Conclusion: High HK2 expression in bladder cancers induced oversecretion of lactate, which was associated with metastatic behaviors through the cancer stem cell formation, EMT promotion and nuclear translocation of phosphorylated NF-κB and Twist1. HK2 may be a novel oncoprotein and play as target for bladder cancer therapy.

Podium-2
Oncology

PD2-1:
ABERRANT EXPRESSION OF IRF6 IN RENAL CELL CARCINOMA

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Purpose: According to our previous results in methylated-CpG island recovery assay (MIRA) and RNA expression array, methylated status of *Interferon regulatory factor 6 (IRF6)* could be observed in most of renal cell carcinoma (RCC) cases, and presented a negative correlation with gene expression, especially in clear cell type of RCCs. The aim of this study is to clarify the clinical significance and role of IRF6 in RCC.
Materials and Methods: 105 pairs of clinical RCC patients and RCC cell lines have involved in the current study. Real-time PCR assay was used to detect the expression of IRF6 on all cases. Western blot assay was performed to detect whether the expression of IRF6 in the 5-aza-2'deoxycytidine treated RCC cells could be restored. The IRF6 gene expression level in normal and RCC tissues were shown by $-\Delta CT$ and applied by the paired-T test.
Results: The variant and lower level gene expression of the IRF6 could be observed in most of RCC cell lines. After cells treated with 5-aza-2'deoxycytidine, the expression of IRF6 was restored. In the real-time PCR of IRF6 in RCC tissues, the mean $-\Delta CT$ was -8.0 in normal tissue and -11.5 in RCC tissue with significantly different ($P = 0.013$).
Conclusion: Our findings demonstrated that the aberrant expression of IRF6 in RCCs was due to methylation. Also, the expression level of IRF6 was higher in normal tissues as compared with tumor tissues. Besides, it has been described that IRF6 could function as a tumor suppressor since it could inhibit tumor invasion and migration in squamous cell carcinoma. Based on these results, we suggest that IRF6 may play an important role in the pathophysiology of RCC. However, further cell viability and correlation with the clinical information should be further analyzed in the future.

PD2-2:
ENHANCED APOPTOSIS BY INHIBITION OF CISPLATIN-INDUCED AUTOPHAGY IN HUMAN BLADDER CANCER CELLS

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Purpose: Cisplatin has been used to treat bladder cancer (BC), however, cisplatin alone is not very effective, and the combinations of gemcitabine/cisplatin is now the first-line chemotherapy. Moreover, bladder tumor exhibits high basal level of autophagy. In this study, we investigated if cisplatin induces more autophagy in human BC cells, and whether inhibition of cisplatin-induced autophagy enhances apoptosis that leads to cancer cell death.
Materials and Methods: The autophagy status in cisplatin-treated RT4 (grade I), 5637 (grade II), and T24 (grade III) human bladder cancer cells was performed by the detection of microtubule-associated light chain 3 form II (LC3-II) and aggregation of LC3 puncta using Western blots and immunofluorescent staining, respectively. Moreover, the formation of autophagolysosome was detected using transmission electron microscopy to confirm the increased number of autophagosomes in cisplatin-treated T24 cells. The cell viability in cells treated with cisplatin with or without the autophagy inhibitor, bafilomycin A1 (BafA1), was accessed by WST-1 cell viability kit. To investigate the signaling pathway involved in cisplatin-induced autophagy, the activation of AKT, ERK, AMPK and MAPK and the inhibition of mTOR in cisplatin-treated cells were detected by Western blot. Induced apoptosis was determined by the detection of cleaved caspase 3, cleaved PARP, the caspase 3/7 activity and the level DNA fragmentation in treated-cells.
Results: The processing of LC3-II was elevated in cells treated with increased concentration of cisplatin, suggesting cisplatin induces autophagy. Detection of autophagy flux (by blocking autophgosome to lysosomes fusion using Baf A1) in 5637 and T24 cells, and the direct observation of autophagolysosome formation in cisplatin-treated T24 cells using TEM further confirmed that cisplatin indeed triggers autophagy. Advanced bladder cancer cells (5637 and T24) were more resistant to cisplatin than RT4, suggesting autophagy acts as a survival mechanism in high grade BC cells. While no response was found in AMPK, the activation of AKT, ERK and MAPK signaling and inhibition of mTOR was detected in cisplatin treated cells. However, pretreatment of specific inhibitors of ERK, MAPK did not attenuated cisplatin-induced autophagy suggests these pathways are not involved in the induction of autophagy. Finally, reduced cell viability and induced apoptosis were detected in cisplatin-treated cells pretreated with autophagy inhibitor suggesting that inhibition of autophagy enhances cancer killing effect of cisplatin in human BC cells.
Conclusion: Cisplatin induces autophagy in human BC cells, and autophagy inhibition enhances apoptosis in cisplatin-treated cells. This study suggests a new therapeutic paradigm for the treatment of bladder cancer.

PD2-3:
FORCED EXPRESSION OF MIR-30A-5P SENSITIZES BLADDER CANCER CELLS TO CISPLATIN VIA TARGETING ATG5 AND BECLIN-1

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Purpose: Autophagy is activated and may contributed to cisplatin-resistance in cisplatin-treated bladder cancer (BC) cells. It is reasonable to speculate that Inhibition of autophagy enhances the anti-cancer effects of cisplatin in BC cells. In this study, we characterized the role of miR-30a-5p, which is down-regulated in BC cells, in the coordination of apoptosis and autophagy by accessing its potential targeting protein, ATG5 and beclin-1 (BECN1).
Materials and Methods: The BC cell lines, 5637 (grade II) and T24 (grade III) and immortalized human uroepithelium cells (SV-HUC-1) were used in this study. To elevate the expression level of miR-30a-5p, a small RNA expression vector bearing matured sequence of miR-30a-5p (pSM-30a) was constructed and transfected into human BC cells. The expression level of miR-30a-5p was detected by stem-loop miRNA qPCR. Protein level of ATG5 and BECN1, both are predicted targets of miR-30a-5p, was accessed by Western blot. Autophagy detection in cisplatin-treated cells was performed by monitoring LC3-II processing by Western blot. Induction of apoptosis in cisplatin-treated cells with or without the over-expressed miR-30a-5p was detected by the detection of cleaved caspase-3 and PARP.
Results: The expression level of miR-30a-5p was elevated up to 8 fold in pSM-30a transfected BC cells according to miRNA qPCR. The autophagy activity in BC cells increased after cisplatin treatment as indicated by the enhanced processing of LC3-II. As ATG5 and BECN1 were predicted targets for miR-30a-5p by TargetScan, forced expression of miR-30a-5p significantly reduced the expression level of ATG5, BECN1 and LC3-II induced by cisplatin. The blockage of autophagy by miR-30a-5p expression or bafilomycin A1 (Baf A1) significantly decreased cell viability and increased apoptosis in cisplatin-treated BC cells.

[#] These authors contributed equally to this work.