Overexpression of CYP11A1 induces G2/M arrest in Caki-1 cell line

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Abstract

Clear cell renal carcinoma is commonly known for its metastasis propensity to outspread to other organs and there are no symptoms in the early stage. Recent studies have shown that deficiencies of CYP11A1 expression can lead to fatal adrenal failure if not treated and are associated with downstream regulation in various cancer types. However, the molecular mechanisms between CYP11A1 and kidney cancer proliferation remained unclear. In this context, normal and renal carcinoma cell lines (Hek293 and Caki-1) were transfected with CYP11A1 to stimulate overexpression. Cell cycle distribution was investigated by flow cytometry. Western blot analyses were performed to search for the related signaling pathways. We observed that CYP11A1 suppressed the expression of cyclin B1 and cyclin-dependent kinase 1 but the cyclin-dependent kinase 2 and 4 were not altered. Cancer cell migration and invasion were suppressed along with epithelial-intermediate metastatic markers snail and vimentin. In addition, CYP11A1 overexpressed Caki-1 cell line resulted in downregulation of cdc2/cyclinB1 complex while increasing in phosphorylation of cdc25c, an upstream signal related to G2/M arrest. We also identified that the ERK/JNK/p38 pathway is an important mechanism for apoptosis in CYP11A1 overexpressed cell-based models. This finding might suggest that a promising new therapeutic target to suppress kidney cancer proliferation but has little effect on normal cells; thus, improving the survival rate of cancer patients.

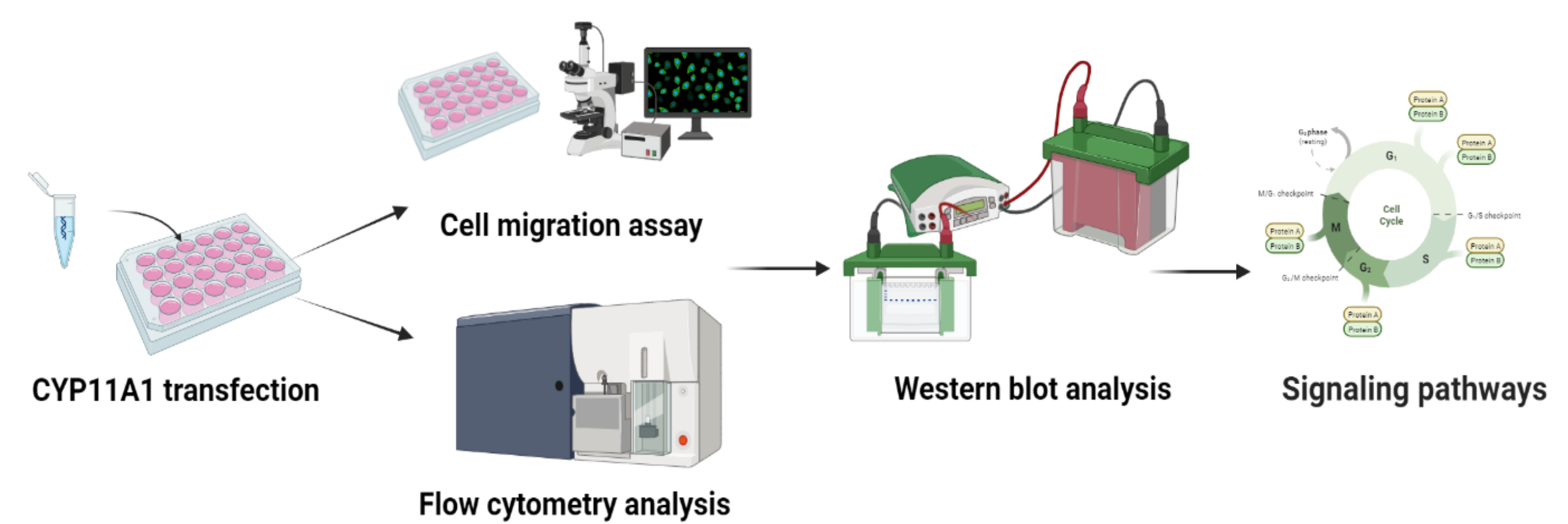**Keywords**: CYP11A1 overexpression, cell cycle, G2/ M arrest, kidney cancer.
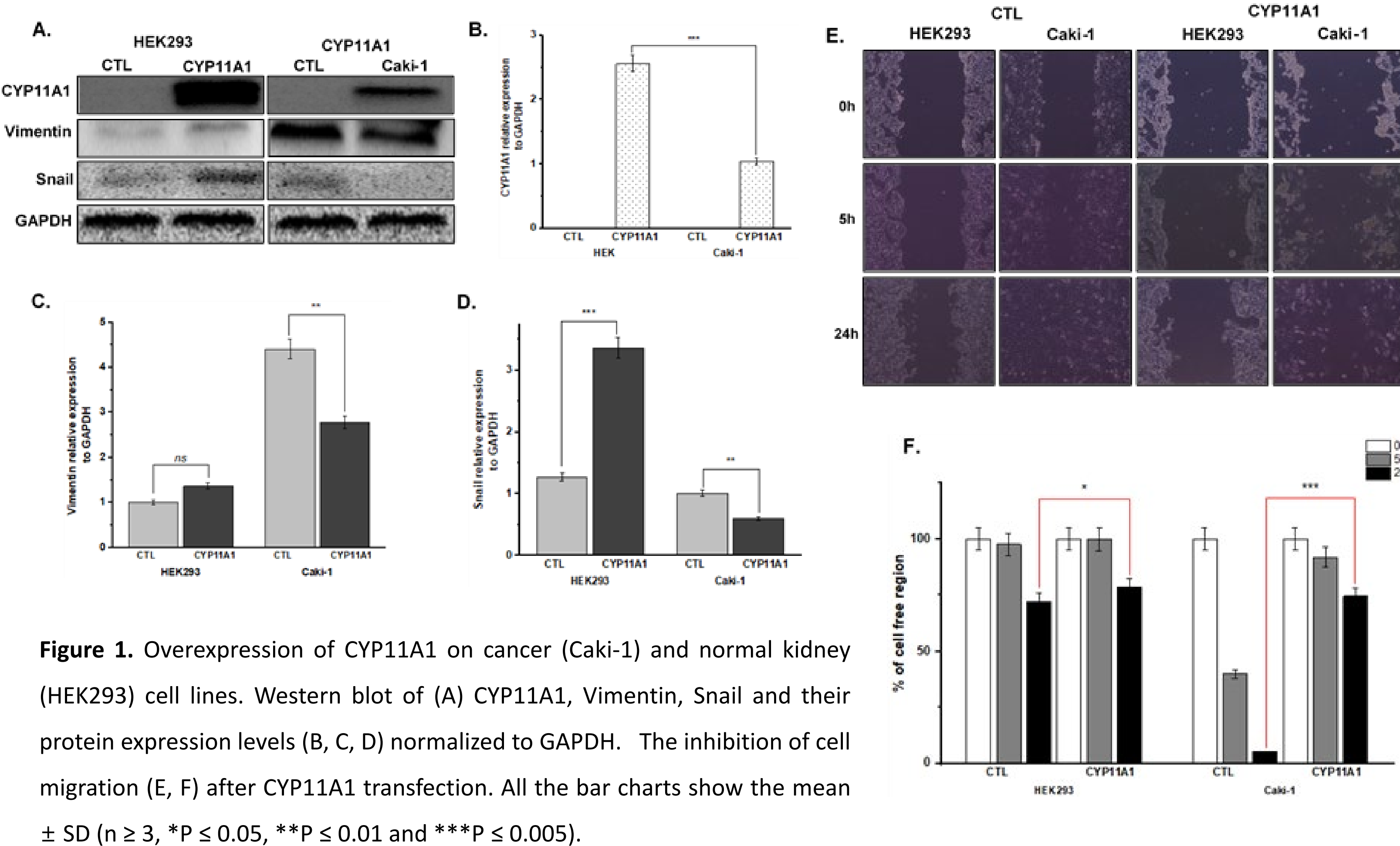
Graphic abstract
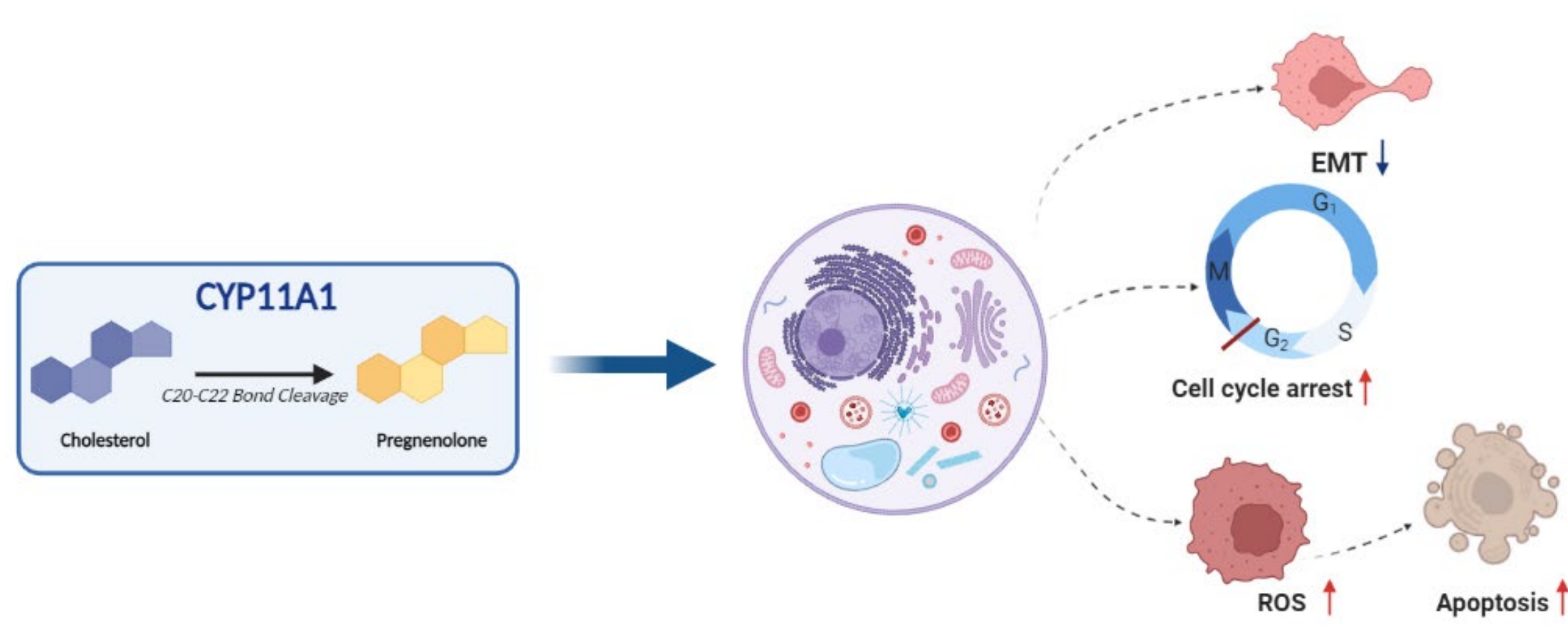
Materials and Methods

- **Cell lines**:

The Human embryonic kidney cell (HEK293) and clear cell renal carcinoma (Caki-1) were used to perform the experiments.

- **Plasmid DNA**:

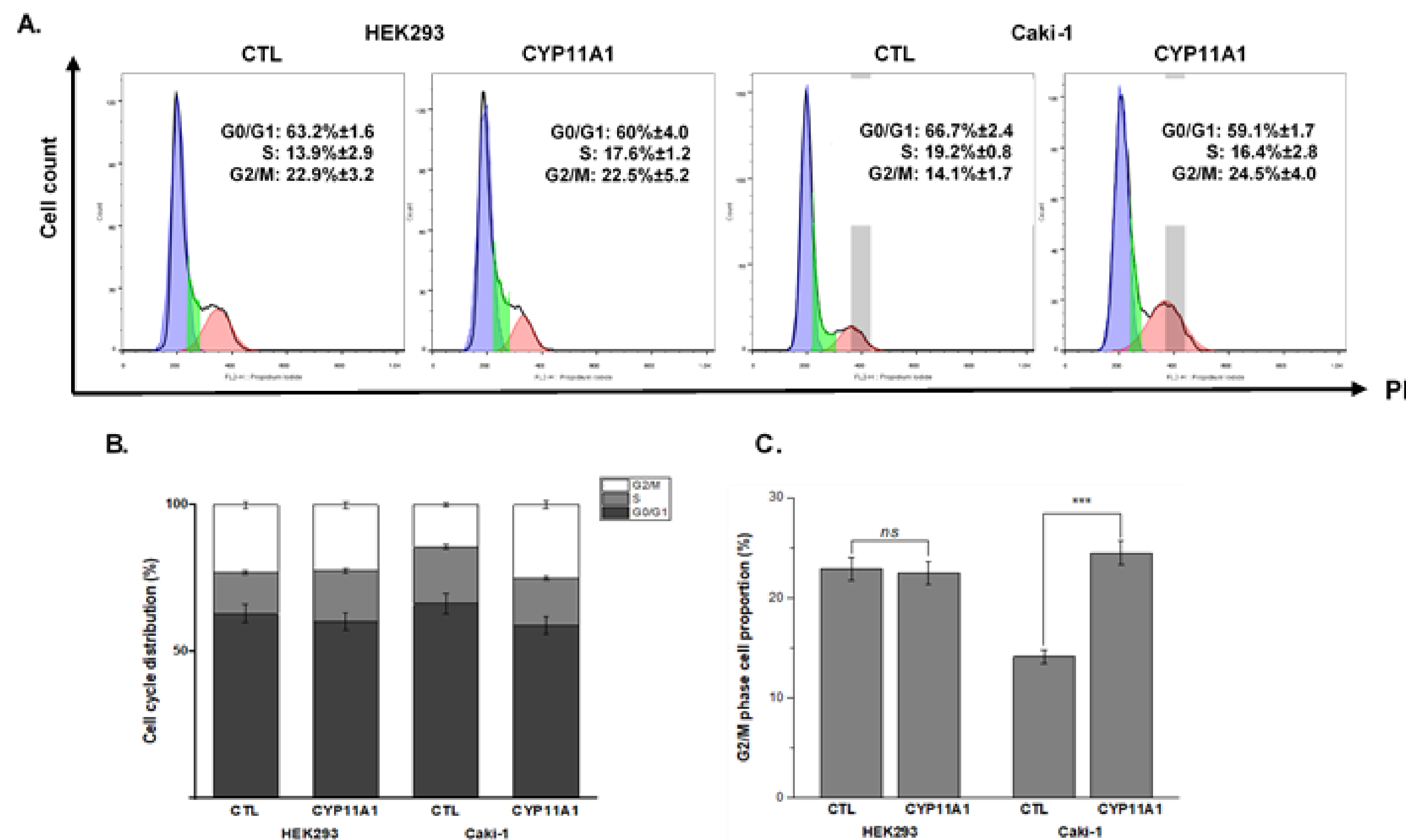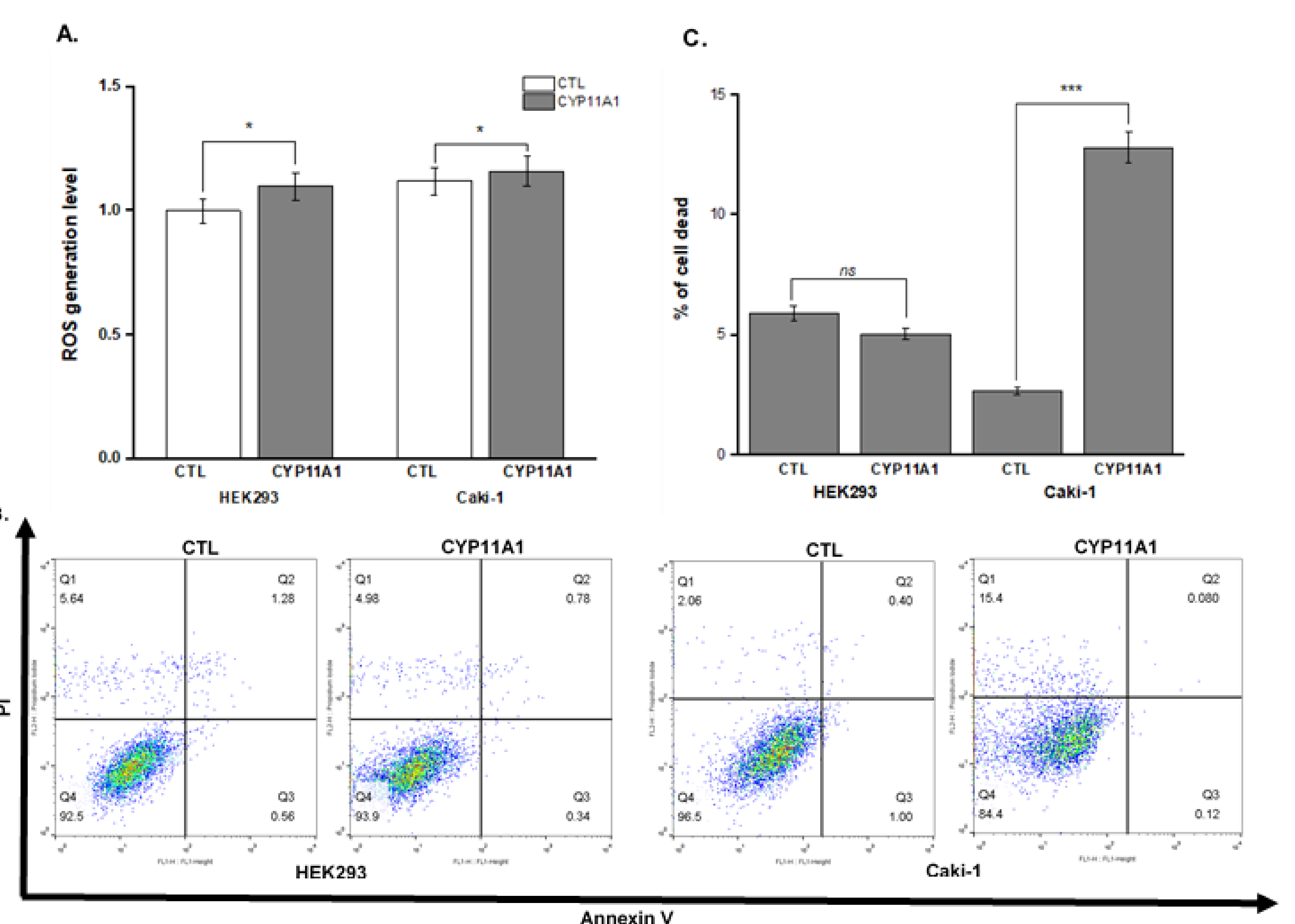CYP11A1 cDNA was obtained from Korea Gene Bank with a pCMV-SPORT5 vector (Clone ID: hMU004796).

Results

1. Overexpression of CYP11A1 inhibits the EMT process

Figure 1. Overexpression of CYP11A1 on cancer (Caki-1) and normal kidney (HEK293) cell lines. Western blot of (A) CYP11A1, Vimentin, Snail and their protein expression levels (B, C, D) normalized to GAPDH. The inhibition of cell migration (E, F) after CYP11A1 transfection. All the bar charts show the mean ± SD (n ≥ 3, *P ≤ 0.05, **P ≤ 0.01 and ***P ≤ 0.005).

2. CYP11A1 overexpression induces G2/M phase arrest in cancer cell line

Figure 2. G2/M phase arrest by CYP11A1-overexpressed cancer cell line. (A) DNA content of PI-stained cells was detected by flow cytometry. The bar charts show (B) the percentage of cells in each phase and (C) the total cell proportion in G2/M phase. All the bar charts show the mean ± SD (n ≥ 3, *P ≤ 0.05, **P ≤ 0.01 and ***P ≤ 0.005)

3. CYP11A1 promoted reactive oxygen species and activated apoptosis

Figure 3. (A) ROS levels were determined by level of hydrogen peroxide, which are generated under conditions of oxidative stress. (B) Apoptosis of HEK293 and Caki-1 cells transfected with CYP11A1 for 24 h was detected using Annexin V-PI double-staining followed by flow cytometry. The bar charts show (C) The percentage of dead cells in each group. All the bar charts show the mean ± SD (n ≥ 3, *P ≤ 0.05, **P ≤ 0.01 and ***P ≤ 0.005)

Conclusion

Renal cell carcinoma (RCC) is a heterogeneous group of cancers arising from renal tubular epithelial cells [1]. Among the top-ten cancers worldwide, Renal Cell Carcinoma (RCC) is the most prominent kidney cancer in adults over 45 years old [2]. Recent studies have shown that deficiencies of CYP11A1 expression are associated with downstream regulation in various cancer types [3].

This research has been conducted with regard to CYP11A1 and RCC, we found that overexpressed-CYP11A1 in Caki-1 cells significantly inhibited RCC migration and decreased levels of epithelial to mesenchymal transition markers, followed by G2/M phase arrest. Our results also observed the increase of ROS level and apoptosis in RCC.

References

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Acknowledgements

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