Alternative approaches for overcoming drug resistance in chemoresistant cancer or overcoming transient silencing effects of siRNA in cancer therapy

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Index

I.
Part 1

Challenges to overcome paclitaxel resistance in cancer therapy

Main issues in chemotherapy are …

Since paclitaxel was discovered,

(1) One of successful anticancer drugs

(2) an antimitotic agent, inducing cell death

Inherent or acquired drug resistance

(1) Reduce cytotoxicity of paclitaxel

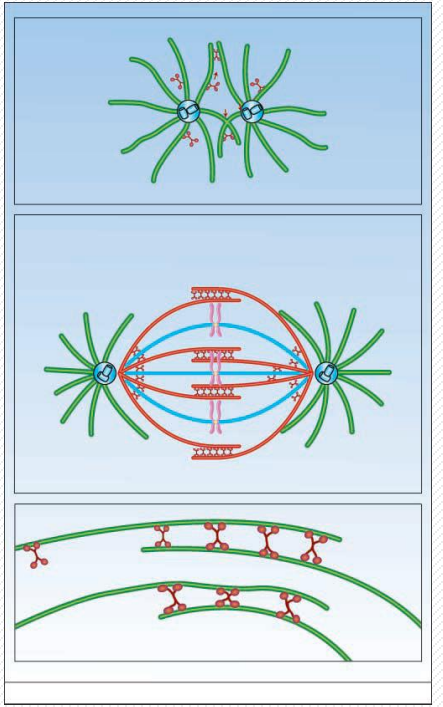
(2) Has limited its use in aggressive cancers due to a relapse of cancer

Since antimitotic agent PTX was discovered in cancer therapy,

(1) Much attention was focused on mitotic arrest
(2) Especially, Kinesin Spindle Protein (KSP)
KSP inhibition results in cell cycle arrest, eventually inducing cell death

Dozens of KSP inhibitors have been developed as anticancer drugs, however

(1) failed to be developed beyond clinical trials
(2) Because a different endogenous kinesin Kif15 can replace the function of KSP in aggressive cancers



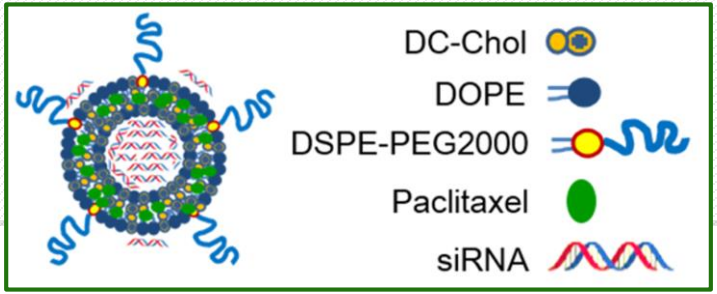
[KSP motors enable microtubules to form mitotic spindle]

Because paclitaxel binds to tubulin and can inhibit Kif15 motility in mitosis,

(1) A potential candidate as a Kif15 inhibitor
(2) "Idea" → a combination of KSP inhibitor and paclitaxel could fully induce mitotic arrest even in aggressive cancers

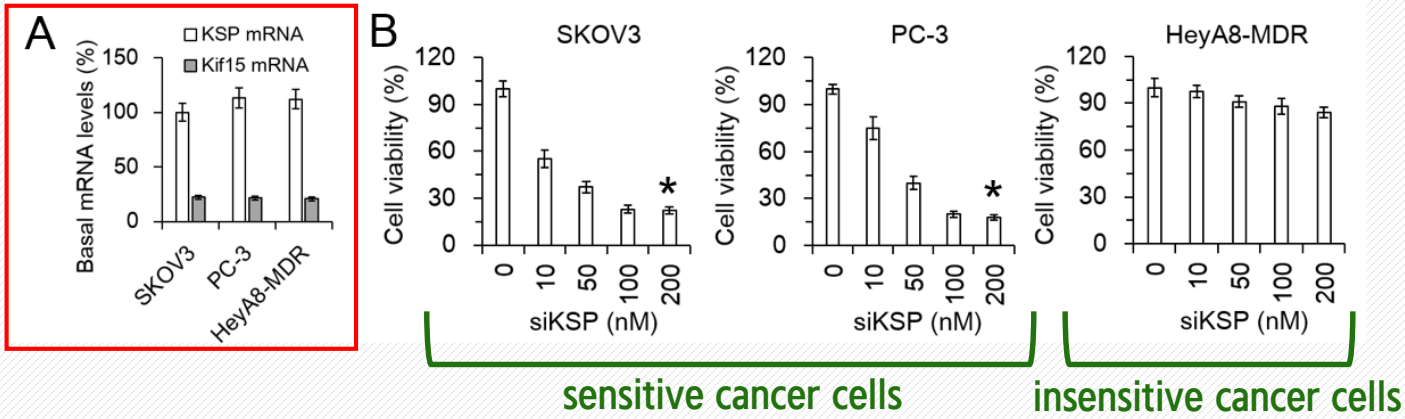
While applying RNAi technology against KSP, we evaluated synergistic antitumor effects of paclitaxel

(1) Target for chemoresistant HeyA8-MDR ovarian cancer
(2) Wished to overcome resistance against KSP inhibitors
(3) Wished to enhance therapeutic effects of paclitaxel in chemoresistant cancers
(4) "PEGylated cationic liposomes" loaded with KSP siRNA and paclitaxel

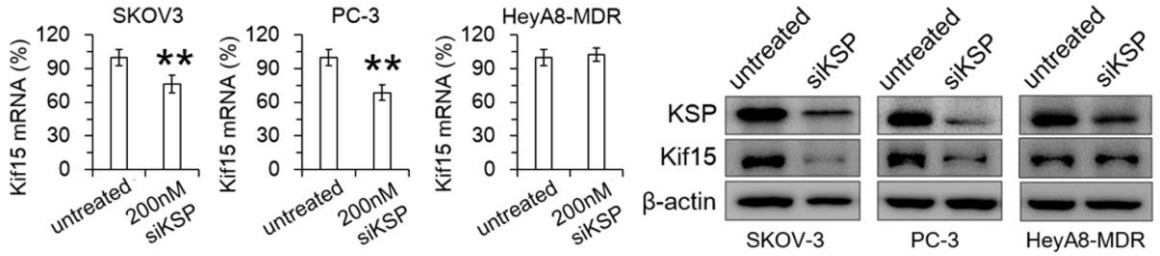


[structure of delivery carrier for PTX and KSP siRNA]

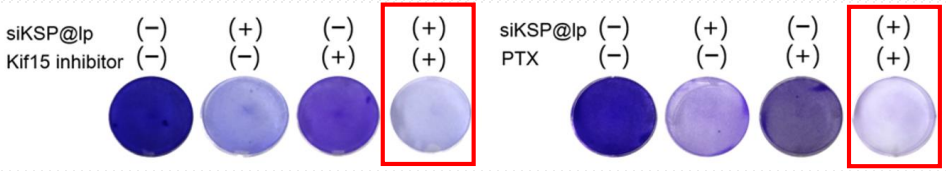
In vitro silencing effects of KSP siRNA in sensitive and insensitive cancer cells



When KSP was silenced, Kif15 expression was reduced in sensitive cancer cells, but not in insensitive cancer cells → implying that Kif15 replaces the function of KSP in HeyA8-MDR cells



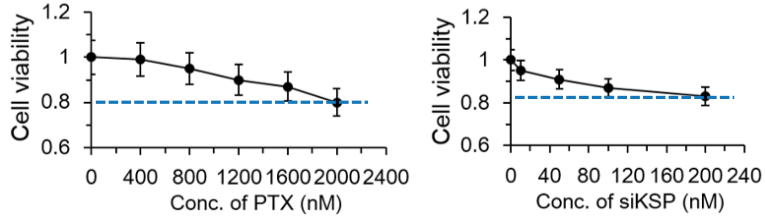
Paclitaxel functions as Kif15 inhibitors



In drug-resistant HeyA8-MDR

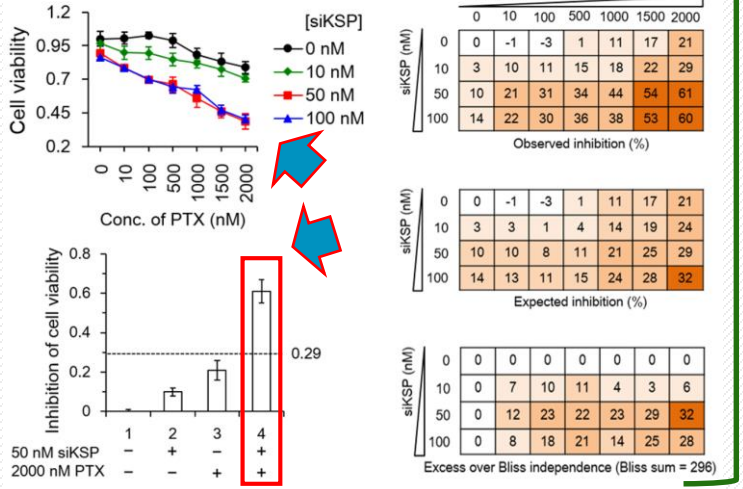
Synergistic antitumor effects of pairwise combinations of KSP siRNA and paclitaxel

→ indicating that a combination therapy can overcome resistance to KSP inhibition and paclitaxel



[Monotherapy of paclitaxel or KSP siRNA alone]

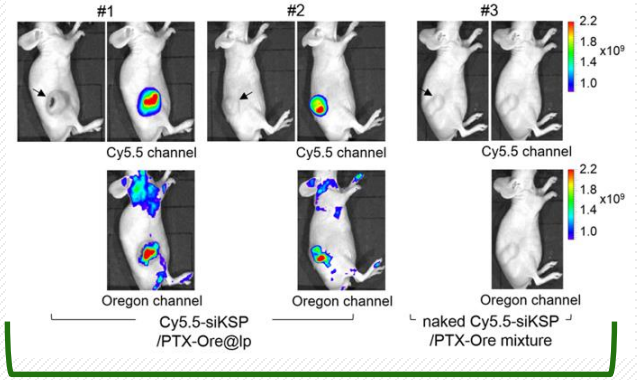
- The most prominent enhancement occurred in a combination of 2000 nM PTX and 50 nM siKSP (61.1%)
- The dotted horizontal line indicates no synergism



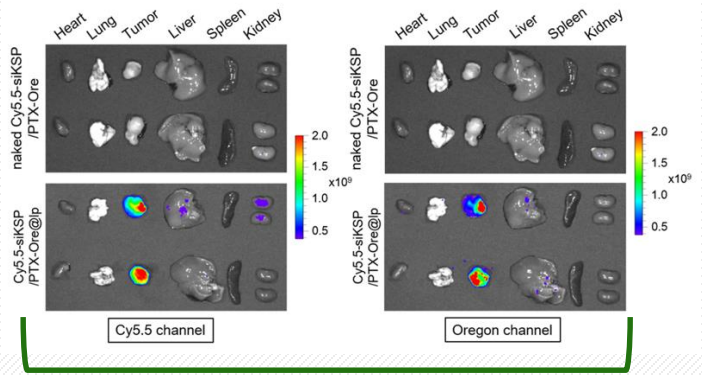
Bliss independence model

[Synergistic antitumor effects of combination of KSP siRNA and paclitaxel]

In vivo biodistribution of siKSP/PTX@lp in HeyA8-MDR-bearing mice



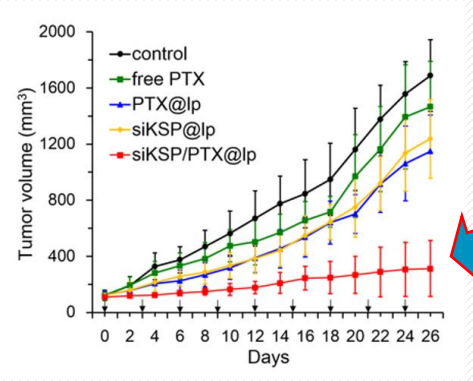
Whole body images

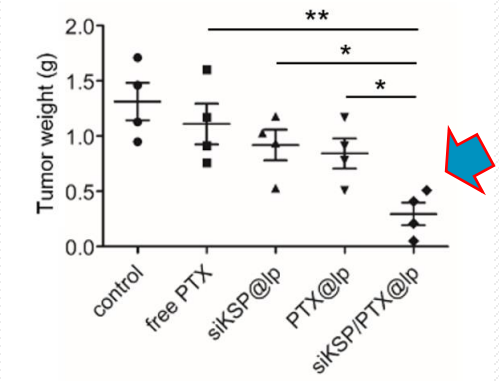


Ex vivo images

[tumor-specific co-delivery of KSP siRNA and paclitaxel]

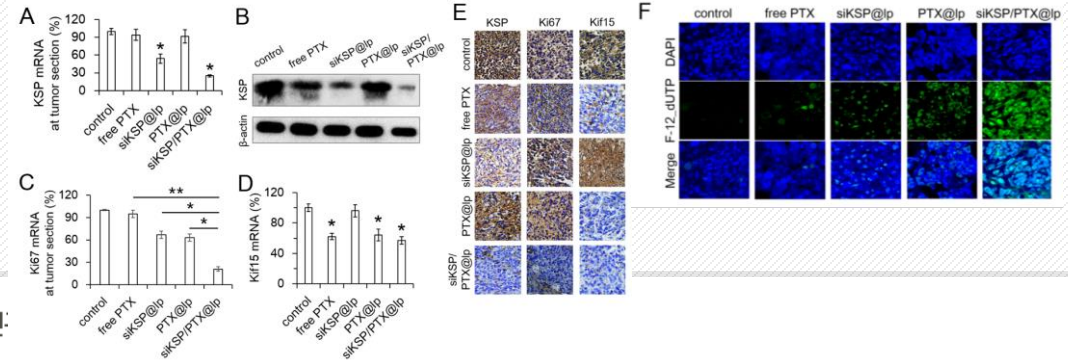
Synergistic antitumor effects of a combination therapy in chemoresistant cell line xenograft



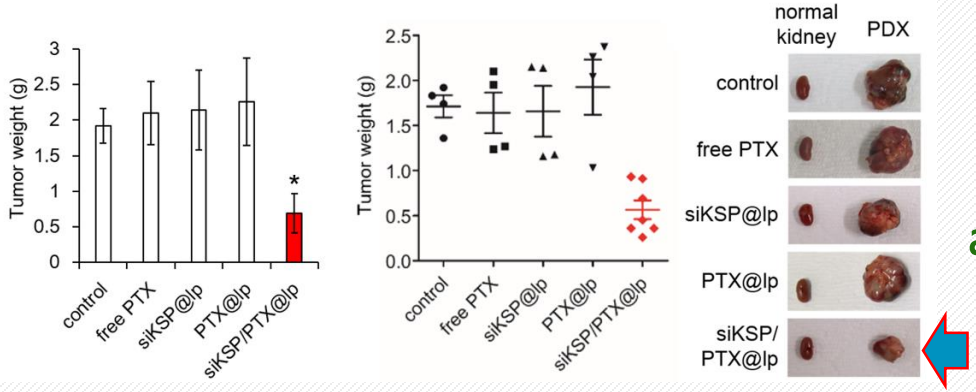


[HeyA8-MDR-bearing mice]

- *Ex vivo* assays show that expression levels of KSP, Ki67, and Kif15 were reduced in tumors
- Apoptosis occurred in tumor tissues



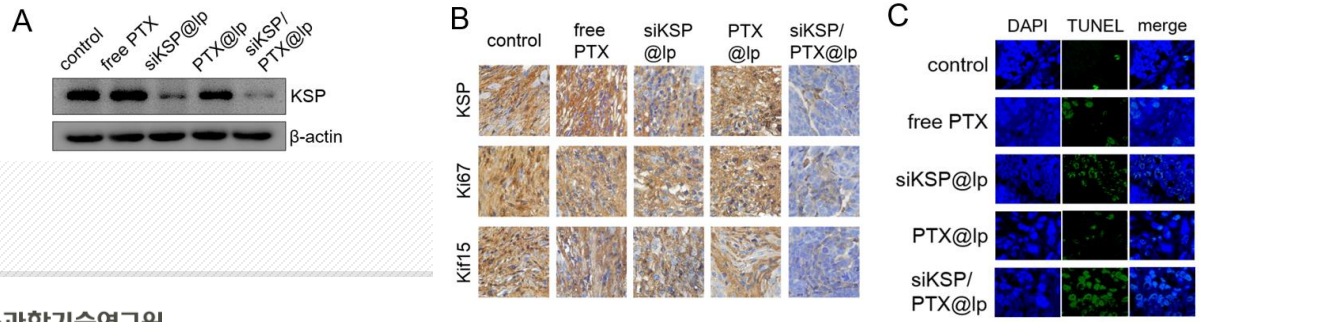
Synergistic antitumor effects of a combination therapy in chemoresistant PDX models



[chemoresistant PDX models]

62-year-old female with high-grade serous adenocarcinoma of FIGO stage IVB representing progressive disease at 5 months after primary debulking surgery followed by adjuvant paclitaxel-carboplatin chemotherapy classified as drug-resistant (OV-40, passage #9)]

- *Ex vivo* assays show that expression levels of KSP, Ki67, and Kif15 were reduced in tumors
- Apoptosis occurred in tumor tissues



IN SUMMARY

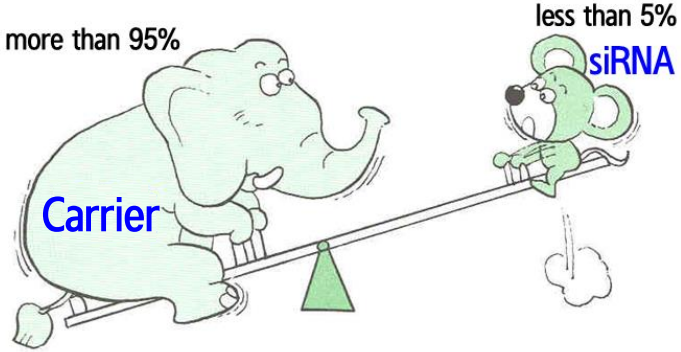
- Despite promising anticancer effects of KSP inhibitors, functional plasticity of kinesin induces resistance against KSP inhibitors, leading to clinical failure
- Paclitaxel is a widely used anticancer drug, but drug resistance has limited its use in the recurrent cancers
- Here, we paired KSP inhibition with microtubule stabilization using KSP siRNA and PTX
- Ultimately, we observed significantly improved therapeutic effects of a combined therapy in the drug-resistance *in vivo* models, including cell line xenograft and PDX models
- This work provides a potential strategy to overcome both resistance to KSP inhibitors and PTX
- This strategy can increase the therapeutic effects of PTX (2 μM) in the drug-resistant cancers (note that 2 μM is very low when compared to 15 ~ 20 μM used in clinical applications)

II.
Part 2

Challenges to overcome transient silencing effects of RNAi in cancer therapy

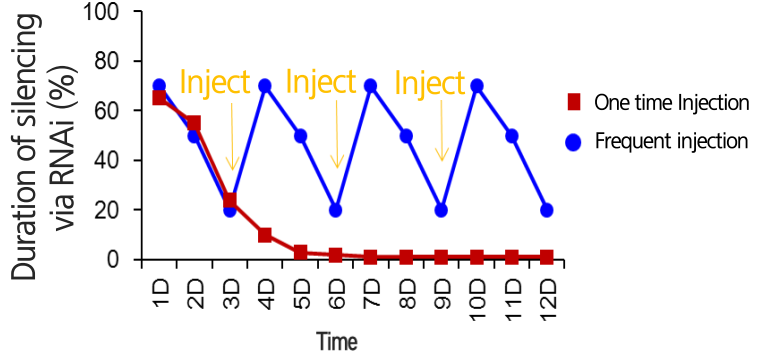
Main issues in RNAi therapeutics are …

- Issue of 'How much?' – how much siRNA can be delivered to sites of interest in body?
- Issue of 'How long?' – how long therapeutic effects can last at the site of interest in patient?



Low loading efficacy & toxicities of carriers themselves → side effects in high dosages → thus, it leads to poor therapeutic effects

How much? issue



Degradation of siRNA in cytoplasm → short duration of therapeutic effects → thus, too frequent injections happen

How long? issue

siRNA delivery carrier : viral & non-viral carriers

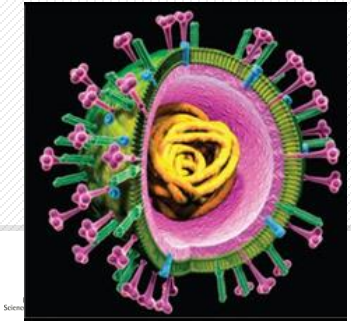
1) Viral carrier – adenovirus, lentivirus

advantage

- Long-term expression of shRNA
- Long-term therapeutic effects

disadvantage

- Insertion into human genome / incurring mutations
- Unsafety (patient death)
- Potentially immunogenic
- Lack of targeting ability



2) AAV (adeno-associated virus) virus

advantage

- No insertion into human genome / No incurring mutation
- Safe
- Low immunogenic

disadvantage

- Lack of targeting ability

Main issues in the use of AAV virus are ...

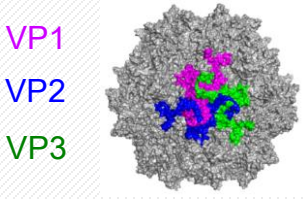
AAV virus has some advantage in RNAi,

(1) long-term expression ability
(2) Low immunogenicity and pathogenicity

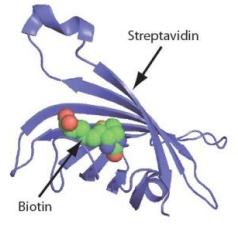
Important factor ;
Redirecting natural tropism of AAV to unique cell surface antigen

(1) Wild type of AAV has broad host tropism
(2) Lack of tissue specificity, limiting clinical use in cancer therapy

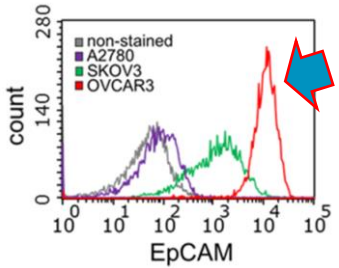
1) Strategy for retargeting AAV vectors



✓ AAV2 virus



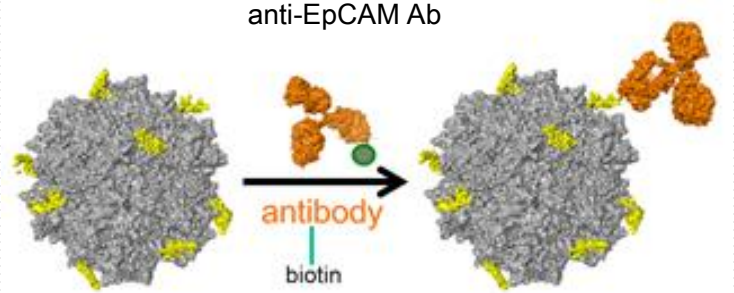
✓ High affinity between streptavidin and biotin

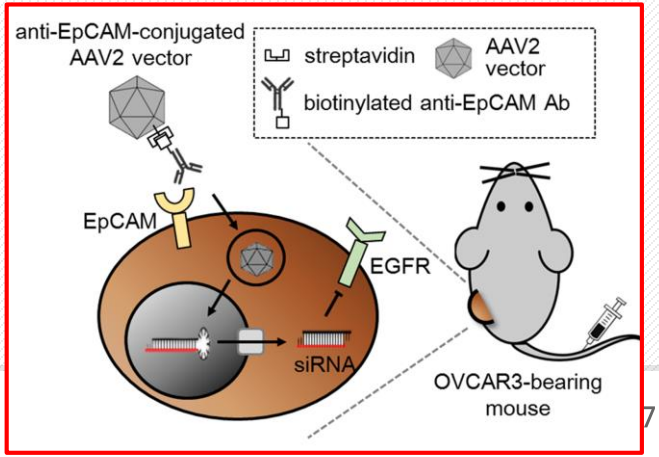


✓ Highly expressed EpCAM proteins at the surface of OVCAR3 cancer

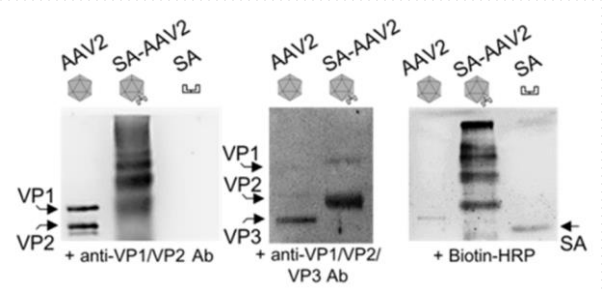
2) Conjugation of streptavidin to one of VP proteins

→ Complexing biotinylated antibody to surface of AAV

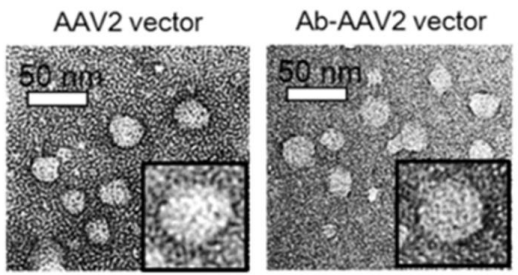




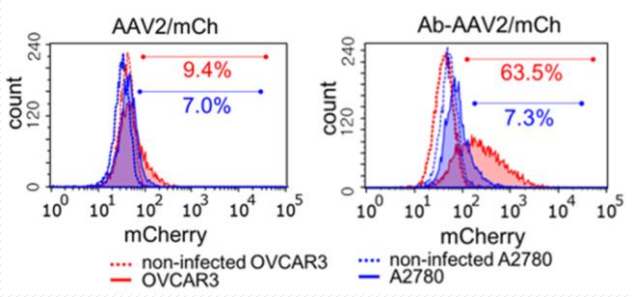
3) Formation of streptavidin-AAV complexes



EDC-NHS coupling reaction between amine residues of capsid and carboxylate residues of streptavidin

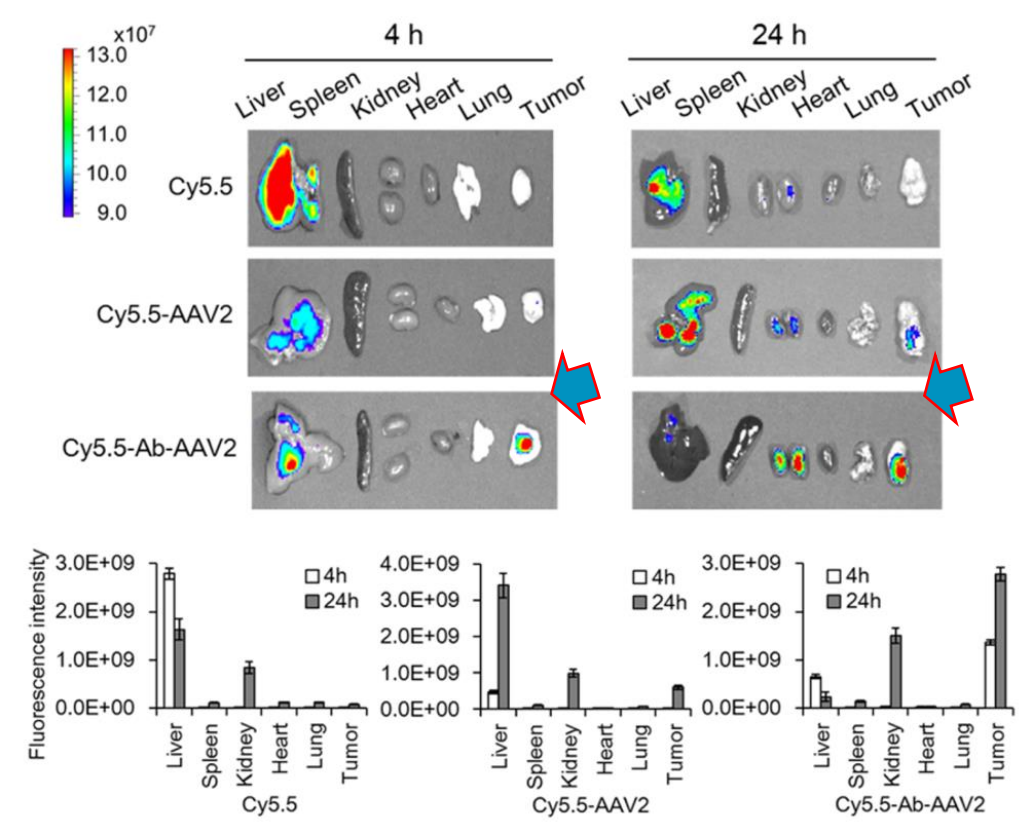


4) EpCAM-mediated transduction of anti-EpCAM-AAV2/mCh

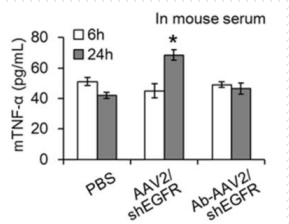


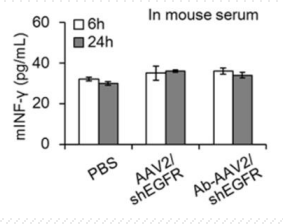
- OVCAR3 cancer – high expression level of EpCAM
- A2780 cancer – low expression level of EpCAM

5) Tumor-selective retargeting ability of anti-EpCAM-AAV2 complexes (I.V. injection)

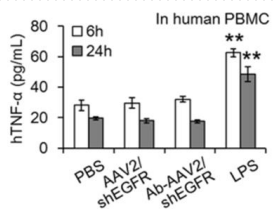


6) Low innate immunogenicity



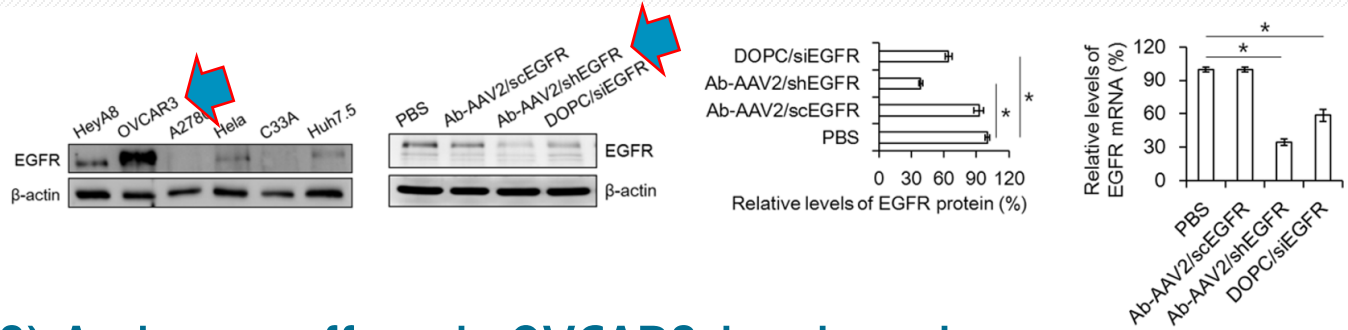


Low serum innate immunogenicity of anti-EpCAM-AAV2/shEGFR

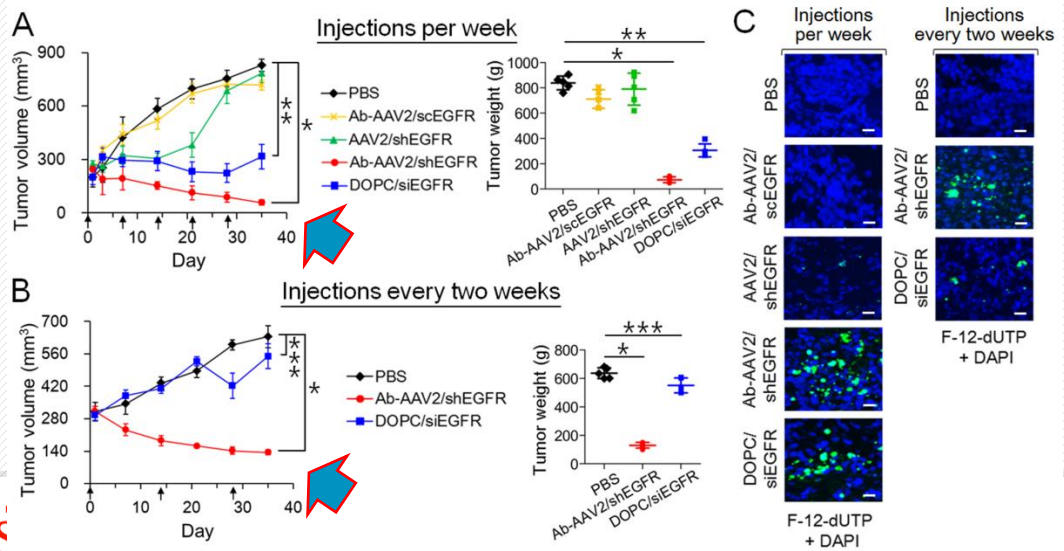


Low *In vitro* innate immunogenicity of anti-EpCAM-AAV2/shEGFR in human PBMC

7) High expression level of EGFR in OVCAR3 ovarian cancer



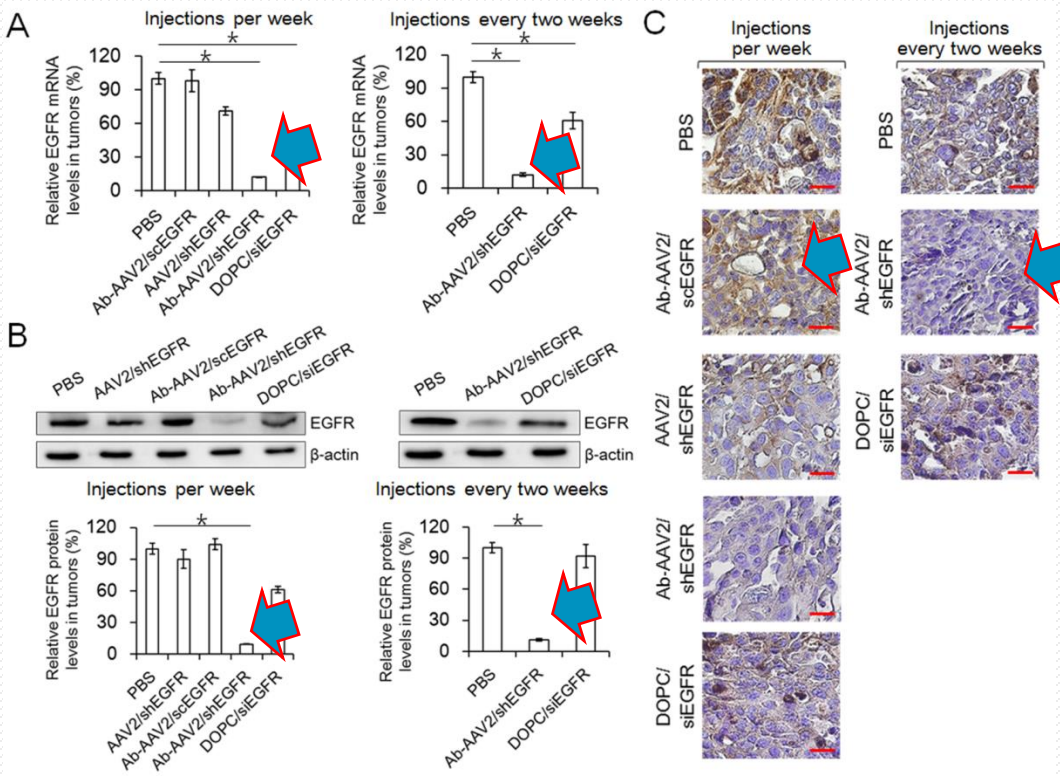
8) Antitumor effects in OVCAR3-bearing mice



[Injections per week]
Ab-AAV2/shEGFR and DOPC/siEGFR show delayed tumor growth

[Injections per two weeks]
Only Ab-AAV2/shEGFR shows delayed tumor growth
→ long-term silencing effects at tumor sites

9) Reduction of EGFR mRNA and protein at tumor tissues



IN SUMMARY

- Despite promising silencing effects of AAV vectors including long-term expression and low immunogenicity, lack of tissue specificity has limited their use in cancer therapy
- Redirecting the natural tropism of AAV vectors is required for cancer therapy
- To redirect AAV tropism, we here exploited EpCAM overexpression and anti-EpCAM antibodies through a SA-biotin bridge
- We observed prominent tumor accumulation of anti-EpCAM-AAV2/shEGFR virus in xenograft
- Ultimately, we observed significant suppression of tumor growth even at the long dosing interval of two weeks
- This work provides a potential strategy to redirect AAV2 vectors to tumors, as well as to overcome transient silencing effects of siRNA *in vivo*

I. Part 1 ;
Challenges to overcome drug resistance in chemotherapy

II. Part 2 ;
Challenges to overcome transient silencing effects of siRNA in cancer therapy

- **Research Partners**

  Prof. Hee Dong Han (건국대 의대)
  Prof. Jeong Won Lee (삼성서울병원) – PDX model
  Prof. Su Hwan Jang (서울아산병원)

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  Sharmin Seraj

Thank you

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