Conclusion: High HK2 expression in bladder cancers induced over-secretion 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.

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 recovery assay (MIRA) and RNA expression array, methylated status of IRF6 in RCC tissues, the mean 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 treated RCC could be restored. The IRF6 gene expression level in normal and RCC tissues were shown by −ΔCT and applied by the paired-T test.

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 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 autophagosome 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 BafA1, 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, AMPK 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.
Conclusion: Our results demonstrate that miR-30a-5p can sensitize BC cells to cisplatin via suppressing ATG5 and BECN1 expression, therefore, increasing miR-30a-5p level in BC represents a novel strategy to enhance the efficacy of cisplatin therapy during cancer treatment.

PD2-4: CHRONIC KIDNEY DISEASE IS ASSOCIATED WITH UPPER TRACT UROTHELIAL CARCINOMA – A NATIONWIDE POPULATION-BASED COHORT STUDY IN TAIWAN

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Purpose: Increased urinary tract malignancy has been reported in end-stage renal disease (ESRD). However, little is known about chronic kidney disease (CKD). This study is designed to explore the association between CKD and upper tract urothelial carcinoma (UTUC).

Materials and Methods: Using Taiwan’s Longitudinal Health Insurance Database, we studied CKD patients between January 2000 and December 2011. The non-CKD controls were selected at a ratio of 4:1 and frequency matched by gender, age group and index date. We used Chi-square test and t-test to analyze the sociodemographic information and comorbidities. Cox regression analysis was used to calculate the hazard ratio (HR) and 95% confidence interval (CI).

Results: The selected cases included 45,321 CKD cases and 181,284 controls. A significantly higher incidence of UTUC was noted in the CKD group (0.22% vs. 0.07%, p<0.001). In univariate analysis, CKD, female gender, age, hypertension, hematuria, repeated urinary tract infection, bladder cancer and ESRD were all associated with UTUC. In multivariate analysis, only CKD, female gender, age, hematuria, bladder cancer and ESRD were significantly associated. The HR for CKD was 1.63 (95% CI: 1.26–2.13). Females had a higher HR of 1.38 (95% CI: 1.11–1.71). After excluding those patients who progressed to dialysis or kidney transplantation, the risk for CKD was still high, with a HR of 1.72 (95% CI: 1.33–2.33).

Conclusion: CKD is a significant factor associated with UTUC. We should pay attention to the possibility of UTUC for CKD patients before they progress to ESRD.

PD2-5: TUMOR CONTACT SURFACE AREA IS ASSOCIATED WITH VOLUME LOSS AND FUNCTIONAL DECLINE AFTER PARTIAL NEPHRECTOMY

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Purpose: We propose a formula of calculate-based contact surface area (CSA). We examined the correlation of contact surface area and renal volume loss and the predictability for renal function after partial nephrectomy.

Materials and Methods: We conducted a retrospective study in patients who underwent partial nephrectomy between January 2012 and December 2014. Based on abdominopelvic CT and MRI, we calculated the contact surface area with the formula "2π × "Radius"Depth"; while resected and ischemic volume (RAIV) was determined by the equation "2πw²+3w(r+d)+6rd"×w×m"³. We evaluated the correlation between CSA, RAIV and perioperative parameters. And we comparatively analyzed the ability of CSA and RAIV to predict the reduction in renal function.

Results: There were 35, 26, and 45 patients receiving OPN, LPN, RPN respectively. The mean±SD contact surface area was 30.7±26.1 cm², and the mean±SD RAIV was 19.1±14.4 cm³. On Spearman correlation analysis we found that CSA and RAIV were highly correlated (coefficient: 0.99, p<0.001). In univariate analysis, BMI (p=0.02) EBL (p=0.001), RAIV (p<0.001), and CSA (p<0.001) significantly affected postoperative renal function. In ROC curve analysis, both CSA and RAIV have good ability to predict more than 10% change of estimated glomerular filtration rate (AUC: 0.86 vs. 0.87). There is no significant difference in AUC between CSA and RAIV. The area difference in PCE10 was 0.002 (p=0.51).

Conclusion: In our study, CSA and RAIV were correlated with several perioperative outcomes and affected post-operative renal function. The ability to predict post-operative renal function between CSA and RAIV was nearly identical. Since CSA was simpler to use, and may possess less interobserver variability in comparison with RAIV, we believe that CSA can represent renal parenchymal loss.

PD2-6: THE IMPACT OF METHYLTIADENOSONE PHOSPHORYLASE (MTAP) DEFICIENCY IN PATIENTS WITH UPPER TRACT UROTHELIAL CARCINOMA

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Purpose: Urothelial carcinomas (UCs) involve recurrent chromosome 9p deletions. Methylthioadenosine phosphorylase (MTAP) on 9p21.3 is a proposed functional tumor suppressor gene. The role of MTAP in upper tract UC (UTUC) is unknown. We aimed to investigate MTAP’s association with disease characteristics and oncologic outcomes in UTUC patients undergoing radical nephroureterectomy (RNU).

Materials and Methods: Using immunohistochemistry, we investigated MTAP expression in 340 UTUC patients treated with RNU from1996–2004, and correlated it with clinicopathologic characteristics and clinical outcomes. Univariate and multivariate Cox regression analyses evaluated the association of MTAP expression with disease-specific survival (DSS) and metastasis-free survival (MeFS).

Results: MTAP was deficient in 119 (35.0%) patients. MTAP deficiency was significantly associated with higher pathologic stage (p<0.001), lymph node metastasis (p<0.001), high grade (p=0.008), vascular invasion (p=0.001), perineural invasion (p=0.001), and higher mitotic rate (p=0.016).Sixty (17.6%) patients died of UTUC and 70 (20.6%) developed metastasis. MTAP-deficient patients demonstrated significantly worse DSS (58.1% vs.89.3%; p<0.0001) and MeFS (54.7% vs.87.9%; p<0.0001) at five years than those with intact expression. MTAP deficiency was independently associated with cancer-specific mortality (hazard ratio [HR]:2.213; p=0.019; 95% confidence interval [CI]:1.141–4.293) and metastasis development (HR:2.867; p<0.001; 95% CI:1.601–5.106).

Conclusion: MTAP deficiency is associated with aggressive cancer phenotype and unfavorable oncologic outcomes, suggesting it may be a new biomarker and provide additional prognostic information in UTUC patients undergoing RNU.

Podium-3 Oncology

PD3-1: TPPP GENE ALTERATIONS AND ITS ROLE IN BLADDER CANCER

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