retrospectively as the derivation cohort to investigate the impact of inflammation markers on overall survival (OS) and cancer-specific survival (CSS). In turn, another independent set of 225 patients were used for validation. Finally, we performed survival analysis in the combined cohort consisting of 420 UTUC patients.
Results: The predictive value of RDW and WBC count on outcome was replicable in different cohorts. Multivariate analysis showed high RDW was independently associated with poor OS ($P < 0.001$), and WBC count was a significant prognosticator for both OS and CSS (both $P < 0.001$). In subgroup analysis, we found the prognostic significance of RDW for OS was limited in organ-confined disease (≤pT2 without pN+). More importantly, a clear survival difference can be demonstrated by combining RDW and WBC count with other known prognostic factors in the risk stratification model.
Conclusion: RDW and WBC count have the advantage of their common accessibility and are useful markers to predict outcome of UTUC in the preoperative setting. RDW and WBC count could provide additional prognostic value and help physicians identify patients at high risk for mortality and formulate individualized treatment strategy.

PD1-4:
ACRIDINE ORANGE EXHIBITS PHOTOTOXICITY AGAINST HUMAN BLADDER CANCER CELLS UNDER BLUE LIGHT EXPOSURE

Yi-Chia Lin [1], Ji-Fan Lin [2], Te-Fu Tsai [1,4], Hung-En Chen [1], Kuang-Yu Chou [1,4], Thomas I-Sheng Hwang [1,3,4]. [1] *Department of Urology, Shin Kong Wu Ho-Su Memorial Hospital, Taiwan;* [2] *Central Laboratory, Shin Kong Wu Ho-Su Memorial Hospital, Taiwan;* [3] *Department of Urology, Taipei Medical University, Taipei, Taiwan;* [4] *Division of Urology, School of Medicine, Fu-Jen Catholic University, Taipei, Taiwan*

Background: Human bladder cancer (BC) cells exhibited a high basal level of autophagic activity demonstrated by accumulating of acridine orange (AO)-stained acidic vesicular organelles (AVOs) in BC cells. In this study, we aim to investigate the cytotoxicity effects of AO on the human BC cell lines under blue-light exposure.
Materials and Methods: To evaluate phtotoxicities of AO toward human bladder cancer cells, we designed and developed a blue-light source equipped with 6 blue-light LED (peak wavelength: 443.7 nm). The AO relocalization in treated-BC cells was recorded using a fluorescence microscopy equipped with a color CCD camerain a real-time fashion. The cell viability was determined using (a) WST-1 reagents (immediately after treatment for 1 hour), (b) continuous quantification with Cytation 5 Cell Imaging Multi-mode reader (Biotek Instruments, Inc., for 24 hours), and (c) time-lapse imaging with a cell imaging recorder (Cytosmart System, Lonza; for 36 hours) in human immortalized uroepithelial (SV-Huc1) and BC cell lines (5637 and T24) treated with indicated concentration of AO with or without blue light exposure.
Results: The AO relocation was clearly monitored by fluorescent microscopy with decreased red fluorescent intensity over exposure duration within 5-15 secounds in BC cells. Treatment ofAO or blue-light exposure alone did not cause a significant decrease of cell viability in BC cells. However, we found that AO exhibited a dose-dependent increment of cytotoxicity toward BC cells with blue-light exposure (AO-PDT). In addition, this phenomenon was more prominent in human BC cell lines compared to SV-Huc1 cells. These results suggested that AO, as a photosensitizer, disrupts acidic organelles in BC cells under blue light irradiation in BC cells.
Conclusion: Blue light irradiation in BC cell treated with AO causes severe cell death. The photodynamic effect can be applied clinically to an existing instrument, namely narrow band image endoscopic system, to deliver blue light. The AO-PDT may serve as a novel therapeutic strategy to reduce recurrence or against human bladder cancer in the future.

PD1-5:
THE URINARY MICROPARTICLE TUMOR-ASSOCIATED CALCIUM-SIGNAL TRANSDUCER 2 AS A BLADDER CANCER BIOMARKER

Chien-Lun Chen [1,2], Petrus Tang [1,2], Chih-Ching Wu [1,2], Ting Chung [1,2], Jau-Song Yu [1,2], Yu-Sun Chang [1,2], Phei-Lang Chang [1,2], Yi-Ting Chen [1,2]. [1] *Department of Urology, Chang Gung Memorial Hospital, Linko, Taiwan;* [2] *Chang Gung University, Taoyuan, Taiwan*

Purpose: In contrast to PSA for prostate cancer, no reliable bladder cancer biomarker is currently widely applicable for the detection and follow-up of bladder cancer. We employed a strategy combining isotopic dimethylation labeling coupled with liquid chromatography-tandem mass spectrometry (LC–MS/MS) to discover bladder cancer biomarkers in urinary microparticles isolated from hernia (control) and bladder cancer patients.
Materials and Methods: The urine specimens of bladder cancer patients and age-matched hernia patients ($n = 81$) were collected in the morning of surgery. The surgically resected bladder tumors were all pathologically identified and determined into 3 groups for comparison; namely, Low-grade/Early stage (LgEs, $n = 40$), High-grade/Early stage (HgEs, $n = 63$), and High-grade/ Advanced-stage (HgAs, $n = 37$).
Results: A total of 107 proteins out of 2964 proteins were identified in this approach as candidate biomarkers. Differences in the concentrations of 29 proteins were precisely quantified by LC–MRM/MS. There were 24 proteins changed significantly ($p < 0.05$) between bladder cancer and hernia. TACSTD2 concentrations measured by LC-MRM/MS were 6.5-fold higher in bladder cancer urinary microparticles than in hernia urinary microparticles. In raw urine specimens ($n = 221$) using ELISA, the area-under-the-curve values of TACSTD2 was 0.80.
Conclusion: Our study revealed that TACSTD2 showed strong association with bladder canLcer in urine specimens, and thus represents a potential biomarker for noninvasive screening for bladder cancer.

PD1-6:
LACTATE PROMOTING CANCER STEM CELL PHENOTYPE AND INDUCING EPITHELIAL-MESENCHYMAL TRANSITION

G.-S. Shieh [1], W.-H. Yang [4], C.-L. Wu [2], A.-L. Shiau [3]. [1] *Tainan Hospital, Dept. of Urology, Tainan, Taiwan;* [2] *National Cheng Kung University Hospital, Dept. of Biochemistry and Molecular Biology, Tainan, Taiwan;* [3] *National Cheng Kung University Hospital, Dept. of Microbiology and Immunology, Tainan, Taiwan;* [4] *National Cheng Kung University Hospital, Dept. of Urology, Tainan, Taiwan*

Purpose: Cancer stem cells were considered to be the genesis of cancer and account for cancer initiation, progression, and recurrence. Studies have highlighted a role for Hexokinase 2 (HK2) in facilitating tumor growth and lactate production, which is downstream product of HK2 reaction in cancer cells. Tumor cells can extrude and shuttle lactate to neighboring cancer cells, adjacent stromal cells, and vascular endothelial cells to induce signaling molecular change. However, in tumor microenvironment, the molecular mechanisms underlying this association of tumor lactate shuttle, HK2 activity and cancer metastases were not well established. In this study, we explored the role of lactate shuttle induced by HK2 in cancer stem cell formation and epithelial-mesenchymal transition (EMT) between bladder cancer cells in vitro and in vivo.
Materials and Methods: The endogenous HK2 in human bladder cancer (TCCSUP, J82 and TSGH8301) and normal (SVHUC) cells was examined by immunoblot and immunofluoscence. Effects of lactate exposure on cell proliferation, morphologic change and cancer stem cell phenotype were analyzed in human bladder cancer cells. Stable HK2-overexpression and –knockdown clones were also examined for their effects on EMT, lactate secretion, NF-κB phosphorylation and CD133 activity in vitro and mouse models. The animal survival and lung metastasis were assessed in a mouse subcutaneous model using TSGH8301 cells with HK2-overexpression clones. All statistical tests were two-sided.
Results: The HK2 expression was significantly higher in bladder cancers compared with normal cells. The urine lactate detection was higher in human bladder cancers than in non-cancer subjects (204.9 vs 54.79 μM; $P < 0.001$). Stable HK2 overexpression induced cell proliferation, and showed morphologic changes with gain of cancer stem cell markers. HK2 knockdown also reduced lactate extrusion in vitro. In response to lactate exposure, nuclear translocation of NF-κB phosphorylation and Twist1 as well as mesenchymal markers was promoted in human bladder cancer cells. In addition, lactate exposure enhanced CD133 activity in vitro. In mice bearing subcutaneous tumors, increased tumor growth and lung metastasis were observed in stable HK2-overexpression cancers compared with mock counterparts (survival, $P = 0.034$). In animal models, HK2-overexpression cancers also induced morphologic change and CD133 activity.

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

Ying-Tzu Chen [1], See-Tong Pang [2], Ying-Hsu Chang [2], Chin-Hsuan Hsieh [2], Cheng-Keng Chuang [2], Wen-Hui Weng [1,*]. [1] *Department of Chemical Engineering and Biotechnology and Graduate Institute of Biochemical and Biomedical Engineering, National Taipei University of Technology, Taipei, Taiwan;* [2] *Division of Urology, Department of Surgery, Chang Gung Memorial Hospital, Chang Gung University, Taiwan*

* Corresponding author.

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

Thomas I-Sheng Hwang [1,3,4], Te-Fu Tsai [1,4], Shan-Che Yang [2], Hung-En Chen [1], Yi-Chia Lin [1,4], Kuang-Yu Chou [1,4], Ji-Fan Lin [2]. [1] *Department of Urology, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan;* [2] *Central Laboratory, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan;* [3] *Department of Urology, Taipei Medical University, Division of Urology, Taipei, Taiwan;* [4] *School of Medicine, Fu-Jen Catholic University, Taipei, Taiwan*

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

Kuang-Yu Chou [1,#], Ji-Fan Lin [2,#], Te-Fu Tsai [1], Hung-En Chen [1], Yi-Chia Lin [1], Thomas I-Sheng Hwang [1,2,3,4]. [1] *Department of Urology, Shin Kong Wu Ho-Su Memorial Hospital Taipei, Taiwan;* [2] *Central Laboratory, Shin Kong Wu Ho-Su Memorial Hospital Taipei, Taiwan;* [3] *Department of Urology, Taipei Medical University Taipei, Taiwan;* [4] *Division of Urology, School of Medicine, Fu-Jen Catholic University Taipei, Taiwan*

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.