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Long non-coding RNA GAS5 Modulates Gemcitabine Efficacy in Bladder Cancer via Regulation of Deoxycytidine Kinase Expression

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Introduction

Gemcitabine is a common used agent for therapy in bladder cancer. Our objective was to characterize the ability of Long non-coding RNA GAS5 to modulate the gemcitabine efficacy.

Methods

Expression of GAS5 was analyzed using RT-qPCR in 30 patients following radical cystectomy and gemcitabine treatment. All subjects signed written informed consent. The study was approved by the Hospital Ethics Boards prior to initiation. The correlation between GAS5 and deoxycytidine kinase (DCK) was further examined by evaluating DCK expression in cells that either overexpressed or knocked down GAS5. Gemcitabine efficacy was identified by CCK-8 assays. DCK and HuR were analyzed by western blotting and immunofluorescence.

Results

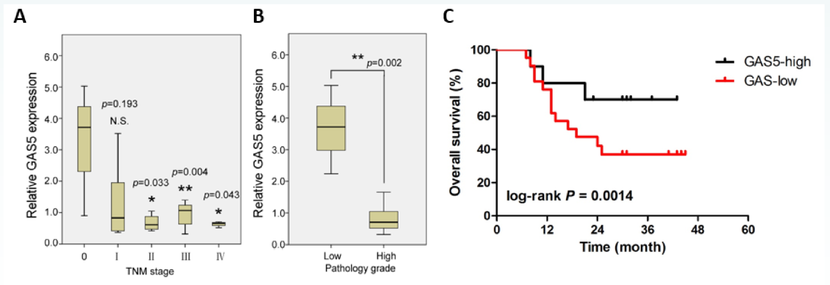

Fig.1 Lower GAS5 expression levels were associated with advanced pathological stages and poor overall survival.

Results

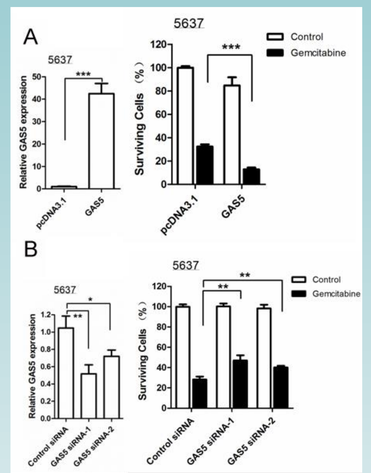

Fig.2 Gemcitabine can dose-dependently increase GAS5 level in bladder cancer cells. （A）GAS5-overexpressing cells displayed an elevated level of cell death induced by gemcitabine. （B） knockdown of GAS5 in bladder cancer cells by small interfering RNA conferred tolerance to gemcitabine-induced cytotoxicity.

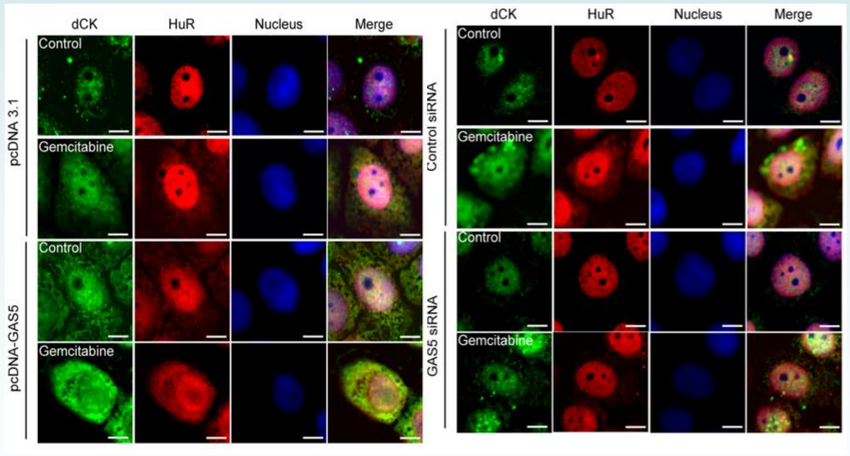

Fig.3 5637 cells were transfected with pcDNA-GAS5 or GAS5 siRNA for 24 h, and treated with 1μM gemcitabine for more 12 h, then stain with dCK antibody (Green), HuR antibody (Red) and DAPI (Blue). Scale bars: 10 μm.

Results

- **GAS5 was significantly downregulated in bladder cancer tissues compared with the paired adjacent non-tumorous tissues. LncRNA GAS5 expression is also lower in T24, 5637 and SW780 compared with the human normal urothelial cell line (Sv-Huc-1).**
- **Lower GAS5 expression levels were associated with advanced pathological stages and poor overall survival. GAS5 level was well correlated with the sensitivity of the cancer cells toward gemcitabine.**
- **Gemcitabine can dose-dependently increase GAS5 level in bladder cancer cells. Of note, pretreated gemcitabine attenuated GAS5-induced with a second dose of gemcitabine.**
- **GAS5-overexpressing cells displayed an elevated level of cell death induced by gemcitabine. Accordingly, knockdown of GAS5 in bladder cancer cells by small interfering RNA conferred tolerance to gemcitabine-induced cytotoxicity.**
- **We experimentally confirmed that GAS5 regulates DCK expression. The proapoptotic function of GAS5 was correlated with promoting HuR nuclear export and the biological consequence of deoxycytidine kinase expression.**

Conclusions

This study shows that long non-coding RNA GAS5 modulates HuR-mediated DCK expression, and GAS5 low expression confers gemcitabine tolerance. The expression of GAS5 may represent a novel companion diagnostic and target to undermine chemotherapy resistance.