT-guided intra-cisterna magna injection of streptozotocin for establishing an Alzheimer's disease model using the cynomolgus monkey (Macaca fascicularis)

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A widely recognized AD model that mimics the pathology of human AD involves the intracerebroventricular (ICV) injection with streptozotocin (STZ). However, ICV injection as an invasive approach has several limitations related to complicated surgical procedures. Therefore, in the present study, we created a customized stereotaxic frame using the XperCT-guided system for injecting STZ in cynomolgus monkeys, aiming to establish an AD model. The anatomical structures surrounding the cisterna magna (CM) were confirmed using CT/MRI fusion images of monkey brain with XperCT, the c-arm cone beam computed tomography. XperCT was used to determine the appropriate direction in which the needle tip should be inserted within the CM region. Cerebrospinal fluid (CSF) was collected to confirm the accurate target site when STZ was injected into the CM. Cynomolgus monkeys were administered STZ dissolved in artificial CSF once every week for 4 weeks via intracisterna magna (ICM) injection using XperCT-guided stereotactic system. The molecular mechanisms underlying the progression of STZ-induced AD pathology were analyzed two weeks after the final injection. The monkeys subjected to XperCT-based STZ injection via the ICM route showed features of AD pathology, including markedly enhanced neuronal loss, synaptic impairment, and tau phosphorylation in the hippocampus.

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P. Neuroscience [P-13]

Amyloid-beta oligomers(AβOs)-induced Alzheimer's disease model in the cynomolgus monkey (Macaca fascicularis) via intra-cisterna magna injection

Jinyoung Won[1#], Jiyeon Cho[1#], Jincheol Seo[1], Jung Bae Seong[1], Hyeon-Gu Yeo[1], Keonwoo Kim[1], Yu Gyeong Kim[1], Lee Wha Gwon[1], Junghyung Park[1*], Youngjeon Lee[1*]

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Till date, researchers have been developing animal models of Alzheimer's disease (AD) in various species to understand the pathological characterization and molecular mechanistic pathways. A widely recognized AD model that mimics the pathology of human AD involves the intracerebroventricular (ICV) injection with amyloid-beta oligomers (AβOs). However, ICV injection as an invasive approach has several limitations related to complicated surgical procedures. In the present study, we applied a customized stereotaxic frame using the XperCT-guided system for injecting AβOs in cynomolgus monkeys, aiming to establish a sporadic AD model. The anatomical structures surrounding the cisterna magna (CM) were confirmed using CT/MRI fusion images of monkey brain with XperCT, the c-arm cone beam computed tomography (CBCT). Cynomolgus monkeys were administered AβOs (100 μg) twice a week for 4 weeks (total 8 times) via intracisterna magna (ICM) injection. The monkeys subjected to XperCT-based AβOs injection via the ICM route showed features of AD pathology, including markedly enhanced synaptic impairment, tau phosphorylation, and astrocyte and microgila activation in the hippocampus CA1 region. These findings suggest a new approach for the construction of Alzheimer's disease models in monkeys and development of therapeutic strategies.

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P. Neuroscience [P-14]

The role of Tat-HPCA protein in oxidative stress-induced neuronal cell death

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Oxidative stress plays a crucial role in neuronal disorders including cerebral ischemic injury. ROS-induced oxidative stress during brain ischemia leads to cell death and irreversible neurological diseases after reperfusion. Hippocalcin (HPCA) protein is expressed in the brain, especially the hippocampal CA1 region. HPCA maintains calcium homeostasis and regulates neuronal survival and death. Since the precise function of HPCA protein in ischemic injury is fully unclear, we investigated the effect of HPCA protein on HT-22 cell damage induced by hydrogen peroxide and in an ischemic animal model using cell permeable Tat-HPCA fusion protein. We examined that transduced Tat-HPCA inhibited cell death, ROS generation, DNA fragmentation and MAPK and apoptotic signaling pathways in H_2O_2-treatment HT-22 cells. In an ischemia animal model, Tat-HPCA showed that transduced into the hippocampus and prevented neuronal cell death and reduced astrocytes and microglia activation. These results indicate that Tat-HPCA may provide an alternative strategy to improve against ischemic injury.

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P. Neuroscience [P-15]

Anxiolytic-like Effect of Inhaled Cinnamon Essential Oil and Its Main Component Cinnamaldehyde in Animal Models

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Aromatherapy is one of the most common safer alternative treatments for psychiatric disorders. Here, we investigated the effects of cinnamon essential oil (CIEO) inhalation on mouse behaviors using behavioral tests. CIEO inhalation showed anxiolytic effects in the elevated plus maze test, as inferred from increased time spent in open arms and decreased time spent in closed arms. The CIEO treatment enhanced social behavior by increasing the total contact number, time spent in the center, distance traveled in the center, and total distance in the social interaction test. However, CIEO inhalation showed no effect in the open field test, tail suspension test, forced swimming test, and Y maze test. Microarray analysis indicated that CIEO treatment downregulated 17 genes and upregulated 15 genes in the hippocampus. Among them, Dcc, Egr2, and Fos are the most crucial genes involved in anxiety-related biological processes and pathways, including the regulation of neuronal death and neuroinflammation. Gas chromatography/mass spectrometry analysis revealed that cinnamaldehyde is the main component of CIEO. Cinnamaldehyde recovered MK-801-induced anxiety-related changes in the electroencephalogram power spectrum in zebrafish. Our findings suggest that CIEO and cinnamaldehyde have an anxiolytic effect through regulating the expression of genes related to neuroinflammatory response and neuronal death.

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P. Neuroscience [P-16]

Attenuation of H2O2-induced hippocampal neuronal cell death by transduced Tat-MDH1

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Malate dehydrogenase 1 (MDH1) is an enzyme that catalyzes the NAD/NADH-dependent conversion of malate and oxaloacetate in many metabolic pathways such as tricarboxylic acid (TCA) cycle and coordinates metabolism between cytosol and mitochondria. Although the function of MDH1 in metabolic disorder has been investigated, its role in ischemia is not well known yet. In the present study, we investigated the effects of MDH1 on cerebral ischemia *in vitro* and *in vivo*. MDH1 was fused to Tat peptides that can cross cell membrane, tissue even blood-brain barrier (BBB) without a specific receptor in order to efficiently penetrate into cells. We showed that Tat-MDH1 transduced into HT-22 cells and it markedly inhibited H_2O_2-induced HT-22 cell death. *In vivo,* immunohistochemical analysis showed that Tat-MDH1 protein transduced into the hippocampal CA1 region and inhibited neuronal cell death in the ischemia animal model. These results demonstrated that transduced Tat-MDH1 markedly protects against oxidative stress-induced hippocampal neuronal cell damage, suggesting that Tat-MDH1 has a potential as a therapeutic agent for oxidative stress-induced neuronal diseases including ischemia.

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P. Neuroscience [P-17]

Investigation of the role of complement receptors in extracellular tau clearance

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Abnormal accumulation of misfolded tau aggregates is a key pathological hallmark of Alzheimer's disease. Pathological tau spreads in the brain, and it is closely correlated with cognitive decline. Therefore, it is important to understand the molecular mechanisms of tau. Microglia regulates the clearance of various pathological proteins, such as α-synuclein, tau, and amyloid β (Aβ). CR3 and CR4, two major phagocytic receptors in microglia, are known to mediate phagocytosis of fibrillar Aβ and α-synuclein, respectively. In this study, we investigated the role of CR3 and CR4 in regulating clearance of extracellular tau.

In this study, we identified that CR4 selectively binds to tau fibrils but not to tau monomers using dot-blot and immunoprecipitation assay. We further demonstrated that silencing of CR4 dramatically reduce the uptake and clearance of tau fibrils. However, silencing of CR4 does not affect the degradation of extracellular tau in the culture media. Moreover, conditioned media form CR4-silenced BV2 culture treated with tau fibrils are more potent in inducing tau aggregation in Tau RD cells compared to the controls. Taken together, our data suggest that CR4 is a novel receptor for tau fibril clearance and may play a critical role in tau spreading.

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P. Neuroscience [P-18]

Mechanosensitive Piezo Channel, PEZO-1, regulates food swallowing in C. elegans.

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Mammalian two PIEZO genes, Piezo1 and Piezo2, of which functions have been shown to be involved in mechanosenation (Coste et al., 2010, Woo et al., 2014, Nonomura et al., 2017). *C. elegans* genome has a single PIEZO gene, *pezo-1,* which encode 14 isoforms (Bai et al., 2020) and function has not been fully determined. To examine *pezo-1* function, we grouped 14 isoforms depending on the mRNA length and observed their expression patterns. The long isoforms are specifically expressed in the pharyngeal-intestinal valve (PI valve), which is predicted to mediate food swallowing (Avery and Thomas, 1997). To examine whether *pezo-1* has role in food swallowing, we fed animals with GFP-microsphere and found that *pezo-1* mutants show excess accumulation of GFP in the intestine lumen. Defects in *pezo-1* mutants are restored by expression of long isoform PEZO-1 or mouse PIEZO1. We next found that the PI valve exhibits calcium transient and the optogenetic activation of PI valve induces the pharyngeal plunge. These results demonstrate that the *C. elegans* PIEZO channel regulates pharyngeal plunge and provides insights to understand the function of the mammalian PIEZO channel shown to be expressed in the esophagus.

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P. Neuroscience [P-19]

Amber extracts suppress neuroinflammation and depressive-like behaviors derived from microglia hyperactivation

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Microglia are emerging as important targets for the treatment of neuropsychiatric disorders. The phagocytic microglial phenotype and the resulting neuroinflammation lead to synaptic loss and neuronal cell death. To explore potential candidates that inhibit microglial hyperactivation, we first investigated ten candidate extracts of traditional Chinese medicine (TCM) using lipopolysaccharide (LPS)-stimulated BV2 microglial cells. Among the candidates, amber extract (AE) was superior; thus, we further investigated its pharmacological activity and underlying mechanisms both in vitro and in vivo. Pretreatment with AE (10, 20, and 40 μg/ml) attenuated the increases in inflammatory factors (nitric oxide and tumor necrosis factor-α), translocation of nuclear factor-kappa B (NF-κB), and phenotypic transformations (phagocytic and migratory) in a dose-dependent manner. These inhibitory effects of AE on microglia were supported by its regulatory effects on the CX_3C chemokine receptor 1 (CX_3CR1)/nuclear factor erythroid-2-related factor 2 (Nrf2) pathway. In particular, intragastric administration of AE (100 mg/kg) considerably improved sickness, anxiety, and depressive-like behaviors in mice subjected to chronic restraint stress (CRS). Our results suggest that AE has strong antineuroinflammatory and antidepressant properties, and the underlying mechanisms may involve not only the regulation of NF-κB translocation but also the normalization of the CX_3CR1/Nrf2 pathway.

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P. Neuroscience [P-20]

Effect of sirtuin-3 preservation on age-dependent locomotive decline

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Aging-related decline in locomotor activity is one of general phenotypes in senescence. Although the locomotor decline can be strongly associated with the decrease in activity of the nigrostriatal dopamine (DA) system, the key molecule to control the decline is not fully understood yet. In present study, we observed that the protein level of sirtuin-3 (SIRT3), which is well-known as an endogenous molecule with anti-aging effects, significantly decreased in substantia nigra (SN) of aged mice compared to young adult mice. To examine whether the loss of SIRT3 expression contributes to the decline in locomotor activity with aging, we administrated adeno-associated virus (AAV) encoding SIRT3 gene into nigral DA neurons of 2-month-old mice and monitored their locomotor activity for up to 20 months. Our results showed that SIRT3 upregulation in nigral DA neurons by AAV transduction inhibited the age-dependent decline of locomotor activity via preservation of tyrosine hydroxylase (TH and p-TH), DA and metabolites in aged mice compared to intact control mice. Therefore, SIRT3 upregulation by AAV transduction in the SN has the resistance to age-related locomotor decline shown in senescence.

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P. Neuroscience [P-21]

Hyperglycemia-induced AhR O-GlcNAcylation upregulates TGM2-induced mitochondrial stress and amyloidogenesis in SH-SY5Y.

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Diabetic encephalopathy (DE) is one of the diabetic complications that caused by impaired control of glucose metabolism. Previous studies have shown that diabetic patients have risk of developing Alzheimer's disease (AD) than healthy people, and there are several similar mechanisms and interactions between AD and DE. Mitochondrial reactive oxygen species (mtROS) induced in the hyperglycemia exacerbate neuronal cell dysfunction. mtROS is generated by Ca2+ release from endoplasmic reticulum (ER) to mitochondria (MT), but the relationship between mtROS and ER-MT connection under hyperglycemia is unclear. So, we aimed the increasing O-GlcNAcylation in the hyperglycemia. Previous studies have shown that O-GlcNAcylation and accumulation of amyloid beta (Aβ) was increased in the AD patients. Therefore, we investigated how amyloidogenesis and increased O-GlcNAcylation affect mtROS in hyperglycemia in SH-SY5Y. First, we demonstrated that increased O-GlcNAcylation is due to increased expression of OGT and checked that APP and BACE1 proteins involved in Aβ production were also increased. And we found that the expression of TGM2 was increased by O-GlcNAcyrated AhR. The upregulated TGM2 expression increased the ER-Mitochondria contact and it caused Ca2+ release from ER to mitochondria. We demonstrated that increased Ca2+ release from ER to MT exacerbates neuronal dysfunctions by causing mtROS.

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P. Neuroscience [P-22]

Increased activation of folate receptor 1 by high glucose inhibits amyloidogenesis by blocking mitochondrial oxidative stress through Nrf2.

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Many researchers are showing many results about the relationship between diabetes in the development of Alzheimer's disease and dementia. Previous studies have shown that folic acid and its receptors are closely related to the pathogenesis of Alzheimer's disease and diabetes. However, the mechanism by which folic acid controls high glucose-induced amyloid beta production in neurons remains not clear. Therefore, we focused on the regulatory effect of folic acid on high glucose-induced amyloid production and related mechanisms. First, high glucose increased APP and BACE1 expression and amyloid-beta production. Folic acid treatment reversed high glucose-stimulated APP and BACE1 expression and mitochondrial active oxygen stress accumulation. And, high glucose decreased Nrf2 expression, recovered by folic acid. We also believe that high glucose increases folate receptor 1 (FOLR1) mRNA expression reduced by OGT inhibitor pretreatment. 5-MTHF (5-methyltetrahydrofolate) treatment did not significantly affect high glucose-induced APP and BACE1 expression. The overall view suggests that folic acid affects amyloid production via the FOLR1 pathway. Thus, we found that folic acid affects amyloidogenesis through the FOLR1 pathway. In our data, activation of FOLR1 by folic acid suppressed mitochondrial oxidative stress and amyloidogenesis in neurons under high glucose conditions.

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P. Neuroscience [P-23]

2-Hydroxypropyl-beta-cyclodextrin exacerbates tau pathology in a mouse model of tauopathy: implication of the pathological role of cholesteryl ester

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Accumulating evidence suggest that the disruption of lipid homeostasis is closely linked to Alzheimer's disease (AD) pathologies. Several evidence reported that cyclodextrin (CD), a cholesterol depleting agent, reduces amyloid beta plaque in AD mouse model. Despite increasing evidence suggesting a pathological link between cholesterol metabolism and tauopathies, the detailed mechanism remains elusive. Here, we investigated the effect of cholesterol on tauopathy in PS19 transgenic mice by injecting 2-Hydroxypropyl-beta-cyclodextrin (HP-CD), one of the CD modified forms. HP-CD dramatically promoted pathological tau accumulation and neurodegeneration in the hippocampus. We further showed that the changes in cholesterol level in the liver and brain. Interestingly, cytosolic cholesteryl ester (CE) was increased by HP-CD. It has been reported that CE is accumulated in the brain of AD patients and AD mouse models, and CE increases tau phosphorylation. Based on these results, we hypothesized that increased cytoplasmic CE might exacerbate tau pathology. To confirm this, we tested the effect of HP-CD in a seed-dependent tau aggregation cell model and found that HP-CD increased tau aggregation. When the effect of HP-CD is inhibited by K-604, increased tau aggregation was reversed. Our data suggest that modulation of cholesterol ester may represent a practical strategy for AD therapy.

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P. Neuroscience [P-24]

MPE ameliorates fatigue via modulating serotonergic and neuronal activity in Flx-induced mice model

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Clinically, pathologic fatigue relates to central fatigue, which is neuromuscular dysfunction due to neurochemical changes. The purpose of this research was to investigate how MPE acts to prevent central fatigue. Male C57BL/6J mice (8 weeks old) were subjected to periodic injections of fluoxetine (Flx) for 28 days. Simultaneously, the mice were administered MPE (0, 50 or 100 mg/kg) or ascorbic acid (100 mg/kg). On the last day, the behavior tests (rotarod, wheel running, nest building, and plantar tests) were performed. And then mouse brain tissue was analyzed for alterations in serotonin-related factors. Flx notably deteriorated exercise performance, sickness behavior, and pain sensitivity like rotarod, wheel running, nest, and plantar tests, respectively. Whereas MPE (50mg/kg) administration significantly ameliorated these alterations. The levels of the main neurotransmitter-related markers such as MAO-a VAMT2 and so on the fatigue were markedly altered by Flx, as a result, neuronal activity was also inhibited in the raphe nucleus. MPE significantly ameliorated these alterations through relevant molecules including brain-derived neurotropic factor (BDNF) and c-Fos. This work provided therapeutic significance for MPE by demonstrating its anti-fatigue properties in an Flx-induced mice model.

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P. Neuroscience [P-25]

Neuroprotective effects of AAV-Rheb(S16H) administration in an inflammatory mouse model of cerebellar ataxia

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Cerebellar ataxia (CA), characterized by impaired motor coordination, can occur as a result of damaged cerebellum. There is no medicine that can fully recover CA symptoms. In the previous study, we reported that the induction of ras homolog enriched in brain (Rheb) activation, which is an important neurotrophic signaling mechanism, could contribute to the neuroprotection in the animal models of neurodegenerative diseases such as Parkinson's and Alzheimer's diseases. However, the effect of Rheb activation in CA is not examined yet. To investigate whether Rheb activation in the cerebellum has neuroprotective potential against a neuroinflammatory condition, we administrated the adeno-associated virus (AAV) containing a constitutively active Rheb [Rheb(S16H)] into the cerebellum and observed the beneficial effects in the lipopolysaccharide (LPS)-induced inflammatory animal model of CA. Our results showed that AAV-Rheb(S16H) transduction in cerebellum inhibited neuroinflammatory responses and preserved pyramidal neurons in the LPS-treated animal model of CA. Therefore, we conclude that the induction of Rheb(S16H) in the cerebellum may be useful to provide neuroprotective effects against inflammatory CA symptoms.

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P. Neuroscience [P-26]

Roles of LIM-HD transcription factors during sensory neuron development

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Sensory neurons receive and deliver a variety of external sensory information, such as pain, temperature, touch and proprioception. During the process of sensory neuron formation originated from migrating neural crest cells, LIM-homeodomain (LIM-HD) transcription factors are known to play a role in generation and maintenance of sensory neurons in early period of sensory neuron development. Nevertheless, the link between LIM-HD transcription factors and individual sensory neuronal subtypes is still unclear. Here we analyzed expression patterns of developmental stages using the published scRNA-seq data of sensory neurons in various developmental stages. As a result, LIM-HD transcription factors are expressed in all sensory neurons, but each gene of the LIM-HD transcription factors is expressed specifically according to the developmental stage and subtype. Immunohistochemistry and in situ hybridization analysis of DRG were also consistent with scRNA-seq data. Currently, transcriptomic analysis using LIM-HD mutant mice is ongoing. This study suggests that the genetic mechanism and function of LIM-HD transcription factors for subdivision of sensory neurons into various subtypes.

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P. Neuroscience [P-27]

Is there a relation between Unexplained fatigue and Cortisol ?: A systematic review

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The cause of fatigue is not yet clearly defined, and due to its multifactorial etiology, it is difficult to address the symptomatology. Then, cortisol levels, known as stress-responsive hormones, were measured differently in people with fatigue symptoms. So, we aimed to quantify the relationship between cortisol level and the characteristics of fatigue. The study selections were commenced by searching PubMed using the keywords fatigue and cortisol as well as several other supplementary search strategies available before December 2022. Results were screened according to inclusion criteria and data on the outcomes of interest were extracted. The initial search generated over 500 articles with 115 studies (3080 participants) meeting the inclusion criteria. A quantitative analysis was conducted to supplement the review, to provide estimates of tools (FSS, MFI-20, BFI, SF-MPQ, VAS) and to determine variation by prognostic factors. The result shows that there is a relatively stable relationship between fatigue severity and cortisol level in a U-shaped pattern. As a conclusion, we suggest cortisol as a biomarker for diagnosing fatigue. To determine and establish the relationship between cortisol and fatigue, further research is required.

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P. Neuroscience [P-28]

Presenilin 2 N141I mutation induces hyperactive immune response through the epigenetic repression of REV-ERBα

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Hyperimmunity drives the development of Alzheimer disease (AD). The immune system is under the circadian control, and circadian abnormalities aggravate AD progress. Here, we investigate how an AD-linked mutation deregulates expression of circadian genes and induces cognitive decline using the knock-in (KI) mice heterozygous for presenilin 2 N141I mutation. This mutation causes selective overproduction of clock gene-controlled cytokines through the DNA hypermethylation-mediated repression of REV-ERBα in innate immune cells. The KI/+ mice are vulnerable to otherwise innocuous, mild immune challenges. The antipsychotic chlorpromazine restores the REV-ERBα level by normalizing DNA methylation through the inhibition of PI3K/AKT1 pathway, and prevents the overexcitation of innate immune cells and cognitive decline in KI/+ mice. These results highlight a pathogenic link between this AD mutation and immune cell overactivation through the epigenetic suppression of REV-ERBα.

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P. Neuroscience [P-29]

CLC-4 Deletion Disrupts Neuronal Development and Synaptic Function: Implications for Autism Spectrum Disorder Pathogenesis

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Mutations in chloride channel 4 (CLC-4) are associated with neurodevelopmental disorders, particularly autism spectrum disorder (ASD), and CLC-4 KO mice exhibit features consistent with ASD. In this study, we performed RNA-sequencing analysis and Gene Ontology (GO) analysis to investigate the genetic and molecular pathways of cells differentiated from knockdown (KD) neural progenitor cells (NPCs) and primary cortical neurons of knockout (KO).

In RNA-sequencing analysis, 2185 genes in KD cells and 97 genes in KO cells were significantly decreased compared to the wild type (WT), and GO analysis showed that genes related to regulation of neuron projection development, synaptic signal, regulation of neuronal synaptic plasticity, and regulation of morphogenesis of a branching structure were significantly decreased in both KD and KO. In particular, 24 genes related to synaptic vesicle transmission and neurogenesis (Synpr, Plk5, Nrgn, Neurod6, Fam107a, Enpp2, Calb2, Bhlh22, and others) were commonly decreased in both KD and KO.

Through the decrease of the aforementioned genes, we can conclude that deletion of CLC-4 may cause synaptic disruption in developing brain and thereby contribute to pathogenesis of neurodevelopmental disorders.

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P. Neuroscience [P-30]

Compound A inhibited LPS-induced neuroinflammation in BV2 cells and microglia-mediated neurotoxicity in HT22 cells.

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In the brain, neuroinflammation is part of the defense against injury or infection. However, Inflammatory cytokines due to microglia over-activity induce neuronal cell death. Neuroinflammatory damage is known to be one of the causes of neurodegenerative diseases.

We investigated the inhibitory effect and mechanism of inflammation and microglia-mediated neurotoxicity of compound A through BV2 and HT22 cells. BV2 cells were treated with LPS (1μg/mL) for 24h following pretreatment with Compound A (0, 5, 10, or 20 uM). No assay, Western blot, and ELISA were performed. The media harvested from BV2 cells were treated to HT22 cells, and Cell viability and Western blot were performed.

LPS treatment induced neuroinflammation for increased inflammatory cytokine in BV2 cells. Also, LPS-treated conditioned medium in BV2 cells induced neuron cell death in HT22 cells. Compound A reduced inflammatory cytokines (NO, TNF-alpha, IL-6) in BV2 cells, and inhibited neuron cell death in HT22.

Our results verified that compound A inhibitory effect on LPS-induced neuroinflammatory of BV2 cells and microglia-mediated neurotoxicity of HT22 cells. We suggest compound A as a candidate for treating brain disorders derived from neuroinflammatory damage.

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P. Neuroscience [P-31]

Identification of picalm effects in TDP-43 overexpressed model

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Transactive response (TAR) DNA-binding protein 43 (TDP-43) is widely expressed nuclear protein which involved in the regulation of gene expression such as RNA splicing, trafficking, stabilization. While shuttling between nucleus and cytoplasm, TDP-43 becomes mislocalized in cytoplasm and aggregated into insoluble inclusion bodies in neurodegenerative disease. Since overexpression of TDP-43 reduces cell viability and increases apoptotic effects, regulation of TDP-43 expression is essential to understand the pathological mechanisms of TDP-43. To identify the factor regulates TDP-43 expression, we investigate variable protein levels in TDP-43 overexpressed toxicity model. Especially, phosphatidylinositol binding clathrin assembly protein (PICALM) expression, which regulates the formation of the clathrin lattice during endocytosis, is reduced in TDP-43 overexpression condition. PICALM was known to associate with protein aggregation in cellular inclusion and involve in various pathological processes. But the molecular mechanism of PICALM related with TDP-43 protein has not been fully elucidated. In addition, we identified that PICALM overexpression decrease endogenous TDP-43 expression level using western blot analysis. Taken together, we suggest that PICALM might be novel factor to regulate TDP-43 pathological expression and the correlation between PICALM and TDP-43 can play a critical role to maintain TDP-43 expression homeostasis intracellular environment.

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P. Neuroscience [P-32]

Augmented Protective Effects of Antioxidant Extracts against H2O2-Induced Blood-Brain Barrier Disruption in the Absence of Metallothionein 3 in Primary Pericyte

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Pericytes are specialized cells found in small blood vessels that help maintain vessel stability, regulate blood flow, and promote the growth of new blood vessels. Metallothionein 3 (MT3) is a protein that regulates cellular metal levels, particularly copper and zinc, and is primarily expressed in the brain. MT3 is involved in protecting against neurodegenerative conditions such as Alzheimer's disease by regulating metal levels and preventing the accumulation of toxic proteins and oxidative stress. This study investigated the role of pericytes and MT3 in regulating the blood-brain barrier (BBB) and their potential protective effects against oxidative stress. An in vitro BBB model was used with primary pericytes obtained from wildtype and MT3-knockout mice, and antioxidant extracts were tested. The results showed that the extracts increased BBB resistance, particularly in knockout pericytes compared to wildtype pericytes, suggesting a protective role of the compounds and highlighting the involvement of MT3 in pericytes.

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P. Neuroscience [P-33]

Protective effects of insect powder on oxidative stress-mediated neuronal apoptosis in a valproic acid-induced autism mouse model

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Autism spectrum disorder (ASD) is a neurodevelopmental condition that causes repetitive behaviors and makes social communication and interaction difficult. This study evaluated the effects of insect powder in a valproic acid (VPA)-induced mouse model of ASD. Neuronal cytotoxicity was measured using western blotting to determine the expression levels of neuron-specific proteins and apoptosis-associated proteins, and Nissl staining of brain tissues. Antioxidant reactivity-associated assays were performed to measure the association between brain damage and oxidative stress. Insect powder significantly protected neurons from oxidative stress and apoptosis in different brain areas, especially in the CA1 and CA3 regions of the hippocampus. These protective effects were demonstrated by restoring the expression of Neu-N and postsynaptic density protein-95, increased levels of glutathione, superoxide dismutase and catalase, and recovery of apoptosis-associated protein expression such as Bax, Bcl-2, cleaved-PARP, and cleaved caspase-3 in autistic mice. Collectively, this study provides a new therapeutic solution to ameliorate ASD using insect powder.

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P. Neuroscience [P-34]

Impairment of NMDA-R in Dscam-Knockout mice express ASD pathology

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[Purpose] This study aims to show NMDA-R dysfunction in heterozygous *Dscam* mutation generates autism spectrum disorder (ASD) symptoms. [Methods] We produced knockout mice with *Dscam* heterozygous mutation to cause ASD symptom. Reciprocal interaction test and three chamber test were conducted in order to observe social interaction. During the task with Nestin-*Dscam* heterozygous knockout mice, we observed ASD symptom such as social interaction deficit. Moreover, the ratio of NMDA-R and AMPA-R-mediated synaptic currents of layer 2/3 pyramidal neurons in the anterior cingulate cortex (ACC) was measured. [Results] Nestin-*Dscam* knockout mice displayed decreased total interaction time in the reciprocal interaction test and had less time interacting with the stranger mouse than WT littermate in the three-chamber test, which is exposed to stranger or object. In addition, there was reduced level of interest to the stranger mouse in knockout mice in knockout mice compared to WT littermates, when they were exposed to the familiar or stranger mouse. The ratio of NMDA/AMPA was also significantly lower in the knockout mice than in WT littermates. [Conclusion] We provide further insight to use ASD-like mouse model, insisting that *Dscam* mutation may cause autistic symptoms with dysfunction of NMDA-R.

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P. Neuroscience [P-35]

Protective effects of insect powder as an effective therapy to improve brain damage in pentylenetetrazol-induced epilepsy in mice

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Epilepsy is a common neurological disorder characterized by recurrent seizures, leading to transient central nervous system dysfunction. This study evaluated the effect of insect powder in pentylenetetrazol (PTZ)-induced mice model of epilepsy. Neuronal cytotoxicity was measured using western blotting to determine the expression level of neuron-specific proteins and apoptosis-associated proteins, as well as using Nissl staining of brain tissues. Antioxidant reactivity-associated assays were performed to measure the link between brain damage and oxidative stress. Insect powder protected neurons from oxidative stress and apoptosis in different brain areas, especially in the CA1 and CA3 regions of the hippocampus. These protective effects were proved by restoring the expression of Neu-N and postsynaptic density protein-95, increased levels of glutathione and superoxide dismutase, and recovery of apoptosis-associated protein expression such as Bax, Bcl-XL, cleaved-PARP, and cleaved caspase-3 in epilepsy mice. Collectively, this study provides new therapeutic bioactive materials from insect to ameliorating epilepsy.

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P. Neuroscience [P-36]

Corticostriatal Sapap3 and serotonergic contributions to cognitive discounting task activity

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Impulsivity trait is defined as the tendency to execute an action without mature consideration and consequences. Such unbridled behavior can impair ideal or long-term goals. Among the various sub-divide core aspects of impulsivity, which refers to choice impulsivity, tendencies to select smaller-immediate rewards over larger-later rewards, is considered one of the domains contributing to impulsivity. Several studies have reported that cortico-striatal synaptic defects, which are induced by the genetic deletion of *Sapap3* (SAP90/PSD95-associated protein 3), evoke behavior abnormalities in rodents, including increased anxiety and repetitive behavior, and these abnormalities of *Sapap3* KO mice are rescued by the serotonin reuptake inhibitor (SSRI). However, how serotonergic systems work differently in mice with Sapap3 deletion is still unknown. Therefore, we hypothesized that deletion of *Sapap3* in the specific brain region alters normal serotonergic action within the cortico-striatal pathway. *Sapap3* conditional knock-out mice were trained to learn sequential delay discounting (DD) and probability discounting (PD) tasks in our custom-made operant chamber. We are able to measure impulsive choice in the *Sapap3* cKO mice. Also, a newly developed genetically encoded serotonin sensor, sDARKEN, will help us visualize the in vivo serotonergic neuromodulation in the prefrontal area during the discounting task.

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P. Neuroscience [P-37]

Glutamine-rich adhesive proteins buffer huntingtin toxicity by promoting cytoplasmic aggregation of mutant huntingtin proteins

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Abnormal accumulation of mutant Huntingtin (mHtt) proteins with an expanded polyglutamine (polyQ) tract is the neuropathological hallmark of Huntington's disease (HD). However, whether the aggregation of cytoplasmic mHtt proteins contributes to neuronal toxicity or an intrinsic self-protective mechanism remains controversial. In this study, we focused on revealing the protective roles of cytoplasmic mHtt aggregates in neurons and investigated detailed molecular mechanisms underlying HD pathology. Using our *in silico* prediction model, we searched for novel cytoplasmic Q-rich adhesive proteins among *Drosophila* endogenous proteins which may facilitate the aggregation of cytoplasmic mHtt proteins. Our genetic screening identified that Q-rich adhesive proteins increased the amount of cytoplasmic mHtt puncta and restored mHtt-induced neuronal toxicity using *Drosophila* HD model. Our findings suggest that these interactors may prevent the accumulation of toxic nuclear mHtt proteins through the facilitated aggregation of cytoplasmic mHtt proteins, acting as intrinsic buffers against nuclear mHtt-derived neuronal toxicity. Together, we believe our studies revealing the neuroprotective roles of cytoplasmic mHtt and their Q-rich adhesive interactors may contribute to a better understanding of HD pathology and developing HD therapeutics.

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P. Neuroscience [P-38]

Ketamine-mediated changes in medial prefrontal cortex neurons abolished in GluN2D knockout mice

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Ketamine has recently emerged as a treatment for depressive disorder due to its fast-acting antidepressant effect in dose-dependent manner. It acts on the N-methyl-D-aspartic acid receptor (NMDAR) as a non-competitive antagonist, and previous study has shown that ketamine-induced hyperlocomotion is no longer present in GluN2D knockout (KO) mice, a subunit of NMDAR. Its physiological mechanism, however, is still elusive. Here, we examined whether ketamine induce physiological changes in medial prefrontal cortex (mPFC) involve GluN2D-containing NMDARs. When sub-aneesthetic dose of ketamine(25mg/kg) was injected to wild-type(WT) mice, the the frequency of spontaneouse excitatory postsynaptic currencs(sEPSC) was increased 1 hour after injection, which was not shown in GluN2D KO mice. On the other hand, changes in he amplitude of sEPSC and paired-pulse ratio(PPR) did not appear in both WT and GluN2D KO mice. Our data propose that ketamine-induced changes in glutamatergic neurons in mPFC via GluN2D-containing NMDARs presumably mediate ketamine-induced hyperlocomotion.

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P. Neuroscience [P-39]

In vivo study of the correlation between synaptic connections among engram cells and memory state.

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In neural networks, ensembles of synapses have a crucial role in cognitive function. Synapses between the CA3 and CA1 are strengthened after the contextual fear conditioning. These synaptic engrams show increased spine density and the larger spine size. Increased connectivity between hippocampal CA3 and CA1 is associated with memory intensity. In this study, we reveal the synaptic dynamics between the CA3 and CA1 by using the two-photon microscopy and in vivo dual-eGRASP technique. By tracing the identical dendrite, we showed that the connectivity between the engram neurons is increased through the synaptogenesis within the Schaffer collateral pathway after fear learning. However, the engram synapses between the CA3 and CA1 disappeared after extinction. We classified synapses into the newly-formed synapses and pre-existing synapses and observed the newly-formed synapses and pre-existing synapses of engram cells are clustered after fear learning. In conclusion, we suggest that the engram-to-engram synapse is a substrate for acquisition and elimination of fear memory.

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P. Neuroscience [P-40]

CBP-Mediated Acetylation of Importin α Mediates Calcium-Dependent Nucleocytoplasmic Transport of Selective Proteins in Drosophila Neurons

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Proteins must be properly located within cells to function correctly, and dysregulation of their nucleocytoplasmic transport (NCT) is linked to neurodegenerative diseases. It is unclear if a shared or selective pathway regulates NCT. Recently, we demonstrated that NCT of TDP-43 is controlled by the intracellular calcium-Calpain-A-Importin α3 signaling pathway in Drosophila neurons, which suggests that dysregulation of this intrinsic regulatory mechanism may lead to mislocalization of TDP-43 in ALS (Park et al., 2020). Here, we investigated that the molecular mechanism regulating NCT of truncated ataxin-3 (ATXN3) proteins, of which genetic mutation leads to a type of polyglutamine diseases, and compared it with that of TDP-43 to determine whether they are regulated in a commonly shared manner in *Drosophila* neurons. We found that truncated ATXN3 showed dynamic changes in subcellular localization during development, and neuronal toxicity occurred upon abnormal nuclear accumulation. NCT of ATXN3 was regulated by intracellular calcium levels and Importin α3, similar to TDP-43. However, NCT of ATXN3 was primarily mediated by CBP, which acetylates Importin α3 to regulate NCT, instead of Calpain-A. These findings demonstrate that CBP-dependent acetylation of Importin α3 is crucial for intracellular calcium-dependent NCT of ATXN3, which differs from TDP-43 regulation in *Drosophila* neurons.

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P. Neuroscience [P-41]

Study on mechanisms underlying recognition of acetic acid in C. elegans

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Animals detect and discriminate countless environmental chemicals for their well-being and survival. *C. elegans* has a highly developed chemosensory system with 32 chemosensory neurons that detect hundreds of water-soluble and volatile molecules (Bargmann, 2006). However, the mechanisms underlying odorant recognition and discrimination are not fully understood. Here, we found that *C. elegans* attracts to acetic acid in the chemotaxis assay and avoids acetic acid in the drop assay. To investigate neuronal mechanisms underlying the acetic acid response, we first examined whether sensory cilia are required for attraction to acetic acid. We found that the cilia-defective *che-2* and *che-3* mutants exhibit defects in acetic acid attraction. Interestingly, we found that pre-exposure to acetic acid appeared to decrease the glycerol avoidance, indicating that pre-exposure of acetic acid influences glycerol signal transduction. Currently, we are investigating which chemosensory neurons mediate attraction to acetic acid.

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P. Neuroscience [P-42]

GABAergic-like dopamine synapses in health and Parkinsonism

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Dopaminergic neurons exist in the midbrain and their axons establish synapses throughout the whole brain. Synaptic transmission at these synapses is crucial for volitional movement and reward-related behaviors, while dysfunction of these synapses causes various psychiatric and neurological disorders. Despite this significance, true biological nature of dopamine synapses remains poorly understood due to difficulties defining functional dopamine synapses at the molecular and physiological levels. Here we show that a significant portion of dopamine synapses are structured and function like GABAergic synapses with marked regional heterogeneity, which we call GABAergic-like dopamine synapses. In addition, GABAergic-like dopamine synapses show lower clustered patterns compared to conventional GABAergic synapses on striatal spiny projection neuron dendrites. Interestingly, 6 weeks knockdown of neuroligin-2, a key postsynaptic protein at GABAergic synapses, unexpectedly does not weaken GABA co-transmission but instead temporarily facilitates it at dopamine synapses in the striatal neurons. As expected, longer periods of neuroligin-2 knockdown (12 weeks) show significantly decreased GABA co-transmission. More importantly, the attenuation of GABA co-transmission precedes deficits in dopaminergic transmission and changes in dopamine synapses in animal models of Parkinsonism. Our findings reveal unknown spatial and functional nature of GABAergic-like dopamine synapses in health and disease.

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P. Neuroscience [P-43]

TET family proteins are dispensable for dopamine neurons in health and Parkinson's disease pathology.

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DNA undergoes demethylation via the oxidation of 5-methylcytosine (5mC), which is mediated by the Ten Eleven Translocation (TET) family of proteins. Notably, 5hmC is highly enriched in the brain than other tissues, the level of which is dynamically regulated during development, aging, and in brain disorders. In addition, accumulating evidence has recently revealed that 5-hmC and TETs play a significant role in synaptic functions, anxiety, addiction, and cognition in several brain regions. Furthermore, TET enzymes have turned out to be essential for diverse types of neurons in health and brain disorders. In this study, by generating triple knockout (TKO) mice of TET family proteins (TET1, 2, and 3) selectively in dopamine (DA) neurons, we investigated the functional roles of TET proteins in the structure and the function of DA neurons, which are pivotal for voluntary movement, reward-related behaviors, and motivation. Here we unexpectedly show that triple knockout (TKO) of all three TET proteins in dopamine (DA) neurons did not lead to significant alterations of neuronal structure and function in normal and pathological conditions. Thus, contrary to the previous reports, TET family enzymes may be dispensable for the structure and function of dopamine neurons in health and Parkinson's disease.

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P. Neuroscience [P-44]

Investigating the Role of the Ventral Hippocampus to Basal Amygdala in Contextual Fear Conditioning

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Contextual fear conditioning (CFC) is a widely used paradigm to study the association between novel stimuli and imminent stress, whereby an animal is exposed to a novel context paired with a foot shock. The hippocampus is a region for learning and memory which plays an important role in acquisition, encoding and consolidation during the association between spatial and fear-related information. While the dorsal hippocampus has been extensively studied, the role of the ventral hippocampus remains unknown. Previous studies have demonstrated the role of ventral CA1 (vCA1) to basal amygdala (BA) during fear learning. In this study, we focused on reactivating engram cells during CFC in vCA1 and BA. Our results showed that exposure to the novel context alone is sufficient to increase the activation of neurons in vCA1, whereas neurons in BA are only activated when mice receive foot shock. Additionally, we are optimizing anisomycin, a protein synthesis inhibitor, to investigate the necessity of synaptic strengthening between vCA1 and BA during fear learning.

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P. Neuroscience [P-45]

PLCγ1 in dopamine neurons critically regulates striatal dopamine release via VMAT2 and synapsin III

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Dopamine neurons are essential for voluntary movement, reward learning, and motivation, whose dysfunction is closely linked to various psychological and neurodegenerative diseases such as Parkinson's disease. Hence understanding the detailed signaling mechanisms functionally modulating dopamine neurons is crucial for the development of better therapeutic strategies against dopamine-related disorders. Phospholipase Cγ1 (PLCγ1) is a key enzyme in intracellular signaling that regulates diverse neuronal functions in the brain. It was proposed that PLCγ1 would be implicated in the development of dopaminergic neurons, while the physiological function of PLCγ1 remains to be determined. In this study, we found that cell type-specific deletion of PLCγ1 does not adversely affect the development and cellular morphology of midbrain dopamine neurons but does facilitate dopamine release from dopaminergic axon terminals in the striatum. This enhancement of dopamine release was accompanied by increased co-localization of vesicular monoamine transporter 2 (VMAT2) at dopaminergic axons. Notably, dopamine neuron-specific knockout of PLCγ1 also led to the heightened expression and co-localization of synapsin III which controls the trafficking of synaptic vesicles. Our findings suggest that PLCγ1 in dopamine neurons could critically modulate dopamine release at axon terminals by directly or indirectly interacting with synaptic machinery including VMAT2 and synapsin III.

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P. Neuroscience [P-46]

Two distinct modes of dopaminergic modulation on striatopallidal synaptic transmission

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Dopamine (DA) and its GPCR receptor control willed movement through D1-direct pathway and D2-indirect pathway within basal ganglia. Although striatopallidal synapses function as a critical gateway of indirect pathway, the physiological functions of dopamine on striatopallidal synapses remain unclear. Here, we seek to understand how DA through nigropallidal pathway modulates striatopallidal synaptic transmission. We revealed that DA is directly released onto the GPe and there is a marked regional heterogeneity of dopaminergic innervation to the GPe. In addition, we found that dopamine D2-like receptors modulate striatopallidal synaptic transmission via two distinct modes, canonical and non-canonical modes. As potential mechanisms behind distinct modes of dopaminergic modulation, we further found that D2 and D4 receptors in GPe subregions differentially regulate striatopallidal synaptic transmission through their differences in subcellular expression and sensitivity. To sum up, these results demonstrate that synaptic information conveyed by indirect pathway can be regulated by DA via two distinct modes, which seem to be determined by anatomical locations of striatopallidal synapses in the GPe. Since structural and functional organization of basal ganglia circuits is critical to understanding both DA-related behaviors, our findings will provide new insights into the overlooked role of dopaminergic modulation on striatopallidal synapses and globus pallidus.

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P. Neuroscience [P-47]

The Influence of Exercise Duration on Inhibiting Apoptosis in MCAo Rats

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Ischemic stroke, one of the world's leading fatal diseases, has a high recurrence and incidence that can lead to severe mortality and disability. In this study, we investigated whether exercise can treat ischemic stroke to prevent recurrence and improve functional impairment. Experimental cerebral ischemia was induced by middle cerebral artery occlusion (MCAo) in rats, and the effect of 10- or 30-minute training for two weeks was evaluated. Following cylinder and rota-rod behavioral tests, we found that motor function was improved compared to the non-exercise group. In addition, the brain infarct volume was decreased after exercise following TTC staining. Further examination of the cell signaling mechanisms involved in the improvement showed that the immune reactivity significantly decreased the expression of the pro-apoptotic protein, Bax, and increased that of the anti-apoptotic protein, Bcl-2. Our results suggest that exercise has a beneficial effect on ischemic stroke for short- or long-term training.

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P. Neuroscience [P-48]

Ethanol extract from Chrysanthemum zawadskii flowers restores inflammation-related neuronal dysfunction by inhibiting the secretion and activity of acetylcholinesterase in microglia

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Although Chrysanthemum zawadskii (CZ) has been widely used in traditional medicine to treat various inflammatory diseases, its effect on neuronal function remains unclear. Therefore, we investigated whether CZ extract had anti-inflammatory effects on microglia and whether these effects could restore inflammation-related neuronal dysfunction. Our data revealed that ethanol extract of CZ showed strong scavenging activity with on DPPH assay and on ABTS assay *in vitro*. In addition, in lipopolysaccharide (LPS)-stimulated murine microglial cell line BV2, CZ extract inhibited formation of reactive oxygen species and nitric oxide, and expression of iNOS and pro-inflammatory cytokines (e.g., IL-1β). Consistently, CZ extract reduced LPS-induced IκBα phosphorylation and nuclear localization of NF-kB, supporting that anti-inflammatory effects may be mediated via inhibition of NF-κB signaling. Interestingly, CZ extract reduced inflammation-induced acetylcholinesterase expression in BV2 cells and directly inhibited its activity *in vitro*. Moreover, three main components of CZ extract, quercetin showed the best anti-inflammatory and antioxidant effects, and linarin showed the strongest inhibitory effect on acetylcholinesterase activity. Furthermore, CZ extract improved cognitive performance in sleep-deprived Zebra fish. Together, these data suggest that CZ extract could reverse inflammatory neuronal dysfunction by alleviating inflammation-associated acetylcholinesterase activity in microglial cells, resulting in improved cognitive performance in brain.

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P. Neuroscience [P-49]

Suppression of LPS-induced iNOS expression in BV2 murine microglial cells by polydeoxyribonucleotide

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Microglia are innate immune cells of the central nervous system and their activation is essential to maintain homeostasis in the brain. Polydeoxyribonucleotide (PDRN), a natural DNA polymer derived from salmon sperm, has anti-inflammatory and anti-oxidant effects through the adenosine A2A receptor. In this study, we investigated PDRN's anti-neuroinflammatory effect by focusing its capability to inhibit lipopolysaccharide (LPS)-induced expression of inducible nitric oxide synthase (iNOS), an inflammatory enzyme, in BV2 cells, a mouse microglial cell line. Treatment with LPS at 1 μg/ml for 8 h up-regulated iNOS expression at the protein and mRNA levels in BV2 crlls. LPS treatment at 1 μg/ml rapidly induced the phosphorylation of JNK-1/2, ERK-1/2, PKB and ERK-5 in BV2 cells. Of note, treatment with PDRN at 100 μg/mL significantly diminished the LPS-induced iNOS expression and phosphorylation of JNK-1/2, PKB, and ERK-5 in BV2 cells. Distinctly, results of pharmacological inhibition studies revealed that treatment with SP600125, an inhibitor of JNK-1/2 or LY290412, an inhibitor of PI3K/PKB could suppress the LPS-induced iNOS expression in BV2 cells. Collectively, these results demonstrate that PDRN inhibits the LPS-induced iNOS expression in BV2 cells by inhibiting JNK-1/2 and PKB signaling pathways.

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P. Neuroscience [P-50]

Application of generative models to heterogenous transcriptome data of AD postmortem brain tissues

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The study utilized generative adversarial networks (GANs) to investigate the early molecular events in Alzheimer's disease (AD) model mouse data, resulting in successful GAN applications. With the development of NGS methods, efforts have been made to analyze bulk RNA-seq of human postmortem brain tissues for neurodegenerative diseases. However, due to the pathological complexity in dementia patients, only dominant common pathological pathways could be identified from heterogeneous NGS data. Therefore, we must provide solutions for two obstacles in applying GANs to human AD RNA-seq data. First, we have to determine the various subtypes in dementia data. Second, the GANs training procedure should be revised to capture heterogenous modes showing complex features for individuals. In this work, we modified the training procedure to show excellent learning performance in the public AD RNA-seq data of Mayo Clinic. Biclustering was used to classify various AD subtypes, and the robustness of biclustering was improved using an ensemble method. The findings contribute to understanding gene expression patterns and their functions and offer a new protocol for exploring heterogeneous human data.

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P. Neuroscience [P-51]

Targeting E3 Ubiquitin Ligase WWP1 rescues Alzheimer's disease through Destabilizing DVL2 via Inhibition of nogo-A-Linked Ubiquitination

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Nogo-A has been extensively demonstrated to play key roles in inhibiting central nervous system regeneration, regulating endoplasmic reticulum formation, and maintaining the integrity of the neuromuscular junction. In this study, an E3 ubiquitin ligase WWP1 was first identified to be a novel interacting partner for nogo-A both in vitro and in vivo. Without adequate treatment, pathological Alzheimer's disease atrophy induced by sustained pressure overload eventually leads to neuronal cell injury. WWP1 (WW domain-containing E3 ubiquitin protein ligase is an important regulator of aging-related pathologies, including cancer, cardiovascular diseases and Alzheimer's disease. However, the role of WWP1 in pressure overload-induced nerve tissue remodeling and brain injury is yet to be determined.To examine the correlation of WWP1 with Alzheimer's disease, we analyzed WWP1 expression in AD mice with brain injury by Western blotting and immunofluorescence imaging. Confocal was performed on WWP1-expressed mice to assess the role of WWP1 in Alzheimer's disease examined by ex vivo, and related cellular and molecular markers were examined. Confocal imaging and coimmunoprecipitation assays were conducted to identify the proteins that interacted with WWP1. Ubiquitination assay an in vivo mouse model was used to explore the mechanisms by which WWP1 regulates neuronal remodeling. Small hairpin RNA targeting WWP1 was administered to investigate its rescue role in 3x Tg AD animal mice. The WWP1 level was significantly increased in the Alzheimer's disease from mouse with Aβ and Tau mutation. The results of ex vivo and histology demonstrated that siWWP1 protected the AD brain from Aβ and Tau-induced brain injury. There was a direct interaction between WWP1 and nogo-A protein. nogo-A was stabilized by WWP1-mediated K27-linked polyubiquitination. The role of WWP1 in pressure overload-induced Alzheimer's disease was mediated by the DVL2/CaMKII/HDAC4 signaling pathway. This study provides rationales as well as a template nogo-A for further design of molecules to intervene in the WWP1-nogo-A interaction which may regulate the nogo-A protein level by controlling its ubiquitination for Alzheimer's disease.Therapeutic targeting WWP1 almost abolished siWWP1 induced Alzheimer's disease, suggesting WWP1 as a potential target for treating Alzheimer's disease and brain injury. We identified WWP1 as a key therapeutic target for pressure overload induced brain nerve tissue remodeling. We also found a novel mechanism regulated by WWP1. WWP1 promotes atypical K27-linked ubiquitin multichain assembly on nogo-A and DVL2 and exacerbates Alzheimer's disease atrophy by the DVL2/CaMKII/HDAC4 pathway.

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P. Neuroscience [P-52]

Intestinal CNMa peptide secretion stimulated by protein deficit activates two distinct pathways in the brain.

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Maintaining protein homeostasis is crucial for the survival and reproduction of animals. Although it is well established that taste receptors recognize dietary protein, it remains unclear how animals detect and respond to protein deprivation. A previous study reported that CNMa peptide is released from enterocytes in the Drosophila midgut during protein deprivation and guides animals to prefer essential amino acids (EAAs) over non-essential amino acids (NEAAs). However, the mechanism by which the CNMa signaling pathway influences the brain to increase protein-specific or EAA-specific appetite during protein starvation remains incompletely understood. Here, we identified two distinct brain regions expressing CNMa receptors that regulate protein or carbohydrate appetite to maximize protein feeding during protein deprivation. We demonstrate that CNMa signaling suppresses carbohydrate feeding by inhibiting glucose response of DH44 neurons in protein-starved flies. Meanwhile, a specific population of ellipsoid body (EB) neurons express CNMa receptor activated by CNMa peptide and increase protein appetite in protein-deprived flies. These findings reveal the interplay between the gut and the brain in nutrient-specific appetite regulation.

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P. Neuroscience [P-53]

CRBN attenuates the neuroprotective activity of Hsp40 chaperones and aggravates Parkinson's disease pathology.

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Cereblon (CRBN), a component of an E3 ubiquitin ligase complex, mediates polyubiquitination and proteasomal degradation of its endogenous substrates. CRBN is involved in multiple biological functions including metabolism, autoimmune and neuronal disorders, and arbitrates diverse effects of IMiDs by interacting with neosubstrates. Here, we identified the roles of CRBN in the progression of Parkinson's disease (PD) through the regulation of certain Hsp40 chaperones of class A and B. CRBN recruits these Hsp40 chaperones, ubiquitinate and destine them for proteasomal degradation. Subsequently, tangled forms of tau and amyloid proteins amass in the neuronal tissue resulting in the progression of PD. Knocking out *Crbn*, on the other hand, improves the availability of the targeted Hsp40 chaperones, reduces the chunks of tau and synuclein in the brains, and hence decreases the neuropathology of PD. LC-MS/MS data and computational analysis also confirm the accumulation of targeted Hsp40 chaperones in the brains of $Crbn^{-/-}$ mice as compared to $Crbn^{+/+}$ mice. We observed a positive correlation between CRBN and PD pathology utilizing cell-based and *in vivo* models of PD generated by methyl phenyl tetrahydropyridine and synuclein aggregates. Our findings highlight the therapeutic potential of CRBN for the management of PD symptoms.

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P. Neuroscience [P-54]

Regulation of Kv2.1 by TMEM178a in neurons

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The voltage-gated K+ channel Kv2.1 localizes to the soma, proximal dendrites, and axonal initial segment (AIS) in mammalian neurons and is clustered at somatic endoplasmic reticulum (ER)–plasma membrane (PM) junctions. $K_V2.1$ clusters mediate the ER Ca^{2+} uptake at the soma of neurons during the electrical activity or after ER Ca^{2+} depletion, but the mechanisms remain unknown. Here, we identified TMEM178a, a negative regulator of store operated Ca^{2+} entry, as a Kv2.1-associated protein using affinity immunopurification and mass spectrometry from rat brain membranes. We found that TMEM178a is an ER-resident transmembrane protein and is N-glycosylated at Asn158 in the predicted extra region. TMEM178a is complexed with Kv2.1 in heterologous cells and hippocampal neurons and its N-glycosylation is necessary for the association with Kv2.1. TMEM178a reduces the clustering of Kv2.1 in the soma and proximal dendrite, but not in AIS of hippocampal neurons. TMEM178a is complexed with STIM1 at basal conditions, but not with Kv2.1. ER Ca^{2+} depletion leads to the dissociation of TMEM 178a with STIM1, and Kv2.1 and to the formation of STIM1 puncta. Our data show that TMEM178a, an associated protein of Kv2.1, might regulate ER Ca^{2+} levels in neurons.

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P. Neuroscience [P-55]

Piezo1 transduces inflammatory pain signals in nociceptors

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Mechanosensation begins with the sensing of pressure by mechanically activated (MA) channels in the nerve endings of dorsal root ganglion (DRG) neurons. Piezo1, a fast-inactivating MA channel, has surfaced to be involved in pruriception. However, the pressure-dependent activation mechanism and its physiological role in mechanical pain remain unidentified. Here, we report that Piezo1 is expressed in a small DRG subpopulation, which is largely positive for TRPV1 rather than MRGPRD, which is known for nociceptors. To investigate the molecular function of Piezo1 in DRG neurons, we reclassified DRG neurons based on the MA current type. The silencing of the Piezo1 gene resulted in two subgroups—intermediately adapting (IA) and intermediately slowly adapting (ISA) responders of DRG neurons. Silencing Piezo1 in mice via specific lumbar DRG-targeted ganglionic injection of shRNA virus reduced tactile pain hypersensitivity in formalin- and carrageenan-dependent inflammation. Piezo1 mediates mechanical pain by acting as a nociceptive MA channel.

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P. Neuroscience [P-56]

Tentonin3/TMEM150C displays independently conserved mechanosensitive ion channel properties

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Tentonin3/TMEM150C (TTN3) is a mechanosensitive ion channel that portrays slowly-inactivating kinetic properties when upon mechanical indentations. The ability for TTN3 to respond from mechanical stimulation has involved with many physiological implications such as proprioception, baroreceptor reflex, and insulin release. Despite these profound findings, the molecular independence and ion channel nature of TTN3 has been challenged by the influence of Piezo1. Although the mechanosensitivity of TTN3 is recovered by application of F-actin polymerizing agent Jasplakinolide in Piezo1-ablated HEK293T (HEK-P1KO) cells, the clarification as an ion channel is still under debate. In order to elucidate ion channel characteristics, first, we managed to express different species of the vertebrate phyla of TTN3 in HEK293T cells and established mechanically activated inward currents. Second, from the species evoking the largest mechanosensitive currents, we were able to reveal single channel recordings of zebrafish (*Danio Rerio*) TTN3 sensitive to stretch and pharmacological inhibition. Lastly, to affirm the distinctive mechanosensitive ion channel characteristic of TTN3 from Piezo1, velocity-current mechanical stimulation and inhibitor experiments were executed. Taken together, the mechanosensitive ion channel TTN3 is responsible for slowly-adapting kinetics, which have been conserved within vertebrates, displays single channel conductance and biophysics different from the Piezo family.

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Q. Organoid [Q-1]

Quantitative and qualitative analysis of neurotransmitters and neurosteroids production from cerebral organoids during differentiation

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In human brain, neurophysiological activity is fulfilled by the movement of neurostransmitters and neurosteroids. So far, similarity between cerebral organoids and actual human brains are evaluated by sophisticated total omics analysis. However, systematic analysis of the production of neurotransmitters and neurosteroids are not yet fully reported. Here we carefully analyze the production of neurotransmitters and neurosteroids over the course of cerebral organoids differentiation. Our data is suggesting that the amount of neurotransmitters and neurosteroids as well as RNA and protein expression related to neurotransmitters and neurosteroids were increased as cerebral organoids matures. Increased electrophysiological activity of human cerebral organoids was also correlated to increased production level of neurotransmitters and neurosteroids. Our study demonstrated that expression levels of neurotransmitters and neurosteroids can be serve as key factors evaluating the maturity and functionality of human cerebral organoids.

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Q. Organoid [Q-2]

Development of human neuromuscular organoid for disease modeling and drug development

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Neuromuscular diseases such as amyotrophic lateral sclerosis are caused by selective death of motor neurons, but their precise etiology and treatment are yet to be discovered. Neuromuscular organoid derived from human induced pluripotent stem cells has the potential to be used as a disease modeling and drug screening platform for various neuromuscular diseases. Previously reported neuromuscular organoid has shown the formation of neurons, skeletal muscle tissue, and neuromuscular junctions, but most of the neurons failed to differentiate into motor neurons. Thus, we have set out to identify additional factors to improve the efficiency of neuromuscular organoid formation. In the current study, we found such a factor with a promoting activity of motor neuron differentiation during neuromuscular organoid formation. With the current modified protocol, neuromuscular differentiation was more efficient, evident by enhanced motor neuron-marker expression and contraction of skeletal muscle at earlier differentiation stages. In addition, the formation of bigger skeletal muscle tissue with synchronous contraction was also observed. The neuromuscular organoid generated by the optimized protocol from this study is a promising in vitro model for neuromuscular diseases by better recapitulation of motor neurons and skeletal muscle tissue.

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Q. Organoid [Q-3]

Study of Prmt1 function in cardiovascular diseases utilizing human iPSC-derived cardiac organoid

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Cardiovascular diseases are the leading cause of mortality worldwide. Upon stress or pathological conditions, cardiac cells undergo remodeling processes associated with inflammation, myocardial fibrosis, and hypertrophy which is one of the major risk factors for heart failure. Many studies led to identification of the key molecular mechanisms underlying cardiovascular function and pathogenesis. Protein arginine methyltransferase1 (Prmt1) is the major Prmt catalizing asymmetric dimethylation of arginine (ADMA) in substrates. The deregulation of Prmt1 is linked with elevated ADMA levels, an endogenous inhibitor of nitric oxide synthase associated with cardiovascular diseases, supporting for a potential pathological role of Prmt1. However, Prmt1 ablation in cardiomyocytes or vascular smooth muscle revealed its critical role in maintenance of cardiovascular function. In the current study, we set out to further investigate the role of Prmt1 in cardiac function using human iPSC-derived cardiac organoid as a model system. The effect of Prmt1 inhibition on the cardiac organoids was characterized via changes in contractile property, cell death, and stress response under pro-inflammatory or hypoxic condition which are the major drivers of cardiac failure in cardiovascular disease patients. Mechanistic studies will follow to reveal the role of Prmt1 in cardiovascular pathogenesis utilizing the human-derived cardiac model.

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Q. Organoid [Q-4]

Probiotics Lactobacillus regulates intestinal mucosal homeostasis by promoting IL-22-mediated epithelial proliferation and IL-10 production from stromal cells

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The regeneration of intestinal mucosal barrier are maintained by continuous differentiation and proliferation of intestinal stem cells (ISCs) under physiological and pathological conditions. However, little is known about the regulatory effect of intestinal microbiota on its ability to epithelial homeostasis and regeneration. In this study, we aimed to investigate the regulatory effect of microbiota on the proliferation of intestinal epithelial cells. We found that one of probiotics, *Lactobacillus Xa* stimulated epithelial cells to secret IL-22 through Nod-like receptor 2 (Nod2) and then induced phosphorylation of STAT3 to accelerate the production of anti-microbial peptides and the proliferation of intestinal epithelial cells, leading to epithelial regeneration. Furthermore, we found that *Lactobacillus Xa* stimulated IL-10 production from intestinal stromal cells of lamina propria through phosphorylation of STAT3, indicating that the probiotics might support the anti-inflammatory responses when the epithelial barrier is disrupted. Moreover, *Lactobacillus Xa* induced secretion of IL-10 from stromal cells to accelerate the proliferation of intestinal epithelial cells. Taken together, these results suggest a novel mechanism by which the intestinal microbiota can support the homeostasis and regeneration of intestinal epithelium.

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Q. Organoid [Q-5]

Establishing the therapeutic strategies for menopausal xerostomia using ovariectomized mice and salivary gland organoids

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Salivary gland (SG) dysfunction leads to xerostomia (dry mouth). Importantly, the prevalence of xerostomia is high in elderly women, implying the correlation between salivary function and estrogen. To understand the etiology of post menopause-associated xerostomia, we investigated the functional alteration of the salivary gland (SG) of ovariectomized (OVX) mouse model. Notably, OVX leads to SG dysfunction with reduced salivary flow, and TGF-β expression in the SG was significantly upregulated after OVX. Next, established mouse and human salivary gland organoids (SGOs) were able to maintain stably and differentiate into mature cells. We found that TGF-β could increase the susceptibility of SG cells against oxidative stress, partially via induction of ferroptosis. To downregulate the oxidative stress in OVX-SG, we administrated extracellular vesicles (EV) isolated from mesenchymal stem cells overexpressing SOD3, one of antioxidant enzymes. Interestingly, treatment of EV-SOD3 could restore the salivary flow rate in OVX mice. These findings suggest a novel insight regarding the pathogenesis and effective treatment strategy of menopausal xerostomia.

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Q. Organoid [Q-6]

Three-dimensional lung tissue inflammatory model

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Three-dimensional (3D) culture system has attracted a great attention since it offers advantages over conventional two-dimensional (2D) culture and animal use. Epithelial cells form continuous layers covering the surface of the airways and alveoli. Basement membrane, which lies underneath epithelial cells, serves as supporting extracellular matrix. To mimic this *in vivo* structure, we utilized 3D co-culture-based model on transwell. Yet instead of commercial polycarbonate membrane, we employed water-tolerant electrospun Poly(vinyl alcohol) (PVA) nanofiber membrane for insert well. PVA was blended with laminin-derived peptides for stronger cell attachment. Lung epithelial cells (MLE-12) were cultured on insert well, fibroblasts as feeder cells were grown on lower chamber. Zonula occludens-1, a tight junction protein, distributed evenly on cells cultured on peptide-blended PVA. The focus stacking result revealed MLE-12 cultured on peptide-blended PVA still remained *in vivo*-like morphology, whereas cells cultured on polycarbonate membrane had 2D-like morphology. To examine the validation of the model, we added bone marrow-derived dendritic cells on lower chamber, followed by challenges. The *ex vivo* model showed more efficiency in the production of pro-inflammatory cytokines compared to 2D culture, suggesting potential application in the field of infectious lung diseases.

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Q. Organoid [Q-7]

Establishment of patient-derived brain tumor organoids with tumor microenvironment

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Despite of remarkable advances in brain tumor research in the past decades, little has changed for patients due to a lack of clinically relevant research models. Current 2D cell lines or organoids cannot reflect biological features of patient tumors since they undergo transformation along culture and consist of only tumor cells without microenvironment. Here, we established brain tumor organoids from patients with meningioma, glioblastoma, and gliosarcoma. Exclusion of enzymatic dissociation-reaggregation steps endowed brain tumor organoids with original histology and tumor microenvironment. We used a liquid media culture system instead of embedding samples into Matrigel, resulting in an easy-to-handle, cost-efficient, and time-saving system. Brain tumor organoids maintained their functionality and morphology after long-term culture and repeated cryopreserving-recovery cycles. The similarities between organoids and their corresponding parental tumors were confirmed by both immnohistochemistry and whole-exome sequencing. Finally, we demonstrated the utility of these organoids as drug screening platforms for predicting therapy response to anti-tumor therapies, including chemotherapy and immune-cell therapy. Taken together, our organoid model overcame limitations of previous research models for studying brain tumor and showed superior resemblance to parental tumors. Thus, our model could facilitate translational research identifying and selecting drugs for brain tumor in the era of precision medicine.

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Q. Organoid [Q-8]

Label-Free 3D High-resolution Analysis of Cellular Differentiation in Live Organoids Using Low-Coherence Holotomography

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Three-dimensional (3D) visualization of live organoids is essential for understanding the physiological functions and cell type diversity. Conventional 3D imaging techniques based on fluorescence have limitations in long-term non-invasive observation. Holotomography offers a potential solution of 3D live organoid imaging by exploiting the refractive index (RI) of a sample as an intrinsic imaging contrast. The dry mass and volumetric information of organoid were measured by translating RI information.

We employed a low-coherence holotomography imaging system to examine the morphological features of early-stage murine small intestine organoids. We acquired 3D RI tomograms of live organoids embedded in Matrigel for a duration of 120 hours, revealing the early differentiation of small intestine organoids, including the formation of a central cyst structure and crypt-like budding structures. The differentiation of enterocytes, goblet cells, and Paneth cells was distinctly identified through the marker-free observation of subcellular structures such as secretory vesicles and granular structures. Furthermore, we observed cellular dynamics, such as mitotic cell division and the translocation and chromatin condensation of apoptotic cells. We also analyzed the volume and dry mass of individual organoids, exhibiting fluctuations due to size oscillation and organoid fusion.

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R. Plant Biology [R-1]

Bioactivity and skin recovery effects of Xanthium strumarium L. fruit extract in human dermal cells and skin reconstructed model

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Xanthium strumarium L. (XS) fruit, as known as cocklebur, has been used as traditional herbal medicine in Asia. The aim of this study is to investigate bioactivity such as anti-oxidant and anti-inflammation and skin protective and recovery properties of XS fruit from Korea (XS-K) and China (XS-C) in human dermal cells, co-culture cells, and skin reconstructed human epidermis (RHE) models. XS-K showed slightly higher anti-oxidant activity than XS-C. XS-K showed slightly higher lipoxygenase inhibition, *i.e.*, more anti-inflammatory activity, than XSE-C. Moreover, XS-K increased cycloocygenase-2 at the molecular level. Treatment with XS-K and XS-C showed skin recovery potential by increasing hyaluronic acid content in dose-dependent manners. In addition, both XS-K and XS-C increased mitochondrial membrane potential in a dose-dependent manner in UVB-irradiated cells. Both XS extracts stimulated mRNA level of collagen but inhibited mRNA level of MMP-1. XS-K showed more effective wound closure rate than XS-C in all models. In conclusion, both XS fruits from Korea and China had anti-oxidant and anti-inflammatory activities, accounting, in part, for protection of mitochondrial membrane potential against UVB-induced apoptosis. Moreover, XS-fruits showed production and degradation of hyaluronic acid and collagen at molecular levels and rapid wound recovery in human dermal cells and RHE models.

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R. Plant Biology [R-2]

Optimization of enzyme assisted extraction of protein from the ginger (Zingiber officinale) leaves for alternative plant protein concentrate

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In this study, enzyme-assisted extraction (EAE) conditions for the yield of proteins from ginger (*Zingiber officinale*) leaves were studied using a three-factor Box-Behnken response surface design. Process parameters affecting the efficiency of EAE, such as sample size (10, 30, and 100 mesh), time (30, 60, and 120 min) and amount of enzyme relative to substrate (0.1, 0.5, and 1.0%), were investigated. The experimental data obtained are high coefficient of determination (R2) 0.9726, 0.9713, and 0.9829. An optimization study using Derringer's desired function methodology was performed, and a combination of individual variables and all independent variables (sample size 100 mesh, time 60 minutes, and enzyme amount 1.0%) was determined as the maximum protein yield of 16.46%, which was confirmed through validation experiments. As a result of the current investigation, the protein content under the optimized conditions was in the order of Mix (Alcalase:protamex, 1:1) > Alcalase > Protamex. Our study provides basic data for the efficient extraction and utilization of ginger leaf low-molecular protein.

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R. Plant Biology [R-3]

Production of active Exendin-4 in Nicotiana benthamiana and its application in treatment of type-2 diabetics

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GLP-1 (Glucagon-like peptide-1) is a peptide that stimulates insulin secretion from the β-cell for glycemic control of the plasma blood glucose level. Its mimetic exenatide (synthetic Exendin-4) with a longer half-life of approximately 3.3–4 h is widely used in clinical application to treat diabetes. Currently, exenatide is chemically synthesized. In this study, we report that the GLP-1 analogue recombinant Exendin-4 (Exdn-4) can be produced at a high level in Nicotiana benthamiana. For high-level expression, we generated a recombinant gene, B:GB1:ddCBD1m:8xHis : Exendin-4 (BGC : Exdn-4). The GB1 domain (B1 domain of streptococcal G protein) was used to increase the expression, whereas a double cellulose binding domain 1 (CBD1), and 8 His residues were included as affinity tags for easy purification. BGC : Exdn-4 was purified by single-step purification to near homogeneity using both Ni resin and microcrystalline cellulose (MCC) beads. Moreover, Exdn-4 without any extra residues was produced from BGC : Exdn-4 bound onto MCC beads by treating with enterokinase. Plant-produced Exdn-4 (Exendin-4) was as effective as chemically synthesized Exendin-4 in glucose-induced insulin secretion (GIIS) from mouse MIN6m9 cells a pancreatic beta cell line.

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R. Plant Biology [R-4]

De novo transcriptome assembly and proteome profiling of the recently formed allopolyploid Tragopogon mirus (Asteraceae) and its diploid parents

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Polyploidy (whole-genome duplication) is recognized as an important evolutionary process in speciation and genome evolution of diverse organisms, particularly plants. However, much of our current understanding of polyploidy is based on analyses of crop species. Here, we examined the transcriptomes of naturally occurring, recently formed, allopolyploid *Tragopogon mirus* and its diploid parents (*T. dubius* and *T. porrifolius*) using Illumina technology. In parallel, we employed iTRAQ LC-MS/MS to investigate the global proteomes of the three species. A total of 480 million 100-bp paired-end reads was generated from leaf transcriptomes of the three species, which corresponds to 34 Gb of sequence. These reads were assembled *de novo* by the Trinity short-read assembler, and this assembly was utilized as a reference for RNA-Seq and proteomic data analyses. Differential gene expression between the allopolyploid and its diploid parents was analyzed and compared at transcriptomic and proteomic levels. This study will provide valuable insights into transcriptomic as well as proteomic changes in recently formed allopolyploids. In addition, the transcriptome data set generated here provides the most comprehensive sequence resource for the *Tragopogon* polyploid system, which enables further studies of gene and protein expression patterns in different tissues and under different conditions.

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R. Plant Biology [R-5]

Optimization of processing technology of Gardeniae Fructus roasted with ginger and its effect on liver protection on 1-naphthyl isothiocyanate-induced cholestasis hepatitis in rats and the AMPK/ PGC1α/p38 MAPK signaling pathway in C2C12 myotubes

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In this study, it was optimized the processing technology of Gardeniae Fructus (fruit of *Gardenia jasminoides* Ellis, Rubiaceae; GF) roasted with ginger (GG) and investigated the hepatoprotective effects in rats before and after processing. We prepared 1-naphthyl isothiocyanate (ANIT)-induced cholestasis hepatitis rat model and carried out the pharmacodynamics analysis of GG extract under the optimal process conditions. We also compared the regulatory effects of GG extract on the AMPK/ PGC1α/ p38MAPK signal pathway in C2C12 myotubes before and after processing. Our results obtained the optimal processing technology of GG as follows: the ratio of ginger to raw GF is 8:1, and the processing condition is at 208°C for 5 minutes. The GG extract protected ANIT-induced damage of liver tissues in rats, and also regulated the AMPK/ PGC1α/ p38 MAPK signaling pathway in muscle cells. These effects were further enhanced after processing GG extract. Therefore, processing of GG extract can help to improve the pharmacodynamics property.

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S. Protein Modification and Regulation [S-1]

Phosphorylation of mitochondrial creatine kinase tyrosine residues rescue from cardiac ischemic injury

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Ischemic cardiomyopathy (ICM)-related heart failure is one cause of cardiovascular disease-related deaths. Ischemic preconditioning (IPC) was found to effectively rescue ICM. IPC occurs when short-time ischemia is applied before ischemia/reperfusion injury (I/R). This application may contribute to rapid changes in protein expression and regulation emphasizing protein function modulation by post-translational modifications. Mitochondria is vital in heart disease progression and is promising target for ICM treatment. We focused on mitochondrial creatine kinase (CKMT2) under I/R injury. We focused on mitochondrial creatine kinase (CKMT2) under I/R injury. E*x vivo* Langendorff system using Sprague-Dawley rat hearts simulated normal perfusion, I/R, and IPC conditions. Samples were subjected to phosphoproteomic analysis. Human cardiomyocyte AC16 cells were used to investigate CKMT2's role through overexpression and how site-directed mutagenesis of CKMT2 phosphorylation sites affects cardioprotection. CKMT2 phosphomutations decreased cell viability, ATP and mitochondrial membrane potential during normoxia and hypoxia while CKMT2 overexpression improved cell conditions. CKMT2 overexpression increased proteins regulatinmitochondrial function which led to cardiac cell protection during H/R in an *in vitro* setting while mutated CKMT2 impaired mitochondrial function proteins. Our study offers a different perspective on cardioprotection during I/R injury through a novel target rather than the conventional method of targeting infarct size reduction.

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S. Protein Modification and Regulation [S-2]

Interaction of KCNQ4 C-terminus and HAP1 regulates surface expression and channel current of KCNQ4

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K^+ homeostasis is essential for auditory function, and KCNQ4 plays a crucial role in K^+ recycling. In this study, we hypothesized that other proteins interact with KCNQ4 C-terminal tail, hence modulating its activity. To find KCNQ4 interactors, we performed Y2H screening with a murine adult inner ear cDNA library as the prey and the C-terminal tail of KCNQ4 as the bait. In addition to known interactor CaM, HAP1 and MMP14 were identified as novel interactors. The direct interaction was confirmed by co-immunoprecipitation and GST pull-down assay. In electrophysiology experiment, we found that the overexpression of HAP1 only decreased KCNQ4-mediated currents. Through surface biotinylation assay, we determined that HAP1 overexpression decreased the surface KCNQ4 expression. Followed endocytic assay confirmed that when HAP1 was co-expressed, time-dependent endocytosis of KCNQ4 did not occur. To determine if HAP1 participates in competitive inhibition of KCNQ4 with CaM, we measured KCNQ4 surface expression under HAP1 and CaM overexpression. When HAP1 was overexpressed alone, the surface expression of KCNQ4 decreased considerably, however the level of downregulation was much milder when CaM was co-expressed with HAP1. These results indicate that HAP1 modulates surface KCNQ4 via interacting with the C-terminal tail of KCNQ4 in the inner ear.

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S. Protein Modification and Regulation [S-3]

The Cys/N-degron pathway mediates inflammation via oxidative stress-induced secretory autophagy

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Cell-to-cell communication is mediated by the secretion of signal-conveying proteins and factors via either the ER-Golgi pathway in basal conditions or secretory autophagy in stress conditions. Under oxidative stress, pro-inflammatory cytokines are exocytosed via secretory autophagy, the molecular mechanisms and key regulators of which have not yet been fully elucidated. In this study, we report that the Cys/N-degron pathway mediates oxidative stress-induced secretory autophagy for inflammatory signaling. The oxidized Nt-Cys-degron of cytokines undergoes ATE1-dependent Nt-arginylation and binds the ZZ domain of the autophagic cargo receptor/N-recognin p62/SQSTM1. Such interaction conformationally and biologically activates p62 in tandem with the multivesicular body-associated CD63, mediating amphisome biogenesis and exosome secretion for Nt-Cys-cytokine release. We developed antagonistic ligands to the p62-ZZ domain, which efficiently inhibited N-degron-dependent secretory autophagy in *in vitro* and organoid models of paracrine inflammation. Overall, our results implicate the Arg-Cys/N-degron pathway as critical for secretory autophagy and inflammatory signaling under oxidative stress.

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S. Protein Modification and Regulation [S-4]

The Arg/N-degron pathway regulates protein ISGylation

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The post-translational modifications of intracellular proteins play a critical role in determining their functions. These processes including SUMOylation, NEDDylation, and ISGylation mediate various metabolic processes such as protein degradation and signal transduction. Among these modifications, ISGylation in which the ISG15 protein binds to substrates is rarely elucidated despite its structural similarity to ubiquitination. In this study, we investigated the mechanism of ISGylation mediated by the Arg/N-degron pathway. ISGylation of global proteins occurred in ATE1 dependent manner. Additionally, the study suggested the E3 ligases ARIH1 and TRIM25 as potential mediators. These E3 ligases contain a ZZ-like domain, which can recognize N-terminal arginine, and they were confirmed to be bound to the N-terminal arginine-mimicking compound. Therefore, it is demonstrated that these E3 ligases may mediate ISGylation by recognizing N-terminal arginine as N-recognin. Overall, this study provides important insights into the mechanisms of ISGylation, which may have significant implications for cellular processes including the immune system and cell survival.

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S. Protein Modification and Regulation [S-5]

The Cys/N-degron pathway mediates proteolysis of muscle actins

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Skeletal muscle-wasting diseases lead to poor quality of life and therefore maintaining muscle homeostasis is important for the well-being of patients. Actin discovered and studied 70 years ago, is one of the most abundant proteins in eukaryotic cells and comprises 15% of total cellular proteins by weight in muscle cells. However, the metabolic turnover of skeletal actin and its contribution to muscle homeostasis, especially in disease settings, have yet to be fully elucidated. N-degrons, especially Arg (R)-degrons, are a recognition signal of both UPS and autophagy pathways and play an important role in the cellular protein degradation system. N-terminal Cys was known as the target of arginylation-dependent degradation in the N-degron pathway but substrates with Cys2 were not well investigated in mammals. In this study, we show that N-terminal Cys carrying isoforms of muscle actin are not only degraded via the Cys/N-degron pathway but also generate R-C^{ox} degrons that act as key determinants in p62-dependent musclephagy, paving the way for pharmacological modulation of the Arg/N-degron pathway to combat muscle wasting dystrophies. A better understanding of Arg/N-degron-dependent degradation of muscle actin will enable the development of promising therapeutic targets for muscle wasting diseases.

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S. Protein Modification and Regulation [S-6]

The Cys/N-degron pathway regulates pexophagy through the N-terminal arginylation of ACAD10

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Peroxisomes play a key role in regulation of dynamic changes in ROS levels via ROS scavenging and production. While producing and detoxifying ROS, peroxisomes are prone to damage, necessitating their timely turnover via autophagy. However, the regulation mechanism of pexophagy under oxdiative stress is largely unknown. In the Arg/N-degron pathway, N-recognins recognize cognate substrates for degradation via the ubiquitin (Ub)-proteasome system (UPS) or the autophagy-lysosome system (hereafter autophagy). Here, we show that the N-degron pathway mediates pexophagy under both normal and oxidative stress conditions. This degradative process intiates when the Nt-Cys of ACAD10 (Acyl-CoA dehydrogenase family, member 10) is oxidized into Cys sulfinic (Cys^{O2}) or sulfonic acid (Cys^{O3}) by cysteamine (2-aminoethanethiol) dioxygenase (ADO). Under sodium nitroprusside (SNP) induced oxidative stress, the Nt-Cys of ACAD10 is chemically oxidized by reactive oxygen species (ROS). These oxidized Nt-Cys is arginylated by arginyltransferase 1 (ATE1), generating the $R\text{-}C^{OX}$ N-degron. $R\text{-}C^{OX}$-ACAD10 marks the site of pexophagy and binds the ZZ domain of p62, recruiting $LC3^{+}$-autophagic membranes. Our results demonstrate that the Cys/N-degron pathway generates an N-degron that regulates pexophagy.

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S. Protein Modification and Regulation [S-7]

YTHDF2 promotes aggresome formation independent of m6A modification

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N6-methyladenosine (m^6A) modification is the most abundant internal RNA modification in eukaryotic cells [1]. YT521-B homology domain family 2 (YTHDF2) protein binds to m^6A-modified mRNA to induce mRNA decay by recruiting CCR4-NOT deadenylase complex [2]. YTHDF2 governs stability of m^6A-modified mRNA through binding to m^6A modification [3]. However, m^6A independent role of YTHDF2 is not studied yet. Here we report that m^6A independent role of YTHDF2, promotes aggresome formation: microtubule-dependent cytoplasmic inclusion body for protein degradation when ubiquitin–proteasome system is impaired or overwhelmed [4]. YTHDF2 leads to efficient aggresome formation through UPF1 binding which is a positive regulator of aggresome formation, and that is not dependent of m6A modification. We also find that YTHDF2 promotes interaction between misfolded proteins and dynein, a motor protein dependent on microtubule, to transport misfolded proteins to aggresome efficiently. We show that YTHDF2 increases the movement of misfolded proteins as well as the circularity of aggresomes using single molecule imaging. Collectively, our data provide compelling evidence that YTHDF2 is a cellular factor that is involved in protein quality control.

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S. Protein Modification and Regulation [S-8]

Cullin-RING E3 ubiquitin ligases are differentially deneddylated by COP9 signalosome subunit 5

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Cullin-RING E3 ubiquitin ligases (CRLs) mediate the proteasomal degradation of various cellular proteins on the subject of cell cycle control, DNA replication, and genome stability. Activation of CRLs is regulated by neddylation through NEDD8-activating, conjugating and attaching enzymes to C-terminal of scaffold cullins (CULs). In contrast to neddylating process, CRLs are also inactivated by deneddylation, occurred by COP9 signalosome (CSN). Here, we found that the deneddylation speed of each CUL is differentially regulated by pevonedistat, a small molecular inhibitor of NEDD8-activating enzyme (NAE). Dose- or time-dependent treatment of pevonedistat revealed rapid deneddylation in most of CRLs including CUL1, CUL3, CUL4A/B and CUL5 whereas neddylated CUL2 is slowly converted to deneddylation form. We showed that the different deneddylation speeds of each CUL were highly correlated with binding strength on CSN5, catalytic core of CSN complex. Immunoprecipitation analysis showed that binding affinity of CUL2 to CSN5 is lower than other CULs. Consistently, Cells released from the CSN5 inhibitor treatment showed that most CULs are rapidly deneddylated, while CUL2 is slowly deneddylated to inactive form. These results provide dynamic insights about differential conversion mechanisms of each CUL deneddylation by CSN complex.

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S. Protein Modification and Regulation [S-9]

Mechanisms of mRNP granule formation in non-dividing and chronologically aged Saccharomyces cerevisiae

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Saccharomyces cerevisiae **forms cytoplasmic messenger ribonucleoprotein (mRNP) granules known as p-body (PB) and stress granule (SG) in response to various stresses. Especially, chronological aging (CA) influences on condensation of PB and SG in *S.Cerevisiae*. But we still don't understand the mechanisms of mRNP granule formation induced by aging stress. In this study, we identified the dynamic changes of SG or PB components induced by CA. Nst1 forms foci from diauxic shift in the PBs and are colocalized with the foci of Dcp2, a major component of PB. We discovered the molecular weight of Dcp2-Egfp is shifted in early stationary phase, suggesting its modification. Also, the physical characteristic of Dcp2-Egfp is changed when cells are under aging stress. It suggests the condensates like PBs become insoluble aggregates during CA. It is known that cell growth defect is induced when overexpressed Nst1 forms condensates. However, the growth defect induced by Nst1 overexpression is suppressed when TIF4631, a SG component, is knocked out. We discovered DCP2-EGFP foci formation induced by Nst1 overexpression is reduced in this TIF4631 deletion mutants. Altogether, our observations suggest that CA may induce the changes in dynamics of PBs and their close connections to SGs.**

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S. Protein Modification and Regulation [S-10]

Digital Degron: A Potential Therapeutic Strategy for Breast Cancer Treatment via Autophagy Induction

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Breast cancer is a complex disease that is difficult to treat, and new therapeutic strategies are needed. In this study, we investigated the role of autophagy induced by glucose deprivation in breast cancer growth. Our results demonstrated that autophagy inhibits breast cancer growth. Furthermore, we identified that JHDM3A regulates stability of Estrogen-related receptor α (ERRα), a transcription factor that promotes breast cancer metastasis, by reducing the methylation of it. Methylation of ERRα induces phosphorylation, leading to degradation. We also found that glucose starvation lowers the expression of JHDM3A in mRNA expression level. Additionally, we observed that glucose starvation up-regulates miR137, the repressor of JHDM3A, and down-regulates JHDM3A. We termed the phenomenon in which methylation leads to a phosphor-degron a digital degron. Based on our findings, we suggest that a novel therapeutic strategy involving the use of glue that induces digital degron may be effective in breast cancer treatment. In summary, our study provides new insights into the complex regulatory mechanisms underlying breast cancer growth and identifies potential therapeutic targets for the treatment of this disease.

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S. Protein Modification and Regulation [S-11]

ROS inhibits tumor suppressor degradation by decreasing the activities of PRMT5 in liver cancer

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Retinoic acid receptor–related orphan receptor α (RORα) is a transcription factor known as a tumor suppressor. Contrastively, Protein arginine methyltransferase 5 (PRMT5) symmetrically dimethylates arginine residues and is regarded as a potential oncogene. Arginine methylation can influence the function and fate of the substrate protein.

Our Findings demonstrate that PRMT5 methylates RORα, and then regulates the interaction between the E3 ubiquitin ligase ITCH and RORα. Namely, PRMT5 can methylate the R37 residue in RORα, causing RORα degradation through poly-ubiquitination. This degradation ultimately causes reduced activation of downstream target genes.

Interestingly, reactive oxygen species (ROS) induced by H2O2 treatment decreased PRMT5 levels and activity, increasing RORα protein levels in HepG2 liver cancer cells. Furthermore, ROS inhibited cancer progression by inducing apoptosis via PRMT5-RORα-ITCH axis.

Our data provide evidence that arginine methylation–dependent degradation of RORα is carried out by PRMT5 and that PRMT5 may serve as a potential target for cancer therapy. In addition, our results suggest that the anti-tumor effect of excessive oxidative stress in liver cancer might be due to PRMT5 dysfunction. We plan to explore this possibility further by monitoring changes in the methylation of other PRMT5 targets, such as p53 and H3R8, following ROS generation.

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S. Protein Modification and Regulation [S-12]

Regulation of auto-deubiquitinating activity of USP35 is required for the mitotic progression

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USP35 plays various roles in cellular functions such as mitophagy, endoplasmic reticulum stress, and mitosis.. However, there are not many studies on the relationship between the regulation of USP35 activity and function. In this study, we show that USP35 forms a homodimer by interaction between its C-terminal domains and exhibits its enzymatic activity through the dimeric state. Indeed, USP35 has no enzymatic activity when it has only a catalytic domain. In addition, USP35 homodimer formed through the C-terminal domain prevents degradation of USP35. CHIP bound to HSP90 interacts with and ubiquitinates USP35. The ubiquitin chain attached to USP35 by CHIP is cleaved by auto-deubiquitination of fully active USP35. USP35 dimer with full activity could deubiuqitinate Aurora B, resulting in the regulation of normal mitotic progression. We believe that our study, which identify the unique homodimeric structure of USP35, the regulation of USP35 activity through it, the auto-deubiquitination function of USP35, and the E3 ligase involved in the degradation of USP35, can help understand the function of USP35.

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S. Protein Modification and Regulation [S-13]

USP14 modulate cancer cell growth independently of fatty acid synthase regulation

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Fatty acid synthase (FASN) is an important enzyme in de novo lipogenesis and is overexpressed in many cancers, playing a critical role in cancer progression by providing a source of excess lipids. Therefore, FASN is an attractive target for cancer therapy. However, FASN inhibitors alone did not show significant anti-cancer effects despite the development of various drugs that inhibit FASN. This study aimed to investigate the potential synergistic effect on cancer cell death by simultaneously inhibiting FASN and USP14, a protein known to maintain FASN levels in hepatocytes. However, co-inhibition of FASN and USP14 did not significantly reduce cancer cell growth compared to inhibition of FASN alone. Contrary to hepatocytes, USP14 rather reduced the protein levels and activity of FASN in cancer cells, although it slightly inhibited the ubiquitination of FASN. In addition, metabolite analysis revealed significant differences between cancer cell treated with the FASN inhibitor and the USP14 inhibitor. These findings suggest that USP14 may not directly target FASN in cancer cells, and contribute to cancer cell proliferation independently of FASN. In conclusion, combining with FASN inhibitors withUSP14 inhibitors may not be an effective anti-cancer strategy.

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S. Protein Modification and Regulation [S-14]

p62-mediated autophagy is essential for coagulation step at wound healing

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Wound is generated by various reasons including injuries, diseases, and surgeries. Preventing further damages caused by the destruction of barrier, the wound healing process is essential. Three steps of wound healing are inflammation, proliferation, and remodeling. At initial step of wound healing, inflammation step, macrophages are recruited and induce inflammation which activates platelet-mediated coagulation. Coagulation can block the wound sites and protect them from infection and rotten. Here we investigated that p62 mediated coagulation at wound. The activation of p62 by autophagy targeted ligand (ATL) accelerated the formation of platelet clots. The ligand also induced autophagy via forming oligomeric bodies implying that p62-mediated sequestration and degradation of targets can promote coagulation during the wound-healing process. Moreover, ATL upregulated pro-inflammatory cytokines such as TNF-a, IL-1, and IL-6 which are associated with platelet recruitment. Overall, this poster highlights the potential for ATL to be utilized in the treatment of wound healing and related injuries.

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S. Protein Modification and Regulation [S-15]

Screening of Anti-RUFY4 (RUN and FYVE domain-containing protein 4) antibodies by phage display technology

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The regulation of osteoclast formation and function is important role in bone restruction and homeostasis. RANKL signalling is essential for the differentiation of osteoclast, and excessive secretion of RANKL can cause many bone-related diseases such as osteoporosis, rheumatoid arthritis and bone metastasis of cancer. RUFY4 is one of the genes induced by RANKL which controls autophagic flux by inducing tethering with autophagosome formation and lysosome. Autophagy dysregulation increases bone loss associated with alteration of osteoclast function and progression of osteoporosis. Therefore, in order to maintain the normal formation and activation of bone cells, autophagy needs to be properly regulated, and tools for the sensitive and specific detection and analysis of RUFY4 are essential for the investigation of autophagy. Here we discovered antibodies that specifically recognize RUFY4 from a human scFv phage library by phage display technology and ELISA screening. An anti-RUFY4 scFv that showed specific binding to murine RUFY4 in immunoblotting assay was converted to immunoglobulin G (IgG) format, expressed in mammalian cells (ExpiCHO-S), and purified. Research involving RUFY4 has been hampered by the lack of high-quality antibodies, and the newly discovered anti-RUFY4 antibody is expected to provide a viable tool for the reliable detection and quantification of RUFY4.

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S. Protein Modification and Regulation [S-16]

Investigating a role of OTUD5 deubiquitinating enzyme at stressed replication forks through regulating FACT histone chaperone

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Proper regulation of replication fork progression is important for genomic maintenance. Subverting the transcription-induced conflicts is crucial in preserving the integrity of replication forks. Various chromatin remodelers, such as FACT histone chaperone and histone deacetylases (HDACs) are known to modulate replication stress. However, how these factors are organized or collaborate is poorly understood. Here, we found that OTUD5 form a complex with FACT to regulate transcription elongation near replication fork. We found that OTUD5 is recruited to forks and interacts with SPT16 which is a component of FACT. Furthermore, we identified that OTUD5 binds to SPT16 through C-terminal disordered region. Depletion of OTUD5 or expressing an interaction-deficient mutant OTUD5 increases FACT-dependent transcription elongation at replication forks resulting in genomic instability. In addition, proteomic analysis showed that HDAC1 and HDAC2, which regulate H4K16 acetylation, are associated with OTUD5, suggesting that OTUD5 coordinates histone acetylation near replication fork to regulate transcription elongation. Consistently, HDAC1 or HDAC2 depletion activates transcription near replication fork. Altogether, OTUD5, FACT and HDAC1/2 form a complex to negatively regulate the FACT-dependent Pol II elongation. Finally, cell line with high FACT activity requires DNA repair pathway. This study identified a new regulation that limits transcription-induced replication stress.

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S. Protein Modification and Regulation [S-17]

AKT and PTF1a signaling regulates development of the GABAergic neurons in the cerebellum

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Akt exerts its effects in the cell by phosphorylating a variety of downstream substrates in both cytoplasm and nucleus. All 3 members of Akt family are expressed in the developing central nervous system (CNS) and are involved in the neural development. Although active Akt promoted neuronal progenitor cells into GABAergic neurons but not glutamatergic neurons in the telencephalon, the roles and molecular mechanisms of Akt signaling in the cerebellar development remain to be determined. In this study, we proposed that PTF1a, a bHLH transcriptional factor, which is known to the most prominent regulator in early cerebellar development, is a new substrate of AKT in developing cerebellum. AKT binds to PTF1a and phosphorylates it at Serine 154. Phosphorylated PTF1a by AKT enhances its transcriptional activity toward Lhx1/5 transcription factors those are responsible for the generation of GABAergic neurons. Moreover, PTF1a phosphorylation by AKT copes it from ubiquitin-proteasomal system (UPS)-dependent degradation, reducing its interaction with FBXW7, rather caged in nucleus. However, AKT inhibitor treatment into the neuronal cells represses nuclear localization of PTF1a, reducing transcriptional activity for GABAergic neuron. Thus, our findings suggest that Akt signaling activates a bHLH transciption factor, PTF1a, leading to induction of GABAergic neurons during cerebellar development.

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S. Protein Modification and Regulation [S-18]

Optimization of Recombinant Homo-tetrameric SOD3 for use as a Therapeutics

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Superoxide dismutase 3 (SOD3) is a homo-tetramer that functions to remove reactive oxygen species and has the potential as a treatment for inflammatory diseases. However, the production of recombinant SOD3 is limited by high oligomerization and the formation of heterogeneous tetramers through inter disulfide bond and proteolytic cleavage. To overcome this limitation, we engineered SOD3's heparin binding domain, resulting in the production of a homogeneous tetramer with stable enzyme activity and a 200% improvement in expression level. Our findings demonstrate the potential of protein engineering to enhance recombinant protein properties, with implications for the development of SOD3 as a therapeutic agent.

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S. Protein Modification and Regulation [S-19]

ErbB3-binding protein 1 (EBP1) acetylation: a novel post-translational modification related to apoptosis

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Besides epigenetic control through histone acetylation, Acetylation is also known to be an important post-translational protein modification, that usually regulates transcriptional level, protein-protein interaction, and functional activity of proteins. ErbB3-binding protein 1 (EBP1) controls cell survival, proliferation, and differentiation through protein modification such as phosphorylation, ubiquitination, and sumoylation. Although recent proteomic studies have predicted EBP1 protein might be modified by acetylation,acetylation on EBP1 has not been elucidated yet. In the current study, we demonstrated that EBP1is able to be modified by protein acetylation. EBP1 acetylation was seemed to be augmented as apoptotic death was occurred by staurosporine (STS) treatment in cells, suggesting anti-apoptotic function of EBP1 is probably involved in its acetylation. Moreover, we found that acetylated EBP1 is diminished by SIRT2, deacetylase. Therefore, we speculated that the acetylation of EBP1 could affect on its cellular activities including apoptosis.

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T. Protein Structure and Function [T-1]

Lactobacillus paracasei aspartate ammonia-lyase is a phenylalanine/tyrosine ammonia-lyase

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Aspartate ammonia-lyase (AAL) catalyzes the reversible conversion reactions of aspartate to fumarate and ammonia. In this work, *Lactobacillus paracasei LpAAL* gene was heterologously expressed in *Escherichia coli*. Besides a recombinant His-tagged LpAAL protein, a maltose-binding protein (MBP) fused LpAAL protein was used to enhance its protein solubility and expression level. Both recombinant proteins showed broad substrate specificity, catalyzing aspartate, fumarate, phenylalanine and tyrosine to produce fumarate, aspartate, *trans*-cinnamate, and *p*-coumarate. The optimum reaction pH and temperature of LpAAL protein for four substrates were measured at 8.0 and 40 °C. The K_m values of LpAAL protein for aspartate, fumarate, phenylalanine, and tyrosine as substrates were 5.7, 8.5, 4.4, and 1.2 mM, respectively. The k_{cat} values of LpAAL protein for aspartate, fumarate, phenylalanine and tyrosine as substrates were 6.7, 0.45, 4.96 and 0.02 s^{-1}, respectively. In conclusion, aspartate, fumarate, phenylalanine and tyrosine are *bona fide* substrates for LpAAL enzyme. Supported by the grant (NSTC 110-2313-B-029-001-MY3) from the National Science and Technology Council, Taiwan.

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T. Protein Structure and Function [T-2]

Recent Progress in the Study of Taste Characteristics and the Nutrition and Health Properties of L-Pyroglutamic acid

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Taste is classified into five types, each of which has evolved to play its respective role in mammalian survival. A taste receptor or tastant is a type of cellular receptor which facilitates the sensation of taste. When food or other substances enter the mouth, molecules interact with saliva and are bound to taste receptors in the oral cavity and other locations. Molecules which give a sensation of taste are considered. Sour taste is one of the important ways to judge whether food has gone bad, and the sour taste receptor (PKD2L1) is the gene behind it.

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T. Protein Structure and Function [T-3]

Human SHBG hinders the entry of zoonotic coronavirus via AXL

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Although vaccines and drugs have been developed to prevent and treat severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, intrinsic host factors involved in the zoonotic transmission of coronaviruses require further attention. SARS-CoV-2 enters cells using one of the host tumor-associated macrophage (TAM) receptors, the tyrosine-protein kinase receptor UFO (AXL). Sex hormone-binding globulin (SHBG) inhibited TAM receptors in a recent study; thus, we investigated whether SHBG restricted coronavirus entry via AXL inhibition. In transgenic (*SHBG*-Tg) mice, the SHBG protein was present in the lavage fluid of the respiratory system, which decreased pulmonary AXL levels. SHBG nebulization could decrease pulmonary AXL levels. Acute intranasal exposure to SHBG interfered with pulmonary mouse hepatitis virus (MHV) entry during *ex vivo* viral inoculation. *In vitro*, SHBG rapidly degraded the AXL protein in DBT cells. AXL suppression by SHBG incubation restricted the propagation of MHV in DBT cells. The virus suppressive effect of SHBG depended on AXL because it was not observed in AXL inhibitor (bemcentinib)-treated and *Axl* knocked down cells. *Axl* inhibition and knockdown inhibited MHV entry and propagation. In pseudotyped SARS-CoV-2 transduction, SHBG reduced viral entry into AXL-enriched A549 cells compared with that in Vero cells lacking AXL. In conclusion, our study reports that SHBG is a novel intrinsic host antiviral factor that reduces coronavirus entry via AXL inhibition, suggesting that SHBG is a human protective protein against a diverse family of coronaviruses.

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T. Protein Structure and Function [T-4]

TLR3 forms a highly organized cluster when bound to a poly(I:C) RNA ligand

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Toll-like Receptor 3 (TLR3) recognizes dsRNA ligands that are derived from virus infection[1]. Upon ligand binding, TLR3 forms homodimer and triggers the recruitment of adaptor proteins to initiate anti-viral immune response. Previous crystallographic studies showed that TLR3 requires a minimum of 40-50 base pair for homodimer formation[2]. However, this short RNA barely activates TLR3 and TLR3 requires ~100 base pair RNA ligand for robust immune responses[3,4]. To obtain structural insights into the length dependency of TLR3 ligands, we determined the cryo-EM structure of TLR3 in a complex with a synthetic RNA with an average length of ~400 base pairs. In the structure, the dimeric TLR3 units are clustered along the dsRNA helix in a highly organized and cooperative fashion with a uniform inter-dimer spacing of 103 angstroms. Our structural observation suggests that ligand-induced clustering of TLR3 dimers is necessary to facilitate TLR3 signaling. TLR3 clustering may enhance the productive ordered assembly of intracellular signaling adaptors and initiates a robust innate immune response.

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T. Protein Structure and Function [T-5]

The effects of Tat-SOD against high-fat diet (HFD)-induced nonalcoholic steatohepatitis in C57BL/6 mice

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Inflammation is known as one of important factor for the induction and promotion of nonalcoholic steatohepatitis (NASH). We have previously reported that antioxidant protein SOD has anti-inflammatory effect against oxidative stress, however the role of SOD in the NASH is not studied yet well. Therefore we prepared cell permeable Tat-SOD fusion protein and investigated the anti-inflammatory effects of this fusion protein in Raw 264.7 macrophage cells and NASH mice model which was generated through a high-fat diet. We found that Tat-SOD transduced into Raw 264.7 cells and significantly reduced LPS-induced cytokines (TNF-α, IL-1β, IL-6) mRNA levels and decreased the activation of MAPK and NF-κB. In NASH mice model, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total cholesterol (TC) levels were increased and cytokines mRNA and NF-κB activation levels were increased. However, AST, ALT, TC, cytokines mRNA levels and the activation of MAPK, NF-κB were markedly reduced in the Tat-SOD treated mice. These results indicate that anti-oxidant enzyme SOD has a protective effect in NASH by suppressing the inflammatory responses and regulating MAPK signaling, suggesting Tat-SOD will be potential therapeutic protein drug candidate for NASH.

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T. Protein Structure and Function [T-6]

A structural vista of phosducin-like PhLP2A-chaperonin TRiC cooperation during the ATP-driven folding cycle

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Proper cellular proteostasis, essential for viability, requires a network of chaperones and cochaperones. ATP-dependent chaperonin TRiC/CCT partners with cochaperones prefoldin (PFD) and phosducin-like proteins (PhLPs) to facilitate the folding of essential eukaryotic proteins. Using cryoEM and biochemical analyses, we determine the ATP-driven cycle of TRiC-PFD-PhLP2A interaction. In the open TRiC state, PhLP2A binds to the chamber's equator while its N-terminal H3-domain binds to the apical domains of CCT3/4, thereby displacing PFD from TRiC. ATP-induced TRiC closure rearranges the contacts of PhLP2A domains within the closed chamber. In the presence of substrate, actin and PhLP2A segregate into opposing chambers, each binding to the positively charged inner surfaces formed by CCT1/3/6/8. Notably, actin induces a conformational change in PhLP2A, causing its N-terminal helices to extend across the inter-ring interface to directly contact a hydrophobic groove in actin. Our findings reveal an ATP-driven PhLP2A structural rearrangement cycle within the TRiC chamber to facilitate folding.

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T. Protein Structure and Function [T-7]

Functional roles of the αd helix of PEX19 in the targeting of peroxisomal membrane proteins.

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Proper targeting and insertion of peroxisomal membrane proteins (PMPs) are critical for maintaining correct peroxisome biogenesis. However, molecular mechanisms underlying PMP targeting through the PEX19-PEX3 complex remain unclear. Here, to dissect the molecular steps of PMP targeting, we used a biochemical reconstitution system with the purified PEX26 protein as a model PMP. Using both FRET-based dissociation assay and turbidity assay, we found that the αd helix of PEX19 plays a key role in maintaining a stable PEX19•PEX26 complex, whereas the chaperone activities of PEX19-WT and PEX19-αd4A (mutation of four hydrophobic residues to alanine) are not largely different. Furthermore, we showed that the cytosolic domain of PEX3 binding to PEX19 activates PEX26 dissociation from PEX19, and the disease mutant PEX3-G138E lacks this activation. A combination of MS analysis and Bpa crosslinking assays revealed a new PEX19 binding site on PEX3; the αd helix of PEX19 directly interacts with the α1 helix of PEX3, suggesting that this interaction could be a prerequisite step for PEX26 release and insertion into the peroxisomal membrane. Therefore, understanding the functional roles of PEX19-αd helix in targeting of PMPs into peroxisome could help elucidate the pathological mechanism of peroxisome biogenesis disorders.

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T. Protein Structure and Function [T-8]

Protein folding intermediate and folding mechanism

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The contribution of hydrophobic residues to the protein folding reaction was studied by using HubWA variant proteins with I and L to V mutation. Folding kinetics of variant proteins was observed to be satisfied by a three-state on-pathway mechanism, U ⇌ I ⇌ N. Three-state folding reaction was quantitatively analyzed and the free energy of folding of each elementary reactions and overall folding reaction, DG^{o}_{UI}, DG^{o}_{IN}, and DG^{o}_{UN}, were obtained. From the ratio of free energy difference between the variant protein and HubWA, $DDG^{o}_{UI}/DDG^{o}_{UN}$, the contribution of hydrophobic residues to HubWA folding was analyzed. The residues which are located in the hydrophobic core between a-helix and b-sheet, I3, I13, L15, I30, L43, I61 and L67, were observed to form relatively solid hydrophobic core in the intermediate state. Residues located at the end of secondary structures and loop, I23, L69 and I36, were observed to form fairly loose hydrophobic interactions in the intermediate state. V17A, L50V and L56V showed to form highly solid but non-natural hydrophobic interactions. It is suggested that these residues prevent the formation of aggregate at the early stage of folding reaction.

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T. Protein Structure and Function [T-9]

Structure-based functional implication of melanocortin receptors

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Peptide hormones, melanocortins, are regulator of variety functions throughout the body, such as thermoregulation, homeostasis, weight control and exocrine secretion. Melanocortin receptors are one of the G-protein-coupled receptor (GPCR) family. There are five subtypes of melanocortin receptors (MC1R-MC5R) that regulate different functions and different expression tissue. Melanocortin-4 receptor (MC4R) has a central role in appetite and energy control regulation. MC4R defects lead to a severe clinical phenotype inducing lack of satiety and early-onset of severe obesity. Melanocortin-5 receptor (MC5R), mainly expressed in secretory epithelium, is involved in controlling exocrine gland secretion. Dysfunction of MC5R leads to secretion disorders, such as sebogenesis. Here, we present the high-resolution cryo-EM structure of human MC4R/Gs/scFv16 complex and MC5R/Gs/scFv16 complex, stimulated by the peptidic agonist. Our structures reveal the binding pocket of melanocortins and the interactions formed between receptor and ligand, playing a crucial role for receptor activation. Present work could provide insights into the investigation of drugs targeting for MCRs, developing new therapeutic strategies to fight against obesity, weight-regulation disorders and acne diseases.

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T. Protein Structure and Function [T-10]

Structural insights into the human somatostatin receptor 3 and 5 complex with somatostatin

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Somatostatin receptor (SSTR) is one of the membrane proteins which included in G protein coupled receptor (GPCR). It is mainly expressed in the nervous system and regulates several signals through interaction with the somatostatin (SST) and G protein. In signaling mechanisms, SSTRs have been reported as one of the important targets in the development of new drugs as they are associated with neurological disorder and metabolic imbalance. Especially, SSTR3 and SSTR5 that linked with various cancers and neurological disorders. However, beside for SSTR2 and SSTR4 among SSTR subfamilies, the structural information in atomic scale upon ligand binding has not been revealed. Here, we report the structure of SSTR3 and SSTR5 complexed with ligand by cryogenic electron microscopy (cryo-EM). By comparison of the SSTR3 and SSTR5 structures, the main residues interacted with ligand molecule in binding sites and conformational changes upon ligand binding were elucidated. With these results of cryo-EM structures of SSTR3 and SSTR5, it will be possible to contribute the development of new drugs based on structure information about SSTR family.

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T. Protein Structure and Function [T-11]

Biochemical and Structural Studies of Urotensin Receptor for Drug Development

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Urotensin II (UII) is a cyclic eleven amino acid neuropeptide binding on Urotensin II receptor (GPR14), class A rhodopsin like G protein-coupled receptor. Human UII (hUII) and receptor are highly expressed on the tissues of myocardium, kidney, and central nervous system as agonist of the receptor. Its activation with $G_{\alpha q/11}$ mainly stimulates Phospholipase C pathway and leads vasoconstriction. The level of UII is increased in several diseases: heart failure, diabetes mellitus, and renal failure. However, the potent drug is not discovered because structural information of receptor has not known yet. Here, we were able to purify the complex of hUII bound to Urotensin II receptor with engineered G protein. Using cryogenic electron microscopy (Cryo-EM), we analyzed the detailed structural information upon ligand binding and obtained the activation mechanism of the receptor. In addition, we determined the solution structure of UII peptide by NMR spectroscopy and demonstrated conformational change upon receptor binding. This study would provide the structural basis of the drug development for UR target in various diseases.

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T. Protein Structure and Function [T-12]

Unique genetic structure of Bombyx mori LC3 protein and fast protein degradation of the cleaved C-terminal part

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Autophagy-related protein 8 (Atg8) is an evolutionarily conserved protein required for the formation of autophagosomes. Insects have genetic diversity. Although many insects' sequences are available, systematic analysis of the ATG8 sequence in insects is undelivered. In this study, we classified and analyzed insect ATG8 genes belonging to the five major orders of insects. As a result, we found that all insect species have at least one ATG8 protein classified into hGABARAP/L1 subfamily, while some species additionally contain ATG8 proteins belonging to LC3 subfamily. Interestingly, among insect ATG proteins, *Bombyx mori* LC3 (BmLC3) protein has a long LC3 protein, in which two LC3 proteins are duplicated within the coding region. These long LC3 genes have been found exclusively in Lepidopteran order in insect. As a further study, we confirmed that BmLC3 is cleaved at G135 and divided into N- or C-terminal BmLC3 by hATG4B in HeLa cells. Cleaved N-terminal BmLC3 can be located in autophagosomes under autophagy induction, while cleaved C-terminal BmLC3 was quickly degraded by both proteasome pathway and the autolysosomal proteolysis pathway. Furthermore, a Tyr residue, the first amino acid in the cleaved C-terminal BmLC3 is a main contributor for fast degradation governed by N-end rule in mammalian cells.

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T. Protein Structure and Function [T-13]

Studies of functional properties of espin 1: Its interaction to actin filaments

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Actin is a multifunctional biomolecule that forms structural bodies like stereocilia. Actin consists of four subdomains (S1, S2, S3, and S4), and the actin filament dynamics are regulated by numerous actin binding proteins with different mechanisms. Espin 1 is an actin bundling protein that is specifically implicated in the elongation and stabilization of stereocilia. However, little is known about the molecular structure and actin regulation mechanism of espin 1; hence, we investigated the interaction between actin and espin 1 through structural data. In this study, we first purified human espin 1 in an E. coli system following a new detergent-free approach and then demonstrated the 2D structure of full-length espin 1 using transmission electron microscopy along with Nickel nitrilotriacetic acid nanogold labeling and 2D averaging using SPIDER. Furthermore, we also determined the espin 1 binding domain of actin using a co-sedimentation assay along with gelsolin and myosin S1. These findings are not only beneficial for understanding the actin binding and bundling mechanism of espin 1, but also shed light on its elongation, stabilization mechanism of actin filament. Furthermore, it can contribute to cure various hearing-related diseases, such as hearing loss and vestibular dysfunction.

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T. Protein Structure and Function [T-14]

Adenosine- 5'- O- (3-thiotriphosphate) inhibits unwinding of duplex RNA by SARS-CoV-2 nsP13 helicase

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The nsP13 helicase plays a crucial role in the replication and transcription of the SARS-CoV-2 by unwinding duplex RNAs with ATP hydrolysis activity in host cells. We developed a fluorescence-based duplex nucleic acids unwinding assay using double-stranded nucleic acid substrates with two fluorophore labels attached to the ends of each nucleic acid strand. As the helicase unwinds the duplex RNA substrates, fluorescence of fluorescein attached in one strand of duplex RNA substrates was enhanced due to abolishment of FRET (fluorescence resonance energy transfer) between two adjacent fluorophores. Using this FRET-based assay of duplex RNA unwinding by nsP13 helicase, the extent of dsRNA and dsDNA unwinding was fluorescently measured in 96-well plate format. A slowly hydrolysable ATP analog, Adenosine-5'-O-(3-thiotriphosphate) (*i.e.* ATP-gS) was identified as effective inhibitor against nsP13 helicase by decreasing rate of duplex RNA unwinding *in vitro*. Both amplitude and kinetic rate of dsDNA and dsRNA unwinding was diminished with increasing amounts of ATP-gS in the reaction of duplex nucleic acids unwinding by nsP13 helicase. This results indicate that ATP-gS inhibits both duplex nucleic acid substrate binding and unwinding of base-paired nucleic acids by nsP13 helicase. Thus, ATP-gS is likely to be developed as antiviral agent against SARS-CoV-2.

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U. Proteomics [U-1]

Ribosomal Proteome Diversification by mRNA Translation in Lung Cancer via Bioinformatics Results

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Lung cancer is the primary cause of cancer-related deaths globally, and the survival rate for advanced lung cancer patients is inadequate. To combat this cancer effectively, various mechanisms need to be researched. Rapidly dividing cancer cells necessitate increased rates of biological energy consumption and biosynthesis, which are partly accomplished by boosting the protein synthesis of ribosomes. Ribosomal proteins (RPs) are a crucial component of ribosomes and have been linked to cancer, including lung cancer. mRNA translation, a tightly controlled mechanism for protein synthesis, is known to be altered by oncogenes to promote cancer development. Through bioinformatics analysis results of two types of lung cancer (lung adenocarcinoma & lung squamous cell carcinoma) from CPTAC, RPs protein expression patterns in cancer are diverse, while mRNA expression patterns of RPs are more conserved. This suggests that ribosomal proteome diversification in lung cancer could be regulated by mRNA translation. In addition, it is worth noting that RPS4Y1 mRNA expression levels were found to be lower in females than in males. However, the sex clustering of RPS4Y1 at the protein level was not as distinct as at the mRNA level in both lung cancers, particularly in lung adenocarcinoma.

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U. Proteomics [U-2]

In situ extracellular labeling of exosome cargo proteins

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The extracellular vesicle exosome mediates intercellular communication by transporting macromolecules such as proteins and ribonucleic acids (RNAs). Determining cargo contents with high accuracy will help decipher the biological processes that exosomes mediate in various contexts. Existing methods for probing exosome cargo molecules rely on a prior exosome isolation procedure. Here we report an in situ labelling approach for exosome cargo identification, which bypasses the exosome isolation steps. In this methodology, a variant of the engineered ascorbate peroxidase APEX, fused to an exosome cargo protein such as CD63, is expressed specifically in exosome-generating vesicles in live cells or in secreted exosomes in the conditioned medium, to induce biotinylation of the proteins in the vicinity of the APEX variant for a short period of time. Mass spectrometry analysis of the proteins biotinylated by this approach in exosomes secreted by kidney proximal tubule-derived cells reveals that oxidative stress can cause ribosomal proteins to accumulate in an exosome subpopulation that contains the CD63-fused APEX variant.

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U. Proteomics [U-3]

Total Proteome Comparison of NASH and Normal Mice Using Nano LC - Mass Spectrometry

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Non-alcoholic fatty liver disease (NAFLD) is a complex disease that encompasses various conditions from simple steatosis to non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis. It's recognized as one of the primary causes of chronic liver disease worldwide. To investigate this further, we used proteomics to compare liver tissues from mice with NASH caused by a high-fat diet (HFD) to that of healthy controls. We collected liver tissue samples from both groups of mice and used nano liquid chromatography-mass spectrometry (nLC-MS) to analyze protein expression changes that might provide insight into the pathogenesis of NASH. Our study revealed significant modifications in the expression of several proteins related to lipid metabolism, oxidative stress response and endoplasmic reticulum (ER) stress response. We observed that proteins involved in lipid biosynthesis, such as alcohol dehydrogenase (ADH) and glutamate oxaloacetate transaminase (GOT2), were upregulated when compared to controls. Furthermore, we observed alterations to ER and oxidative stress proteins in NASH mice such as endoplasmic reticulum chaperone (HSPA5) and glutamate dehydrogenase 1 (GLUD1). Overall, our findings shed some light on the molecular processes driving NASH and highlight their intricate connection between lipid metabolism, oxidative and ER stress in its pathogenesis.

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U. Proteomics [U-4]

Comprehensive proteomic profiling and identification of 14-3-3 protein zeta as a potential diagnostic biomarker for Acute Myocardial Infarction

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Acute myocardial infarction (AMI) is a multifaceted syndrome influenced by the functions of various extrinsic and intrinsic pathological processes, which is also the leading cause of most cardiac death worldwide. This study aims to investigate the secretome protein profile of induced-hypertrophy cardiomyocytes to identify next-generation biomarkers for AMI diagnosis and management. Hypertrophy was induced successfully for immortalized human cardiomyocytes (T0445) by 200 nM ET-1 and 1 μM Ang II. The protein profile of hypertrophied cardiomyocyte secretomes was analyzed by nano LC-MS/MS and differentially expressed proteins that have been investigated by Ingenuity Pathway Analysis. Our data reported a significant elevate of 32 proteins (>1.4 fold) while there are 17 proteins (

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U. Proteomics [U-5]

Molecular cloning and characterization of plasmodium vivax hexose transporter

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Plasmodiums are the most important cause of malaria, which kills above one million children each year. They lost many biosynthetic pathways and became reliant on their hosts as a nutrient source. They take up nutrients from their host cell through membranous transporters.

To increase understanding of hexose transport in plasmodium vivax, we have carried out cloning and characterization of hexose transporter 1 (PvHT1).

The PvHT1 gene from NCBI database was isolated using RT-PCR. The PvHT1 cDNA consisted of 1509 base pairs that encoded a 502 amino acids. Hydropathy analysis suggested 12 putative membrane-spanning domains. When expressed in *Xenopus laevis* oocytes, PvHT1 mediated the transport of [^{3}H]deoxy-D-glucose (ddGlu) in an expression time- and incubation time-dependent manner without sodium dependency. The PvHT1 showed no exchange mode of glucose by efflux experiments. Concentration-dependency results showed saturable kinetics following Michaelis-Menten equation. The Eadie-Hofstee equation analysis revealed a Km value of 294.1 mM and Vmax value of 1,060 pmol/oocyte/hr for [^{3}H]ddGlu. The inhibition experiments showed strong inhibitory effect by glucose, mannose and ddGlu and mild effect by methyl–d-glucose.

These results may give information for the molecular biological study of carbohydrate metabolism in Plasmodium vivax.

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U. Proteomics [U-6]

Developing functional annotation method of uncharacterized proteins using structure based prediction tool and interactome analysis

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To gain a better understanding of the biological mechanisms underlying human diseases, investigating protein functions is crucial. In this study, we developed a filtering algorithm to select specific gene ontology (GO) terms using the GO map generated by I-TASSER/COFACTOR. The selected GO terms showed a higher average precision-recall score, particularly for the cellular component term, compared to the total predicted GO terms. We then applied this algorithm to the C11orf52 gene, which is located on chromosome 11 and is one of the uPE1 genes. To validate our approach, we used immunoprecipitation methods with three different peptide tags (Myc, Flag, and 2B8) in HEK 293T cell lines and identified 79 candidate proteins that are expected to interact with C11orf52. We then selected three partner candidates, namely DSG1, JUP, and PTPN11, based on our expectation of their interaction with C11orf52, and confirmed their colocalization at the cell-cell junctions through coimmunofluorescence experiments. Based on a comprehensive analysis of our bioinformatic predictions, we expect that C11orf52 is related to the Wnt signaling pathway via DSG1-mediated protein-protein interactions.

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U. Proteomics [U-7]

Glycoproteome Profiling of Gallbladder Cancer Tissue using GlycoProteome Analyzer (GPA)

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GlycoProteome Analyzer (GPA) is a tandem mass spectrometry-based database search software for the identification and quantification of glycoproteins, including a mapping system for complex N- and O-glycoproteomes (Sci. Rep., 2016, Nat. Methods., 2021). Recently, we upgraded GPA to enable TMT labeling-based quantitative analysis. In this study, we demonstrated the efficiency of the upgraded GPA using a human serum sample and applied it to investigate differences in protein glycosylation between normal and tumor gallbladder tissues. We studied the variations in glycan types and compared their expression levels. We observed an increased proportion of hybrid type glycans and fucosylation in complex type glycans, as well as a decrease in biantennary glycans and sialylation in gallbladder tumor tissues. In addition, we investigated the potential biological significance of the observed changes in glycosylation patterns by performing functional enrichment analysis of the identified glycoproteins. Our results suggest that altered protein glycosylation may play a role in tumor progression and metastasis in gallbladder cancer.

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U. Proteomics [U-8]

Glycopeptide enrichment using ZIC-HILIC Chromatography in Human serum

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Glycosylation is a critical post-translational modification in mammalian cells that affects protein function and regulation. However, analyzing protein glycosylation poses challenges due to low abundances of glycoproteins and low mass sensitivities of glycopeptides, requiring glycopeptide enrichment processes. In this study, we developed a glycopeptide enrichment method using a ZIC-HILIC column in high-performance liquid chromatography (HPLC). We confirmed the enrichment of glycopeptides and examined the characteristics of peptides and glycan compositions at each fraction. Furthermore, we discovered that sialylated glycopeptides eluted earlier in ZIC-HILIC chromatography, while fucosylated glycopeptides eluted later. Using this approach, we identified 493 unique N-glycopeptides and 48 unique O-glycopeptides from 96 glycoproteins.

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U. Proteomics [U-9]

Proteomic and glycoproteomic profiles of human hepatocellular carcinoma organoids

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Organoids have emerged as a novel tool and promising ex vivo 3D culture model in medicinal research. However, the compositions of organoids have not been fully studied, and their proteome and glycoproteome have yet to be compared to the original tissue. In addition, the limited sample amount of organoid can be challenging to obtain high-quality mass spectrometric data. In this study, we generated proteomic and glycoproteomic profiles at the microgram-scale (1-5 μg) of protein amount from hepatocellular carcinoma organoids. We identified a total of 2,099 proteins in global proteomics, which were involved in the metabolism of RNA, nucleobase-containing small molecule metabolic process, and carbon metabolism. These functions were consistent with the major metabolic functions of liver tissue. In the glycoproteomic approach, we identified 41 glycoproteins from 159 glycopeptides, including important therapeutic targets and biomarkers of hepatocellular carcinoma (HCC), such as EGFR and AFP, respectively. We compared glycan compositions between HCC tissue and HCC-derived organoids and found that they were similar. This finding suggests that organoids share similar characteristics with actual tissues.

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U. Proteomics [U-10]

I-GPA: Site specific intact N- and O-glycopeptide analysis platform for the characterization of glycoprotein

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Post-translational glycosylation plays an important role in protein folding and stability, enzymatic function, inflammation, cancer development, and so on. Therefore, the glycosylation sites and glycan structures need to be thoroughly interpreted for the development of effective therapies. We characterized the site-specific N- and O-recombinant glycoproteins via liquid chromatography-tandem mass spectrometry analysis and the Integrated GlycoProteome Analyzer (I-GPA) platform. This platform can automatically analyze N- and O-glycopeptides, including their glycan composition and amino acid sequences, using various tandem mass spectra (CID, HCD and EThcD) without releasing glycans. Oxonium and b/y ions were easily presented in the HCD spectrum, while B/Y ions providing glycan structure information were observed in the CID spectrum, and c/z ions allowing precise localization were observed in the EThcD spectrum. To obtain a high-quality EThcD spectrum, it is important to generate a highly charged precursor ion during ionization. Since the N-glycopeptide IFAASKNTTEKE_5_4_1_2, which was generated from IL-4 having two lysines and an N-terminal, its glycosylation site was precisely assigned by the c/z ions in the EThcD spectrum from its four charged precursor ions. O-glycopeptides such as TPLPPTSAHGNVAEGETKPDPDVTER_1_1_0_1 from hemopexin were identified for their peptide sequences, glycan compositions and O-glycosylation sites from HCD, CID, and EThcD spectra respectively.

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U. Proteomics [U-11]

Collective protein analysis of various human 3D spheroids exposed to particulate matter

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Particulate matter (PM) is mainly inhaled into the human body through respiratory system, translocate to blood circulation, and eventually penetrates various organs. To elucidate the effects of PM on human health and diseases, human 3D models have been used, providing more relevant in vivo situations. However, few studies have reported on the comprehensive effects of PM on various organs simultaneously, despite the fact that it can affect all organs of the human body. In this study, we examined collective protein changes of human 3D spheroids using eight human primary cells from various tissues. To identify possible diagnostic markers, the up-regulated proteins upon DPM treatment were analyzed and common canonical pathways were extracted using ingenuity pathway analysis. We found that several secretory proteins involved in LXR/RXR activation pathway were upregulated. To use these proteins as possible diagnosis markers or therapeutic targets, further studies are in progress to identify the underlying mechanisms.

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U. Proteomics [U-12]

Spectra-Sum Method for Protein Quantification using LC-MS/MS and TMT labeling Data

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There are two methods used to calculate data for protein quantification using LC-MS/MS with TMT labeling. Spectra-sum method is calculated as a sum of the intensities of TMT report ions in spectra level. In other hand, Spectra-ratio method is calculated as a median of the ratio intensities with the global reference ion. In order to find a best quantification method, we prepared Hela standard peptides with TMT-6-plex labeling of difference protein content ratio as 0.5:0.5:1.0:1.0:2.0:2.0. First, we identified 3,684 proteins with FDR < 1% as spectra and global protein level using Integrated Proteomic Pipeline and ProteinInferencer. Of 30 replicated experiment of day by day, 16,384 is median number of identified spectra. We calculated the ratio value between the different protein content samples using the TMT reporter ions. A spectra-sum method resulted in 87% of proteins with coefficient variance (CV) < 30% and 0.07 standard deviation (SD). The other hand, in a spectra-ratio method, 45% proteins have CV less than 30% and 0.11 SD. It is indicated that spectra-sum method is more accurate quantification for a greater number of proteins.

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U. Proteomics [U-13]

Secretome analysis of lung cancer cells acquired resistance to EGFR tyrosine kinase inhibitor

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Drug resistance to targeted anticancer reagent is a major hurdle in cancer therapy. Tumors are composed of heterogeneous cells that respond to drugs differently and communications between heterogeneous cell populations in tumors are associated with drug resistance. In order to investigate mechanisms that underlie cell-cell communications contributing drug resistance, we analyzed differential secretome between parent EGFR mutant non-small cell lung cancer cell lines (PC9 and HCC827) and the corresponding cells (PC9ER and HCC827ER) with acquired resistance to EGFR tyrosine kinase inhibitor, erlotinib. Tryptic peptides obtained from conditioned media were labeled with tandem mass tag (TMT) reagents, fractionated using high pH HPLC, then analyzed in LC-MS/MS. 11 and 68 proteins are significantly increased and decreased in PC9ER/PC9 and HCC827ER/HCC827, respectively. Pathway analysis revealed that extracellular matrix organization and cell-cell adhesion pathways were enriched in the identified proteins from both cell lines. We found MUC18 was significantly upregulated in both resistance cells (PC9er and HCC827ER). Given its important role in regulating cell proliferation, movement and invasion we propose that the mechanism related to MUC18 may provide novel and deep insights into mechanism of drug resistance as well as mechanism by which resistance cells regulate tumor microenvironment.

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V. RNA Biology [V-1]

Exploiting CVB3 3C Protease Mutants for Therapeutic Intervention of Coxsackievirus B3 Infection

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Coxsackievirus B3 (CVB3) is a prevalent human enterovirus linked to severe illnesses such as myocarditis, meningitis, and pancreatitis. Despite the significant health burden caused by CVB3 infection, current therapeutic options are limited, highlighting the need for novel antiviral strategies. In this study, we investigated the characteristics of CVB3 and identified the 3C protease (3Cpro) as a promising target for CVB3 treatment. Our research revealed that 3Cpro is a key mediator of cell death induction, contributing to the pathogenesis of CVB3 infection. Consequently, we generated a series of CVB3 3Cpro mutants and assessed their potential to attenuate viral replication and virulence. Our ongoing experiments aim to determine the extent to which these 3Cpro mutants can neutralize the virus at the molecular level. Should these results prove successful, they will provide valuable insights into the development of innovative therapeutic interventions for CVB3-associated diseases. In summary, our study underscores the potential of targeting CVB3 3Cpro for the development of novel antiviral treatments. Positive outcomes from our experiments with 3Cpro mutants could significantly contribute to improving the clinical management and prognosis of patients affected by CVB3 infection.

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V. RNA Biology [V-2]

Development of novel genetic resources for target of RNAi to prevent Fusarium head blight

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The ascomycete fungus *Fusarium graminearum* is an important plant pathogen causing Fusarium head blight (FHB) in major cereal crops such as wheat, barley, and rice. In addition to destructive yield losses, this fungus contaminates grains with mycotoxins (trichothecenes and zearalenone), which pose serious threats to human and animal health. RNA interference (RNAi), mediator of gene silencing, is a convenient tool for the identification and characterization of gene function. Recently, it has widely been explored for its potential applications in plant disease management. Previous studies demonstrated that host-induced-gene-silencing (HIGS) targeting fungal *CYP51* genes is an efficient method to inhibit fungal growth and virulence of *F. graminearum*. However, only a few genes such as *CYP51, CNB1,* and *MYO5* were introduced into RNAi targets for the development of FHB control strategy. In *F. graminearum,* we have published the results of functional studies on 709 transcriptional regulators, 102 cytochrome P450 monooxygenases, and 31 peroxidases through genome-wide functional genetic analyses. Based on datasets, we selected six transcriptional regulator genes suitable for *F. graminearum* suppression. Until now, six transcriptional regulator genes were tested for inhibitory effects against *F. graminearum.* This study will provide novel genetic resources for target of RNAi to prevent devastating plant diseases.

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V. RNA Biology [V-3]

Liposome-based mRNA cancer vaccine improves mRNA delivery and induces anti-tumor responses

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mRNA cancer vaccine is a robust anti-cancer therapy platform that uses mRNA to encode tumor-specific antigens and elicits potent antigen-specific T-cell responses essential for anti-tumor immunity. However, mRNA is highly unstable and susceptible to degradation by nucleases *in vivo*. Thus, efficient delivery systems are required to develop mRNA-based cancer vaccines. Lipoplexes are mRNA delivery vehicles that enhance mRNA's stability, transfection efficiency, and expression by forming core-shell structured nanoparticles and can enter the cells through lipid rafts-mediated endocytosis. In this study, we developed cationic liposomes and selected the optimized lipoplex-mRNA vaccines based on the N/P ratio, physiochemical characteristics, and mRNA stability. Then, we demonstrated that our lipoplex improved the durability and delivery of mRNA, leading to high mRNA expression *in vitro* and *in vivo* systems. In addition, ovalbumin (OVA) encoding mRNA lipoplex induced more OVA-specific IFN-γ-secreting T cells than OVA protein with adjuvant in the mouse model. Furthermore, in a B16F10-OVA murine melanoma model, OVA encoding mRNA lipoplex potently delayed tumor growth and prolonged tumor protection and survival. Our study suggests that the mRNA-lipoplex with cationic liposome can be a potent mRNA cancer vaccine.

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V. RNA Biology [V-4]

Identification of a novel splice donor gain variant of Filamin C in dilated cardiomyopathy patient

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Dilated cardiomyopathy (DCM) is a major cause of heart failure and leads to cardiac transplantation. The condition is familial in ~50% of cases, and genetic variants residing in over 40 genes have been found to case DCM. Filamin C (FLNC) is an actin cross-linking protein that provides structure for the sarcomere in cardiomyocytes. Using whole genome sequencing with SpliceAI algorithm, we identified an FLNC splicing variant lead to abnormal splicing and may mediate pathogenicity via haploinsufficiency. To assess impact of this variant, we conducted an minigene-based *in vitro* splicing assay. A normally spliced minigene-derived transcript that excludes intron sequence is depicted. But, FLNC variants that create a novel splice donor gain inappropriately delete 5 base pairs from the 3′ exon. To validate direct splicing assay *in vivo*, agarose gel electrophoresis of endogenous FLNC transcripts from DCM patient specific induced pluripotent stem cells was performed, and as a result, the same results as minigene assay was shown. Therefore, patient iPSCs will be differentiated into cardiomyocytes to measure cardiac function through MEA and calcium transient assay, and if the phenotype is abnormal, the relationship between the FLNC gene and DCM will be identified by gene correction with the CRISPR/cas9 system.

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V. RNA Biology [V-5]

piR-54265 increases viability of colorectal cancer cells by regulating tumorigenesis-related genes

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Colorectal cancer (CRC) is one of the main causes of cancer-related deaths. Currently, surgical removal accompanied by the administration of anticancer drugs such as 5-fluorouracil (5-FU) and oxaliplatin is used for treatment. Many studies have provided evidence that targeting non-coding RNAs (ncRNAs) can increase the therapeutic sensitivity of anticancer drugs. In this context, we focused on finding novel ncRNAs that may contribute to CRC tumorigenesis. Although PIWI-interacting RNA (piRNA) has been reported to be involved in the progression of numerous diseases, the association of piRNA with the development of CRC is still unclear. We infected CRC cell lines with recombinant lentivirus to overexpress piR-54265, which is considered a potential biomarker in CRC. We confirmed that piRNA-54265 improved the viability of CRC cells. In addition, the effect of piR-54265 on the regulation of mRNA level expression of genes related to tumorigenesis was analyzed. Moreover, we investigated the effect of piR-54265 on drug sensitivity by co-treatment with 5-FU, a standard anticancer drug for CRC. The results of this study imply that piRNA can potentially serve as a new molecular target contributing to CRC progression and further investigation is needed for its correlation with drug sensitivity.

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V. RNA Biology [V-6]

Validation and prediction of microRNA genes by high-throughput assays and machine learning

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Maturation of canonical microRNA (miRNA) is initiated by DROSHA which cleaves the primary transcript (pri-miRNA). Over 1,800 miRNA loci are annotated in humans, but they have largely not been validated for their biogenesis mechanism. Here we performed *in vitro* processing on a full set of human pri-miRNAs (miRBase v21) followed by sequencing. Only 758 pri-miRNAs are confidently processed by DROSHA, while the majority may be non-canonical or false entries. To delineate the characteristic features of canonical pri-miRNAs, we analyzed their secondary structures using SHAPE-MaP, a high-throughput RNA structure probing approach. Our SHAPE-based structures differed significantly from computationally predicted structures, highlighting the importance of incorporating experimental data in miRNA structure prediction. By integrating the structures with the processing data, we identified novel elements that promote processing and functionally classified the sequence and structural features of pri-miRNAs. Finally, we developed a computational model that classifies confident DROSHA substrates out of non-miRNA sequences with high accuracy (>0.98 AUC). We screened the human transcriptome and viral genome with this model and found hundreds of sequence segments that could be processed by DROSHA. Our comprehensive analysis of pri-miRNA processing and structures provides new insights into miRNA regulation and practical benefits for small hairpin RNA design.

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V. RNA Biology [V-7]

Wnt/MicroRNA double-negative feedback regulation balances proliferation to facilitate the differentiation of adipocyte precursors

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The type 2 cytokine interleukin (IL)-4 induces the proliferation of bipotential adipocyte precursors (APs) in white fat tissue and primes these cells for differentiation into beige adipocytes, which are specialized for thermogenesis. However, the underlying mechanisms have not yet been comprehensively examined. Here, we identified six microRNA (miRNA) genes upregulated upon IL-4 stimulation in APs, miR-322, miR-503, miR-351, miR-542, miR-450a, and miR-450b; these are encoded in the H19X locus of the genome.

These miRNAs shared a large set of target genes, of which 381 genes were downregulated in mRNA expression upon IL-4 stimulation and enriched in Wnt signaling pathways. Among these genes, several putative targets of the six upregulated miRNAs were validated to be repressed by their corresponding miRNAs. In addition, the Wnt activator, LiCl decreased the expression of these miRNAs in APs, indicating that Wnt signaling−related genes and miRNAs form a double-negative feedback regulatory loop. Furthermore, we observed that these six miRNAs exhibit a suppressive effect on the proliferation of APs, a phenomenon that was alleviated upon the activation of the Wnt pathway. Our results suggest that H19X-encoded miRNAs facilitate the transition of APs from proliferation to differentiation in the IL-4-mediated regulation.

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V. RNA Biology [V-8]

Multi-omics approach to gene expression control of beige adipocyte differentiation

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Beige adipocyte differentiation induces massive cellular remodeling, accumulating mitochondria and generating multilocular lipid droplets. Although transcriptional and epigenetic regulations for particular genes underlying the dynamic changes have been thoroughly studied, it is still unclear how translational regulation contributes to the differentiation from preadipocyte to mature beige adipocyte. We hence combined ribosome profiling with RNA-seq and mass spectrometry and simultaneously analyzed transcriptome, translatome, and proteome. In our data, translatomes provide better estimates to explain gene-to-protein production than transcriptomes, indicating compelling translational control. Interestingly, tens of genes in several critical signaling pathways of beige adipocyte differentiation are highly upregulated at the RNA level but translationally tuned downward, and their protein levels may be further limited by protein degradation. Additionally, the assessment of translational activities for mitochondria-related genes revealed the exceptional downregulation in OXPHOS genes, which can be linked to a coordinated assembly of OXPHOS complex subunits generated from cytoplasmic or mitochondrial translations. Last, codon-usage analysis unveiled specific ribosome stalling on glutamate codons that are caused by a decrease of glutamate level in mature beige adipocytes. Translation of glutamate-codon enriched genes exhibited attenuation of ribosome codon reading, which can serve as an indicator of metabolic shift, an altered balance of glutamine/glutamate metabolites upon beige adipocyte differentiation. Collectively, our study provides clues and resources to the understanding of post-transcriptional regulation during beige adipocyte differentiation.

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V. RNA Biology [V-9]

Purification of in vitro transcribed RNA using mesoporous silica as adsorbent

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As we have noticed the capacity of mRNA vaccine against SARS-CoV-2, mRNA holds great promise for the treatment of a wide range of diseases. Increasing interest in mRNA preparation warrants reliable methods for purifying mRNA by eliminating surplus reagents, such as immunogenic double-stranded RNA (dsRNA). In this study, we developed a new method for eliminating dsRNA from *in vitro* transcribed RNA. As solid support as an adsorbent in chromatography, mesoporous silica is superior to spherical silica due to its larger surface area than the spherical silica. We carried out chromatographical purification of mRNA, in which various polyamines were employed as reagent for facilitating binding of mRNA to mesoporous silica. Among the polyamines we tested, spermidine showed the superior capability to induce the binding of mRNA to silica matrix via electrostatic interaction. The silica-adsorbed mRNA was readily eluted using salt, EDTA, and UREA by disrupting electrostatic interaction and hydrogen bonding between mRNA and silica matrix. Importantly, as the EDTA concentration increased in eluant, proportion of dsRNA in mRNA eluted from the silica matrix was decreased. Thus, our mesoporous silica-based purification method entailing spermidine-based mRNA adsorption and preferential elution of ssRNA with EDTA presents a reliable way for the preparation of non-immunogenic mRNA.

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W. Stem Cell Biology [W-1]

Determination of adipogenesis stages of Human Umbilical Cord derived Mesenchymal Stem Cells (UC-MSC) by Three-dimensional label-free Tomography

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The process of adipogenesis is highly regulated and complex, involving significant changes in gene expression, cytoskeletal organization, and cell morphology. In this process, lipid droplets (LDs) play a crucial role in lipid metabolism and storage. However, the adipogenesis of human umbilical cord-derived mesenchymal stem cells (UC-MSC) is not well studied as compared to bone marrow or adipose-derived MSCs, and no live cell study that provides morphological biomarkers related to different stages. To address these gaps, we used optical diffraction tomography (ODT) to determine the stage of adipogenesis in UC-MSC. We induced adipogenesis in human UC-MSCs and analyzed the expression of differentiation, mesenchymal stem cell, and self-renewal markers, along with Oil Red O (ORO) staining and ODT. Results showed downregulation of Pref1, CD90, and SOX2, indicating markers to distinguish MSCs, preadipocytes, and immature adipocytes, while PPAR-γ and FABP4 were upregulated, indicating markers to distinguish immature from mature adipocytes. Additionally, in ODT image analysis, quantitative measures of LDs (number, volume, mass, and refractive index) and cellular structure reorganization distinguished the morphological properties of different stages of adipogenesis. Overall, our study identified distinct biomarkers of adipogenesis stages in UC-MSCs by 3D label-free live cell imaging, providing potential applications in biomedical and clinical settings.

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W. Stem Cell Biology [W-2]

Long Range Inter-Chromosomal Interaction of Oct4 Distal Enhancer Loci Regulates ESCs Pluripotency

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Nuclear architecture underlies the transcriptional programs within the cell to establish cell identity. As previously demonstrated, long-range chromatin interactions of the *Oct4* distal enhancer (DE) are correlated with active transcription in naïve state embryonic stem cells. Here, we identified and characterized extreme long-range interactions of the *Oct4* DE through chromosome conformation capture and cross-validation with a novel CRISPR labeling technique we developed to identify lethal giant larvae 2 (*Llgl2*) and growth factor receptor-bound protein 7 (*Grb7*) as putative functional interacting target genes in different chromosomes. We showed that the *Oct4* DE directly regulates the expression of *Llgl2* and *Grb7* in addition to *Oct4*. Expression of *Llgl2* and *Grb7* closely correlates with the pluripotent state, where knockdown of either results in loss of pluripotency, and overexpression enhances somatic cell reprogramming. We demonstrated that biologically important interactions of the *Oct4* DE can occur at extreme distances that are necessary for the maintenance of the pluripotent state.

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W. Stem Cell Biology [W-3]

Generation and characterization of an enhanced green fluorescent protein-tagged CDH1 knock-in human induced pluripotent stem cell line using CRISPR/Cas9 system

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Extracellular matrices (ECM), proteoglycans and growth factors are essential to maintain the pluripotency and normal growth characteristics of human pluripotent stem cells (hPSCs) in feeder-free culture. Among them, the ECM provides an adhesive substrate for cell migration of hPSCs and regulates epithelial-mesenchymal transition (EMT), a well-characterized cellular process during embryonic development. E-cadherin is an adjacent homophilic cell-cell adhesion protein that is essential for hPSC adhesion and colony formation and its expression changes are the first signal in the initiation step of EMT. Therefore, in order to confirm the effect of regulating the signaling mechanism of E-cadherin on the undifferentiated state of hPSCs, we introduced the EGFP-tagged CDH1 gene into the AAVS1 locus of human induced pluripotent stem cells (hiPSCs) using the CRISPR/Cas9 system. The engineered cell line expresses functional CDH1-EGFP fusion and shows normal cell morphology. In addition, it maintains karyotypically normal and remains pluripotent state. Since this EGFP-tagged hPSC line shows fluorescence in the cell memebrane, it is expected that morphological alterations can be confirmed in real time depending on the culture conditions. Therefore, we propose that this engineered cell line can be a valuable tool for stem cell research, particularly in the field of cell-cell interactions in hPSCs.

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W. Stem Cell Biology [W-4]

Radially Grid Patterned PCL/col/CA scaffolds coupled with COMP-Ang1 for Regulation of Angiogenesis and Stem Cell Recruitment in Bone Repair

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The maintenance of sufficient vascularization in order to support the formation of new bone is one of important principles underlying BTE approaches. Great efforts to induce adequate vessel growth into engineered scaffolds have been dedicated to develop strategies to deliver therapeutic agent like COMP-Ang1 for the increased angiogenesis capacity. Another approach in BTE is the appropriate use of MSCs with the ability to continuously regenerate and osteogenic differentiate. Because of insufficient natural recruitment of MSCs, endogenous cell recruitment strategies based on a combination of a cell-free scaffold with a chemoattractant such as SDF-1 can provide osteoinductive cues for the promotion of in situ bone regeneration. However, this strategy lies in excessive costs and optimal factor doses of chemoattractant. We demonstrate a novel approach as a step forward to resolving these limitations by combining strategies of the angiogenesis and the recruitment of stem cells. The use of the approach was assessed by utilizing a radially grid patterned PCL/col/CA fibrous scaffold coupled with COMP-Ang1. The goal was achieved by SDF-1 released from vascular endothelial cells was increased by CA. We reported the combination of COMP-Ang1 and CA sequentially results in marked angiogenesis and stem cell recruitment within the developed scaffold.

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W. Stem Cell Biology [W-5]

ETV4 is a mechanical transducer linking cell crowding dynamics to lineage specification

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Dynamic changes in mechanical microenvironments, such as cell crowding, regulate lineage fates as well as cell proliferation. Although regulatory mechanisms for contact inhibition of proliferation have been extensively studied, it remains unclear how cell crowding induces lineage specification. Here, we found that a well-known oncogene, ETV4, serves as a molecular transducer that links mechanical microenvironments and gene expression. In a growing epithelium of human embryonic stem cells (hESCs), cell crowding dynamics is translated into ETV4 expression, serving as a pre-pattern for future lineage fates. A switch-like ETV4 inactivation by cell crowding derepresses the potential for neuroectoderm differentiation in hESC epithelia. Our findings suggest that cell crowding dynamics in a stem cell epithelium drives spatiotemporal lineage specification using ETV4 as a key mechanical transducer.

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W. Stem Cell Biology [W-6]

Stress-resistant translation secures protein synthesis and pluripotency under cellular stress

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In the growing human embryonic stem cell (hESC) colonies, cells face different microenvironments depending on their geometrical positions that generate chemical or mechanical stress. However, how cellular stresses spatially remodel mRNA translation in the hESC epithelium remains unclear. In response to stresses, cells rapidly shut down protein synthesis by phosphorylation of eIF2α, a key translation initiation factor, which is called the integrated stress response (ISR). In this study, we found that differential ISR occurs in a naturally growing colony of hESCs with cells in the center showing lower protein synthesis rates. Although ISR induces cell cycle arrest, we observed active cell division and proliferation in the center of hESC colonies. Surprisingly, a compensatory elevation of eIF2A protein, a stress-resistant alternative translation initiation factor, was observed in the cells with high ISR. Genetic manipulation revealed that the compensatory increase in eIF2A is essential for hESC self-renewal and differentiation. Using Ribo-seq, we found that eIF2A-dependent translation is widespread in hESCs and eIF2A targets include cell cycle-related genes. Furthermore, SOX2, a core pluripotency gene, was identified as a key target of eIF2A, suggesting the significance of eIF2A. Overall, our work identified that eIF2A-mediated stress-resistant translation is active in hESCs while safeguarding pluripotency.

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W. Stem Cell Biology [W-7]

The Therapeutic Potential of Mesenchymal Stem Cells in Attenuation of Sjögren Syndrome

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Sjögren's syndrome (SS) is a progressive autoimmune disease distinguished by a predominant infiltration of lymphocytes in exocrine glands, leading to loss of function. Mesenchymal stem cells (MSC) are a promising tool for autoimmune diseases due to their immunosuppressive and anti-inflammatory properties. This study aimed to illuminate the effect of MSC on the attenuation of SS. MSCs (Catholic Master Cells) were injected into the conjunctiva or the lacrimal glands of 22-week-old NOD/LtJ female mice and sacrificed after 7 days. Clinical phenotype of dry eye was observed by corneal stain scores and tear secretion measurements. Conjunctival goblet cell staining by PAS, foci infiltration in lacrimal glands, inflammatory gene expression levels, and immunofluorescence staining of autophagy markers in glands were observed. The results demonstrated that clinical phenotypes were alleviated in mice treated with MSCs compared to the control group, and tear secretion as well as goblet cell density showed recovery. Foci infiltrations in the lacrimal glands and inflammatory cytokine gene expression levels in the MSCs-treated group decreased compared to the control group. Autophagy markers ATG5 and LC3BII also decreased in the lacrimal glands of MSCs-treated mice. Together, the results demonstrate MSC is a promising candidate for the treatment of SS dry eyes.

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W. Stem Cell Biology [W-8]

The Effect of MSCs Exosomes in Sjögren Syndrome Murine Model

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Sjogren's syndrome (SS) is a systemic autoimmune disease causing dryness of the eyes and mouth due to excessive lymphocyte infiltration in exocrine glands. Exosomes are membrane-bound extracellular vesicles and known to have immunosuppressive and anti-inflammatory functions. The effect of exosomes on the attenuation of Sjogren's syndrome was observed with a SS murine model. Exosomes obtained from MSCs (Catholic Master Cells) were injected into the conjunctival sac once and sacrificed after 7 days in 17-week-old NOD/LtJ female mice, or administered daily as an eyedrop for 14 days, then sacrificed. The effects of MSCs exosomes were examined by tear secretion levels, corneal staining score, PAS staining of goblet cells, infiltration of foci in the lacrimal glands, gene expression levels of inflammatory cytokines, and immunofluorescence of autophagy markers. Administration of exosomes demonstrated attenuations of clinical phenotypes and goblet cell recovery in the conjunctiva. Infiltrations of foci, gene expression levels of inflammatory cytokines, and autophagy markers ATG5 and LC3BII in the lacrimal glands showed statistically significant decreases in mice treated with exosomes compared with the control group. This study demonstrated amelioration of dry eye symptoms with the administration of exosomes in a SS animal model, suggesting promising therapeutic potential in SS dry eye treatment.

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W. Stem Cell Biology [W-9]

Tacrolimus ameliorates high glucose-induced mitochondrial fission by inhibiting NFATC1 in human umbilical cord blood-derived mesenchymal stem cells.

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Umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs) have potential as cell therapy due to their ability to self-regeneration and multi-potential differentiation, and also as a treatment for diabetic patients. However, high glucose decreased the cell viability of UCB-MSCs by stimulating mitochondrial ROS (mtROS) through intracellular stress. Recent studies have shown that excessive calcium level is a major risk factor for high glucose-induced mtROS. Thus, we assumed that tacrolimus (calcineurin inhibitor) would protect against cell death by regulating high glucose-induced NFATC1 in UCB-MSCs. Therefore, we investigated the effect of tacrolimus in UCB-MSCs exposed to high glucose. High glucose increased mtROS, apoptosis, O-GlcNAcylated protein expression and nuclear translocation of NFATC1 in UCB-MSCs. However, the translocation of NFATC1 in the nuclear was reduced by ST045849, an OGT inhibitor. Mitotracker results showed that high glucose induced mitochondrial fission. In addition, the high glucose increased protein and mRNA expression of *DRP1*. In high glucose, the treatment of tacrolimus reduced mtROS and DRP1 expression level by reducing the activity of NFATC1. Finally, Mdivi-1, inhibitor DRP1, reduced the level of mtROS and apoptosis under high glucose. In conclusion, these results suggest that tacrolimus suppressed high glucose-induced cell death by suppressing NFATC1-mediated mitochondrial fission and mtROS.

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W. Stem Cell Biology [W-10]

Mesenchymal stem cells target microglia via galectin-1 production to rescue aged mice from olfactory dysfunction

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Olfactory loss has been considered as the earliest complication for the aging process while underlying mechanisms and therapeutic strategies remain unclear. We investigated whether the immunomodulatory action of mesenchymal stem cells (MSCs) can rescue the olfactory impairment in old mice. The intranasal delivery of MSCs limited microglial activation and neuronal apoptosis in the olfactory bulb (OB), leading to improvement in olfaction. Notably, old astrocytes could not prevent excessive microgliosis because the endogenous production of Galectin-1 (Gal1) was not sufficiently upregulated in the aged brain. MSCs and their culture supernatant (MSC-CM) could regulate the direction of microglial differentiation by impeding the polarization towards the pro-inflammatory M1 type; notably, a selective Gal1 inhibitor could hinder this phenomenon, indicating that Gal1 is involved in immunomodulation exerted by MSCs. Also, microglial activation within the OB upon LPS infusion was attenuated by MSC-CM in a Gal1-dependent manner. Our study demonstrates the therapeutic benefit of MSCs on age-related olfactory dysfunction and suggests Gal1 as a key mediator of the anti-inflammatory action of MSCs.

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W. Stem Cell Biology [W-11]

Establishment of human induced pluripotent stem cell-derived cardiomyocytes from patients with doxorubicin-induced cardiotoxicity.

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Doxorubicin (DOX) is an antineoplastic drug used for tumors such as lymphoma and breast cancer. However, the severe cardiotoxicity associated with the doxorubicin treatment limits its clinical use. Recently, patient-specific human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) provide an attractive research platform for disease mechanisms, drug screening, and disease modeling. We recruited 2 patients with breast cancer who had been treated with doxorubicin and showed doxorubicin-induced cardiotoxicity (DIC) phenotype. We generated hiPSCs from their peripheral blood mononuclear cells and confirmed the expression of pluripotency markers. Then, the hiPSCs were differentiated into cardiomyocytes, and expressed high levels of cardiac markers, indicating successful cardiac differentiation. The cardiac function and electrophysiology of patient-specific CMs were evaluated compared to normal CMs during doxorubicin treatment with dose- and time-dependent using a multi-electrode array (MEA) system. The MEA results showed lower spike amplitude, shorter beat period and increase beat period irregularity in patient-specific CMs. As a result, we were able to establish cell lines that clearly showed the DIC phenotype derived from patients. Using these cell lines, we would like to screen natural compounds that can improve cardiac function in DIC patients and study the associated molecular signaling.

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W. Stem Cell Biology [W-12]

Reciprocal enhancement of SARS-CoV-2 and influenza virus replication in human pluripotent stem cell-derived lung organoids

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Co-infection of SARS-CoV-2 and FLUAV causes severe respiratory failure and increased mortality. We developed a relevant lung model system to study this co-infection and found that hiAT2 organoids were susceptible to infection by both viruses, leading to severe lung damage. Infection with a single virus increased susceptibility to other virus infections and upregulated respective cell entry receptors. RNA sequencing showed hyperactivation of proinflammatory and immune-related signaling pathways and cellular damage in coinfection. Our findings shed light on the molecular mechanisms underlying enhanced infectivity and severity and may aid in developing therapeutics for prevention and management of such cases.

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W. Stem Cell Biology [W-13]

Transcriptional activation of endogenous Oct4 via the CRISPR/dCas9 activator ameliorates Hutchinson-Gilford progeria syndrome in mice

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Partial cellular reprogramming via transient expression of Oct4, Sox2, Klf4, and c-Myc induces rejuvenation and reduces aged-cell phenotypes. In this study, we found that transcriptional activation of the endogenous Oct4 gene by using the CRISPR/dCas9 activator system can efficiently ameliorate hallmarks of aging in a mouse model of Hutchinson-Gilford progeria syndrome (HGPS). We observed that the dCas9-Oct4 activator induced epigenetic remodeling, as evidenced by increased H3K9me3 and decreased H4K20me3 levels, without tumorization. Moreover, the progerin accumulation in HGPS aorta was significantly suppressed by the dCas9 activator-mediated Oct4 induction. Importantly, CRISPR/dCas9-activated Oct4 expression rescued the HGPS-associated vascular pathological features and lifespan shortening in the mouse model. These results suggest that partial rejuvenation via CRISPR/dCas9-mediated Oct4 activation can be used as a novel strategy in treating geriatric diseases.

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W. Stem Cell Biology [W-14]

Effects of integrin alpha 2 on the immunomodulating potential of human bone marrow mesenchymal stromal cells

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Human mesenchymal stromal cells (MSCs) are excellent candidates for regenerative medicine due to its pluripotency and paracrine functions. Here, we evaluated the way of improving 'MSC-ness' using in vivo like culture condition composed of functional polymers. Human bone marrow-derived MSCs were cultivated either on two-dimensional (2D) based regular tissue culture polystyrene or functional polymer based three-dimensional (3D) culture. As a result, 3D culture increased the expression of stemness marker including Oct4 and Nanog in MSCs. Also, COX2 and HO1 which are related to immunomodulatory function of MSCs were increased. Moreover, MSCs on 3D culture elevated the secretion of PGE_2 and highly suppressed TNF alpha from LPS stimulated splenocytes. Finally, intravenously injection of MSCs on 3D significantly reduced the expression of Iba1 and GFAP in brain of mouse neuroinflammation model, compared to the intravenous injection of MSCs on 2D.

Taken together, these results demonstrated that in vivo mimetic 3D culture provides MSCs beneficial microenvironment improving immunomodulatory function, eventually suggesting that engineered MSCs can be utilized as an effective source for cell therapy.

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W. Stem Cell Biology [W-15]

Surface conjugation of microspheres carrying anti-fibrotic or cell-protective drugs on mesenchymal stem cells potentiates the therapeutic efficacy against pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is an interstitial lung disease with a poor prognosis characterized by progressive pulmonary fibrosis. Current treatments for pulmonary fibrosis have been reported to slightly delay the progression of fibrosis, therefore, promising drugs that can significantly reduce or reverse fibrosis should be developed. Mesenchymal stem cells (MSCs) have been reported to have an anti-fibrotic effect by producing various paracrine factors, and MSCs therapy could be an alternative treatment. In this study, we aimed to investigate the anti-fibrotic efficacy of MSCs with anti-fibrotic or cell-protective drugs using surface microsphere-conjugating techniques. We found that MSCs reduced TGF-β1-induced fibrosis of lung fibroblasts, determined by the expression of α-SMA, collagen and fibronectin. Furthermore, we found that anti-fibrotic or cell-protective drugs enhance the anti-fibrotic effect through the elevation of HGF and PGE2 production from MSCs. Co-treatment of these drugs with MSCs reduced fibrosis *in vitro*. In addition, MSCs conjugated with drug-loaded microspheres exhibited higher efficacy against fibrosis compared to sole treatment of MSCs or drugs. These results suggest that MSCs conjugated with microspheres on their surface can exert improved therapeutic efficacy against pulmonary fibrosis.

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W. Stem Cell Biology [W-16]

Suppression of lipid accumulation in the adipocyte differentiation of 3T3-L1 preadipocytes and human adipose stem cells by XMD8-92, an inhibitor of ERK-5

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Obesity is linked to excessive preadipocyte differentiation. Little is known about the expression and phosphorylation ofextracellular signal-regulated kinase 5 (ERK5) and its pharmacological inhibition of by XMD8-92, an ERK-5 inhibitor, in preadipocyte differentiation. Interestingly, treatment with XMD8-92 at 4 mM led to a marked suppression of lipid accumulation during 3T3-L1 preadipocyte differentiation with no cytotoxicity. XMD8-92 treatment at 4 mM strongly reduced the phosphorylation levels of ERK-5 during 3T3-L1 preadipocyte differentiation. Moreover,XMD8-92 treatment at 4 mM caused the reduced expression and phosphorylation levels of CCAAT/enhancer-binding protein-α (C/EBP-α), peroxisome proliferator-activated receptor gamma (PPAR-γ), fatty acid synthase (FAS), perilipin A, and signal transducer and activator of transcription-3/5 (STAT-3/5) in 3T3-L1 preadipocyte differentiation. However, treatment with XMD8-92 at 4 mM had no lipolytic effect on mature 3T3-L1 adipocytes, as evidence by no glycerol release and no hormone-sensitive lipase (HSL) phosphorylation on S563.Importantly, treatment with XMD8-92at2 or 4 mMcould inhibit lipid accumulation during the differentiation of human adipose stem cells (hASCs) into adipocytes. Collectively, these results demonstrate that XMD8-92 has strong anti-adipogenic effects on the adipocyte differentiation of 3T3-L1 preadipocyte and hASCs, mediated through control of the ERK-5, C/EBP-α, PPAR-γ, STAT-3/5, FAS, and perilipin A.

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W. Stem Cell Biology [W-17]

MiR495-3p attenuates neuronal apoptosis in rats with ischemic stroke through activation of the Wnt and MAPK pathway

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Ischemic stroke accounts for over 80% of the stroke and the population is gradually increasing. Neurons and blood vessels are impaired after ischemic stroke, and damage to the BBB is one of the major outcomes of ischemia. EVs could play important roles in an ischemic stroke, however, the mechanisms involved in EVs-derived miRNA remain unclear. In this study, we examined the neuronal cell viability treated of miRNA495-3 in oxygen-glucose deprivation (OGD), which neuronal ischemia-reperfusion injury in HT22 cells. As a result, WNT1, GSK3b, and JNK are upregulation, affecting the WNT pathway. To understand the therapeutic effects of the exosome, they were injected into the ventricle of the transient MCAo rat after 3 days. Following behavior tests, we found that motor function was improved compared to the sham group. In addition, the brain infarct volume was decreased with miRNA 495-3 treatment. We hypothesized that injecting miR495-3p into the ischemic brain can weaken apoptosis and regulate neurogenesis by WNT and MAPK mechanisms. By injecting of MCAo model with miR495-3p, the protein level of MEK2, MK4, JNK, RLK, and p38a are upregulated. Our results suggest that miR495-3p has a beneficial effect following reduced cell death through the MAPK mechanism.

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W. Stem Cell Biology [W-18]

Anti-inflammatory effects of dental mesenchymal stem cells

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Adult stem cells not only have the ability to differentiate into various tissues, but also have anti-inflammatory and immunomodulatory effects, so they have excellent disease healing ability. Among them, it is emphasized that oral stem cells also need to understand the biological function of inflammation and immune response. Inflammation is mainly caused by macrophages residing in tissues, forming a protein complex called NLRP3 inflammasome, which appears when the secretion of important cytokines such as IL-1β increases. Through this study, we tried to show that oral stem cells suppress macrophages' inflammasome activity and exhibit anti-inflammatory effects. It was confirmed that the inflammatory environment was induced by establishing a joint culture system of macrophages and stem cells, reducing the expression of inflammatory cytokines IL-1, 6, 8 and marker CD11b. LPS stimulation formed an inflammasome protein complex in macrophages, and extracellular ATP and mitochondrial ROS activated the inflammasome to cleavage procaspase-1 to cascase-1 and eventually produced IL-1b, IL-18. The results show anti-inflammatory effects in macrophages and stem cell co-culture systems.

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W. Stem Cell Biology [W-19]

Increased sensitivity of endothelial cell from HHT disease modeling derived from gene modified human pluripotent stem cells

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Hereditary Hemorrhagic Telangiectasia (HHT) is genetic vascular disorder that results from arteriovenous malformations without capillary maturation, leading to abnormal connections between arteries and veins. HHT is caused by mutations in several genes, including Endoglin, which is known as an essential endothelial cell co-receptor of the transforming growth factor β (TGF-β).

This study established an HHT disease model using PSCs through gene editing of the Endoglin gene. The results demonstrated that the ENG+/- cell line, which exhibited reduced protein levels of Endoglin, also showed decreased proliferation ability in endothelial cells compared to control cells. Additionally, we confirmed that endothelial cells derived from the ENG+/- hPSCs exhibited increased sensitivity to TNF-α and PM2.5 treatment, while no significant differences were observed in terms of smooth muscle cells.

Furthermore, blood vessel organoids were generated from ENG+/- and WT-hPSCs, and the blood vessel organoids from the ENG+/- cell line showed increased sensitivity to TNF-α and PM2.5 treatment. These findings suggest that blood vessel organoids from the ENG+/- cell line represent a more physiologically relevant model of HHT disease, which can be used to investigate the underlying mechanisms of HHT pathogenesis and future therapeutics.

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W. Stem Cell Biology [W-20]

HLA DR genome editing with TALENs in human iPSCs produced immune-tolerant dendritic cells.

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Although human induced pluripotent stem cells (iPSCs) can serve as a universal cell source for regenerative medicine, the use of iPSCs in clinical applications is limited by prohibitive costs and prolonged generation time. Moreover, allogeneic iPSC trans-plantation requires preclusion of mismatches between the donor and recipient human leukocyte antigen (HLA). We, therefore, generated universally compatible immune non-responsive human iPSCs by gene editing. Transcription activator-like effector nucleases (TALENs) were designed for selective elimination of HLA DR expression. The engineered nucleases completely disrupted the expression of HLA DR on human dermal fibroblast cells (HDF) that did not express HLA DR even after stimulation with IFN-γ. Teratomas formed by HLA DR knockout iPSCs did not express HLA DR, and dendritic cells differentiated from HLA DR knockout iPSCs reduced CD4+ T cell activation. These engineered iPSCs might provide a novel translational approach to treat multiple recipients from a limited number of cell donors.

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W. Stem Cell Biology [W-21]

MKRN1-mediated modulation of AMPK activity is crucial for pluripotency acquisition and maintenance

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Somatic cell reprogramming involves a significant metabolic conversion that is essential for successful pluripotency acquisition. While recent studies have identified a shift from oxidative phosphorylation to glycolysis as a critical factor for pluripotency acquisition, the molecular mechanisms underlying metabolic conversion remain unclear. In this study, we investigated the process of metabolic conversion using metabolic and genetic analyses during somatic cell reprogramming. We found that the process of somatic cell reprogramming itself conflicts with the mechanism that maintains energy homeostasis of somatic cells, and successful somatic cell reprogramming requires inhibition of AMP-activated protein kinase (AMPK) activity. We found that among the regulators of AMPK activity, MKRN1 was specifically expressed in SSEA4, TRA-1-60 positive cells. We identified that MKRN1 directly regulates the activity of AMPK and contributes to the acquisition of pluripotency through inhibiting energy homeostasis of the somatic cell, suggesting that MKRN1-mediated metabolic conversion is critical for successful somatic cell reprogramming. Our study also revealed that NANOG plays an important role in regulating the expression of MKRN1 during somatic cell reprogramming. By shedding light on the molecular mechanisms governing metabolic conversion in somatic cell reprogramming and identifying potential targets for improving its efficiency, our study provides valuable insights into this field.

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W. Stem Cell Biology [W-22]

Potential effects of MSC-derived exosomes treated with blueberry extract in ischemic brain injury

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Ischemic strokes caused by thromboembolic occlusion of the cerebral artery, account for more than 80% of all strokes. Ischemic brain injury also results in a loss of mitochondrial function and reducing ATP synthesis. Mesenchymal stem cells (MSC)-derived exosomes have been studied to recover nerve damage after ischemia, enhance vascular neural production, and have therapeutic effects in stroke models. According to many studies, the therapeutic use of blueberries inhibits TNF-a, IL6, and TRL4, which affects inflammatory control and immune action. In this study, we morphology and NGS analyzed the blueberry-treated MSC exosome (B-Exo). Results showed that the gene expression of ATP synthesis, such as Complex I (ND1, ND4L, and ND5), Complex III (CYTB), Complex IV (COX2), and ATP synthase is upregulated. Whether B-Exo affected an increase of neuronal cell viability in ischemic brain injury *in vitro* and *in vivo*. The results showed a significant decrease in the treatment of miR-5787 in the oxygen-glucose deprivation (OGD) group in HT22 cells, suggesting that B-Exo treatment promoted viability and decelerated cell apoptosis. It was confirmed that B-Exo treatment reduced the infarct area in the MCAo model. In conclusion, the B-Exo has a therapeutic effect via the regulation of inflammation and ATP synthase.

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X. Vascular Biology [X-1]

Trelagliptin, a DPP-4 anti-diabetic drug, dilate rabbit aorta by activation of Kv channels and SERCA pumps

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We investigated the vasodilatory effects of trelagliptin and its related mechanisms using rabbit aortic rings. Trelagliptin induced vasodilation in a dose-dependent manner. Pretreatment with ATP-sensitive K^+ channel inhibitor, large-conductance Ca^{2+}-activated K^+ channel inhibitor, and inwardly rectifying K^+ channel inhibitor did not affect the vasodilatory effect of trelagliptin. However, pretreatment with the voltage-dependent K^+ (Kv) channel inhibitors significantly attenuated the vasodilatory effect of trelagliptin, suggesting that the vasodilatory effect of trelagliptin is associated with Kv channel activation. Although pretreatment with Kv1.5 and Kv2.1 subtype inhibitors did not affect the response to trelagliptin, pretreatment with a Kv7.X subtype inhibitor effectively reduced the vasodilatory effect of trelagliptin. Furthermore, SERCA pump inhibitors also significantly attenuated the vasodilatory effect of trelagliptin. These effects, however, were not affected by pretreatment with Ca^{2+} channel inhibitors, adenylyl cyclase/PKA inhibitors, guanylyl cyclase/PKG inhibitors, or removal of the endothelium. From these results, we concluded that the vasodilatory effect of trelagliptin was associated with the activation of Kv channels (primary the Kv7.X subtype) and SERCA pump.

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X. Vascular Biology [X-2]

Inhibition of voltage-dependent K+ currents of rabbit coronary arterial smooth muscle cells by the atypical antipsychotic paliperidone

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Paliperidone, an atypical antipsychotic, is widely used to treat schizophrenia. In this study, we explored whether paliperidone inhibited the voltage-dependent K^+ (Kv) channels of rabbit coronary arterial smooth muscle cells. Paliperidone reduced Kv channel activity in a concentration-dependent manner with a half-maximal inhibitory concentration (IC_{50}) of 16.58 ± 3.03 μM and a Hill coefficient of 0.60 ± 0.04. It did not significantly shift the steady-state activation or inactivation curves, suggesting that the drug did not affect the gating properties of Kv channels. In the presence of paliperidone, application of 20 repetitive depolarizing pulses at 1 and 2 Hz gradually increased inhibition of the Kv current. Further, the recovery time constant after Kv channel inactivation was increased by paliperidone, indicating that it inhibited the Kv channel in a use- (state)-dependent manner. Its inhibitory effects were reduced by pretreatment with a Kv1.5 subtype inhibitor. However, pretreatment with a Kv2.1 or Kv7 inhibitor did not reduce its inhibitory effect. We conclude that paliperidone inhibits Kv channels (mainly Kv1.5 subtype channels) in a concentration- and use (state)-dependent manner without changing channel gating.

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X. Vascular Biology [X-3]

Omarigliptin-induced vasodilation via activation of SERCA pump and Kv Channels in rabbit aorta

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We investigated the vasodilatory effect of omarigliptin, an oral antidiabetic drug in the dipeptidyl peptidase-4 inhibitor class using phenylephrine (Phe)-induced pre-contracted aortic rings. Omarigliptin dilated aortic rings pre-constricted with Phe in a dose-dependent manner. Pretreatment with the voltage-dependent K^+ channel inhibitor 4-aminopyridine significantly attenuated the vasodilatory effect of omarigliptin, whereas pretreatment with the inwardly rectifying K^+ channel inhibitor Ba^{2+}, ATP-sensitive K^+ channel inhibitor glibenclamide, and large-conductance Ca^{2+}-activated K^+ channel inhibitor paxilline did not alter its vasodilation. Pretreatment with the SERCA pump inhibitors thapsigargin and cyclopiazonic acid significantly reduced the vasodilatory effect of omarigliptin. Neither cAMP/PKA-related signaling pathway inhibitors nor cGMP/PKG-related signaling pathway inhibitors modulated the vasodilatory effect of omarigliptin. Removal of endothelium did not diminish the vasodilatory effect of omarigliptin. Furthermore, pretreatment with the nitric oxide synthase inhibitor L-NAME or small-conductance Ca^{2+}-activated K^+ channel inhibitor apamin, together with the intermediate-conductance Ca^{2+}-activated K^+ channel inhibitor TRAM-34, did not influence the vasodilatory effect of omarigliptin. In conclusion, omarigliptin induced vasodilation in rabbit aortic smooth muscle by activating voltage-dependent K^+ channels and the SERCA pump independently of other K^+ channels, cAMP/PKA- and cGMP/PKG-related signaling pathways, and the endothelium.

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X. Vascular Biology [X-4]

Lurasidone Blocks the Voltage-Gated Potassium Channels of Coronary Arterial Smooth Muscle Cells

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Lurasidone is a second-generation antipsychotic drug used to treat schizophrenia, mania, and bipolar disorder. The drug is an antagonist of the 5-HT_{2A} and D_2 receptors. No effect of lurasidone on the voltage-gated K^+ (Kv) channels has yet been identified. Here, we show that lurasidone inhibits the vascular Kv channels of rabbit coronary arterial smooth muscle cells in a dose-dependent manner with an IC_{50} of 1.88 ± 0.21 μM and a Hill coefficient of 0.98 ± 0.09. Although lurasidone (3 μM) did not affect the activation kinetics, the drug negatively shifted the inactivation curve, suggesting that the drug interacted with the voltage sensors of Kv channels. Application of 1 or 2 Hz train steps in the presence of lurasidone significantly increased Kv current inhibition. The recovery time after channel inactivation increased in the presence of lurasidone. These results suggest that the inhibitory action of lurasidone is use (state)-dependent. Pretreatment with a Kv 1.5 subtype inhibitor effectively reduced the inhibitory effect of lurasidone. However, the inhibitory effect on Kv channels did not markedly change after pretreatment with a Kv 2.1 or a Kv7 subtype inhibitor. In summary, lurasidone inhibits vascular Kv channels (primarily the Kv1.5 subtype) in a concentration- and use (state)-dependent manner by shifting the steady-state inactivation curve.

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X. Vascular Biology [X-5]

An optimized herbal medicine containing Scutellaria baicalensis Georgi, Alisma orientale Juzepzuk, and Atractylodes japonica Koidzumi has potent antiplatelet and antithrombotic activities

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Platelet-derived thrombosis is important in the pathogenesis of cardiovascular diseases. HTB is an optimized herbal medicine including *Scutellaria baicalensis*, *Alisma orientale*, and *Atractylodes japonica*. It is widely used in traditional medicine due to its anti-inflammatory and antioxidant effects. However, its antiplatelet and antithrombotic activities have not been completely validated. The current study aimed to examine the inhibitory effect of the novel herb formula HTB against platelet activation and thrombus formation. The antiplatelet activities of HTB via platelet aggregation, granule secretion, reactive oxygen species generation, and intracellular calcium mobilization were evaluated. Moreover, the antithrombotic effect of HTB via $FeCl_3$-induced arterial thrombus formation *in vivo* in mice was assessed. The inhibitory effect of HTB against primary hemostasis was investigated based on tail bleeding time. HTB treatment significantly inhibited glycoprotein VI-mediated platelet aggregation, granule secretion, reactive oxygen species generation, and intracellular calcium mobilization. Biochemical studies revealed that HTB inhibited glycoprotein VI-mediated platelet signal transduction during cell activation. Further, its antioxidant effect might be derived by reducing the phosphorylation of the $p47^{phox}$/Hic5 axis signalosome. These results suggest that HTB can be an effective therapeutic agent against thrombotic diseases.

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X. Vascular Biology [X-6]

Speckle pattern-based random number generators using blood flow

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The development of Random Number Generator (RNG) using a speckle pattern plays a key role in security for generating cryptographic keys. Recently, the speckle-based RNG focused on affordability and portability. However, to generate random numbers, they obtained statics speckle patterns by passing a laser beam through a volumetric scattering medium, which can hamper toward low cost and acquisition of random numbers with high speed. In this study, we introduced blood flow-inspired RNG that can generate 5 MHz of random numbers with a microfluidic device after processing two passes tuple von Neumann with a speed of 1.25 KHz. To guarantee randomness and unpredictability, we used the NIST (national institute of standards and technology) randomness statistical test suite for the confirmation of the randomness of this system. The designed microfluidic device can generate random numbers with an only small amount (5 μl) of a blood sample to capture speckle patterns. Blood flow-inspired RNG using whole blood will have a new potential toward low cost and high output in various fields, including cryptography, computer security, and data encryption.

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X. Vascular Biology [X-7]

EGT022, an RGD-containing recombinant disintegrin, inhibits VEGF-induced angiogenic process via targeting integrin β3 in endothelial cells.

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EGT022, an RGD-containing recombinant disintegrin from human ADAM metallopeptidase domain 15 (ADAM15), was developed to replace a functional snake venom disintegrin. EGT022 has been reported to stimulate vascular maturation of retinal blood vessels with promoting pericyte coverage by binding to integrin αIIbβ3 in platelet. Although previous studies indicated that the several RGD motif-containing disintegrins inhibit angiogenesis, the effect of EGT022 on Vascular endothelial growth factor (VEGF)-induced angiogenesis has not been determined yet. In this study, we evaluated the anti-angiogenic function of EGT022 on VEGF-induced endothelial cells. EGT022 was able to significantly inhibit angiogenesis including proliferation, migration, tube formation, and permeability in HUVEC cells. We also demonstrated that EGT022 directly binds to integrin αvβ3, dephosphorylated integrin β3, and inhibited phosphorylation of VEGF receptor-2 (VEGFR2). Moreover, EGT022 inhibited phosphorylation of Phospholipase C gamma1 (PLC-γ1) and activation of Nuclear Factor of Activated T-cell (NFAT), downstream signal pathway modulators of VEGF in HUVEC cells. These results clearly showed that EGT022 plays an anti-angiogenic role on endothelial cells, as a potent integrin β3 antagonist.

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X. Vascular Biology [X-8]

Influence of pro-inflammatory chemokines and cytokines induced by shear stress in monocytes/macrophages

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Endothelial cell loss is associated with the accumulation of monocytes or macrophages beneath the arterial surface, where cells are susceptible to mechanical stimuli such as shear stress. However, the effect of mechanical stimulation on monocytic cells have not yet been sufficiently described. To evaluate whether mechanical stress affects monocyte function, we investigated the expression of inflammatory molecules and surface proteins, whose levels change with shear stress in human THP-1 cells. Shear stress increased the inflammatory chemokine CCL2, enhancing the transcriptional and protein level shifts of monocytes and TNF-α and IL-1β. We identified that the surface levels of heat shock protein 70 (HSP70), HSP90, and HSP105 increased using mass spectrometry-based proteomics, which was confirmed by western blot analysis, flow cytometry, and immunofluorescence. Treatment with HSP70/HSP105 and HSP90 inhibitors suppressed the expression and secretion of CCL2 and monocytic cell migration, suggesting an association between HSPs and inflammatory responses. We also demonstrated increased HSP90 immunoreactivity and coexistence and colocalization of CD68-positive cells in atherosclerotic plaques of *ApoE* deficient mice fed a high-fat diet and human femoral artery endarterectomy specimens. These results suggest that monocytes/macrophages affected by shear stress polarize to a pro-inflammatory phenotype and increase surface protein levels associated with inflammatory responses.

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X. Vascular Biology [X-9]

Local delivery of sirolimus and rosuvastatin alleviates neointimal hyperplasia in rabbit injury models by modulating inflammation and AKT/mTOR/NF-κB signaling

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Coronary artery bypass grafting(CABG) is an effective treatment for obstructive coronary artery disease, but further studies are needed to explore long-term patency rates. In this study, we evaluated the potential therapeutic effect of a perivascular microneedle system that co-delivers sirolimus and rosuvastatin(SIR+RSV). The study was conducted on rabbit injury models divided into two groups: a SIR+RSV group and a control group. The efficacy of the local delivery of SIR+RSV was evaluated at three different time points, namely hyper-acute stage(Week 1), acute stage(Week 2), and chronic stage(Week 4). Our findings showed that the SIR+RSV group had a significant reduction in neointimal formation compared to the control group at the chronic stage. Moreover, the SIR+RSV group downregulated inflammatory factors IL-6, IL-1β, and TNF-α at the acute stage and decreased inflammation and proliferation-related protein AKT, NF-κB, and mTOR expression at the chronic stage, suggesting that the co-delivery of sirolimus and rosuvastatin through the microneedle system has the potential to reduce inflammation and inhibit neointimal hyperplasia through AKT/NF-κB/mTOR signaling, providing an additional therapeutic approach for patients with coronary artery disease.

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X. Vascular Biology [X-10]

A Novel PDK1 Inhibitor, JE89, Suppresses Tumor Angiogenesis and Growth via Blocking AKT Signaling Pathway

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Antiangiogenesis has emerged as a promising therapeutic strategy for cancer treatment, and our previous genetic study suggested that inhibiting the PH domain of 3-phosphoinositide-dependent kinase 1 (PDK1) could be a viable approach to suppressing pathological angiogenesis. In this study, we synthesized a novel PDK1 PH domain inhibitor, JE89 and demonstrated its antiangiogenic effects both in vitro and in vivo. We found that JE89 effectively inhibited VEGF-induced proliferation, migration, invasion and tube formation of HUVECs without apparent toxicity. Moreover, our Western blot analysis to investigate the underlying mechanism of JE89 revealed that the compound specifically suppressed PDK1/Akt pathway upon VEGF stimulation without affecting other VEGFR2 downstream signaling pathways. In addition, JE89 significantly suppressed VEGF-mediated vascular network formation in Matrigel plug assay. Importantly, oral administration of JE89 reduced microvessel density in tumor tissue, ultimately leading to retarded tumor growth in lung cancer A549 xenograft model. Taken together, our data determine that JE89 exerts antiangiogenic activity by suppressing PDK1/Akt signaling pathway. These findings suggest that JE89 may have great potential as a novel antiangiogenic therapy for the treatment of cancer.

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X. Vascular Biology [X-11]

Inducible endothelial Prmt1 ablation causes endothelial dysfunction associated with pulmonary thromboembolic hypertension

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The pulmonary endothelium plays a vital role in regulating vascular homeostasis, vascular tone, leukocyte trafficking, and the barrier function. Endothelial dysfunction is a significant instigating factor for pulmonary vascular pathogenesis. Protein arginine methyltransferase 1 (Prmt1) is responsible for asymmetric di-methylation of substrates on arginine residues and is implicated in a variety of cellular processes, including cardiovascular function. In current study, we investigated the function of Prmt1 in endothelial cells using mice with inducible endothelial-specific Prmt1 ablation (KO). Endothelial Prmt1 ablation in adult mice for eight weeks caused lethality accompanied by pulmonary thromboembolic hypertension. Prmt1 ablation for two weeks resulted in multiple abnormalities in the KO lungs, such as elevated inflammation, disruption of endothelial junctions, and increased apoptosis, indicating a crucial role of Prmt1 in pulmonary endothelial homeostasis. Single cell sequencing and ATAC analysis revealed that Prmt1 ablation transformed quiescent endothelial cells into an activated cell state, contributing to a chronic inflammation phenotype in KO mice.

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X. Vascular Biology [X-12]

The inhibitory effect of CU06-1004 on colitis-associated colorectal cancer mouse model

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Colitis-associated cancer (CAC) is a type of cancer that develops due to long-term exposure to chronic intestinal inflammation and fibrosis. Previous research has shown that CU06-1004, an endothelial dysfunction blocker, can attenuate acute colitis by inhibiting inflammation. In this study, we aimed to investigate the role of CU06-1004 in a colorectal cancer model. To induce the CAC mice model, we used azoxymethane (AOM) and dextran sulfate sodium (DSS). Our results showed that CU06-1004 treatment inhibited inflammation, as evidenced by disease activity index (DAI) scores, colon length, and histological analysis. Moreover, CU06-1004 treatment suppressed tumor growth by downregulating β-catenin expression in AOM/DSS-induced mice. Furthermore, administration of CU06-1004 suppressed the levels of inflammatory cytokines (TNF-α, IL-1β, and IL-6) in the serum, reduced the infiltration of immune cells (F4/80+macrophages and CD177+neutrophils), and decreased the expression of the fibrosis marker αSMA in the colon of AOM/DSS-induced mice. Our findings suggest that CU06-1004 has the potential to suppress tumor formation in the colon by reducing inflammation and fibrosis. Therefore, CU06-1004 may be a promising therapeutic candidate for the treatment of inflammation and colitis-associated tumorigenesis.