Purpose: Mitochondria are the powerhouses of cells. Mitochondrial C-Raf is a potential cancer therapeutic target, as it regulates mitochondrial function and is localized to the mitochondria by its N-terminal domain. However, Raf inhibitor monotherapy induced S338 phosphorylation of C-Raf (pC-Raf^{S338}) and impede therapy which is urged to be overcome in clinical practice.
Materials and Methods: We analyzed the growth-inhibition effect of Sorafenib and GW5074 combination therapy on multiple normal and cancer cell lines by MTT assay *in vitro* and its anticancer effect with xenograft and orthotopic metastasis animal models *in vivo*. The molecular mechanisms involved in the regulation of mitochondrial function have been investigated by means of western blotting analysis, fractionation, immunoprecipitation assays and FACS analysis. The protein expression in cancer tissues and normal counterparts was examined by immunohistochemistry assay.
Results: This study identified the interaction of C-Raf with S308 phosphorylated DAPK (pDAPK^{S308}), which together became co-localized in the mitochondria to facilitate mitochondrial remodelling. Combined use of the Raf inhibitors sorafenib and GW5074 had synergistic anti-cancer effects *in vitro* and *in vivo*, but targeted mitochondrial function, rather than the canonical Raf signaling pathway. C-Raf depletion in knock-out MEF$^{C\text{-}Raf\text{-}/\text{-}}$ or siRNA knock-down ACHN renal cancer cells abrogated the cytotoxicity of combination therapy. Crystal structure simulation showed that GW5074 bound to C-Raf and induced a C-Raf conformational change that enhanced sorafenib binding affinity. In the presence of pDAPK^{S308}, this drug-target interaction compromised the mitochondrial targeting effect of the N-terminal domain of C-Raf, which induced two-hit damages to cancer cells. First, combination therapy facilitated pC-Raf^{S338} and pDAPK^{S308} translocation from mitochondria to cytoplasm, leading to mitochondrial dysfunction and ROS generation. Second, ROS facilitated PP2A-mediated de-phosphorylation of pDAPK^{S308} to DAPK. PP2A then dissociated from the C-Raf-DAPK complex and induced profound cancer cell death. Increased pDAPK^{S308} modification was also observed in renal cancer tissues, which correlated with poor disease-free survival and poor overall survival in renal cancer patients.
Conclusions: Besides mediating the anti-cancer effect, pDAPK^{S308} may serve as a predictive biomarker for Raf inhibitors combination therapy, suggesting an ideal pre-clinical model that is worthy of clinical translation.

IPD25:
HIGHER EXPRESSION OF ACETYL-TUBULIN MAY INDUCE DOCETAXEL RESISTANCE IN PROSTATE CANCER CELLS

Yu-Hua Lin [1], Yu-Chuan Lu [2], Chao-Yuan Huang [2], Ying-Fang Su [3], Tzyh-Chyuan Hour [3], Jyun-Hong Wu [3], Hung-Lin Wu [3], Chi-Yu Lu [3], Shiu-Dong Chung [5,6], Wen-Jeng Wu [4], Shu-Pin Huang [4], Yeong-Shiau Pu [2]. [1] *Division of Urology, Department of Surgery, Cardinal Tien Hospital, New Taipei City, Taiwan;* [2] *Department of Urology, National Taiwan University Hospital, Taipei, Taiwan;* [3] *Institute of Biochemistry, Kaohsiung Medical University, Kaohsiung, Taiwan;* [4] *Department of Urology, Kaohsiung Medical Hospital, Kaohsiung, Taiwan;* [5] *Division of Urology, Department of Surgery, Far Eastern Memorial Hospital, New Taipei City, Taiwan;* [6] *School of Medicine, Fu-Jen Catholic University, New Taipei City, Taiwan*

Purpose: Docetaxel-based chemotherapy has generally been considered as one of the effective treatments for prostate cancer. Unfortunately, clinical treatment with docetaxel often encounters a number of undesirable side effects, including drug resistance. Therefore, it has become essential to identify molecular events that may be associated with the development of docetaxel resistance. Tubulin isoforms have been previously examined for their resistant ability to docetaxel in many cancers, but their real mechanisms remained unclear. In this study, we evaluated the feasibility of employing docetaxel as a cytotoxic agent for PC3 cells and to examine the role of acetyl-tubulin in docetaxel-resistant prostate cancer.
Materials and Methods: A series of docetaxel-resistant PC/DX cell subclones, were established by chronically exposing PC3 to progressively increased concentrations of docetaxel. Herein, we characterized the docetaxel-mediated cytoxicity and molecular events in PC3 and PC/DX cells by MTT assay, Western blotting, RT-PCR, real-time PCR and flow cytometry analysis.
Results: Our results showed that levels of acetyl-tubulin, α-tubulin, β-tubulin, γ-tubulin, and βIII-tubulin were significantly higher expression in PC/DX25 than in parental PC3 cells by Western blotting analysis. PC/DX with greater resistance to docetaxel had higher levels of acetyl-tubulin and MCAK (mitotic centromere associate kinesin) than in PC3 cells. The expression of acetyl-tubulin and MCAK was gradually increased by docetaxel in a dose- and time-dependent manner in PC3 cells. Histone deacetylase 6, a deacetyl enzyme of tubulin, mRNA and protein levels were significantly decreased in PC/DX25 than in PC3 cells. PC3 increased the chemoresistant ability to docetaxel by HDAC6 knockdown and Tubastain A (HDAC6 inhibitor), respectively. Conversely, PC/DX25 reversed the sensitivity to docetaxel by MCAK knockdown. Notably, MCAK knockdown in PC/DX25 induced cell cycle G2/M phase arrest after docetaxel treatment by flow cytometry analysis. Overexpression of Plk1 in PC3 increased the cell survival rate and resistance to docetaxel. Interestingly, we also found an evident up-regulation of acetyl-tubulin protein expression after recombinant epidermal growth factor treatment in a time-dependent manner in PC3 cells.
Conclusion: Up-regulation of acetyl-tubulin may play an important role in docetaxel-resistant prostate cancer.

IPD26:
SULF1 OVEREXPRESSION IS A POOR PROGNOSTIC FACTOR IN PATIENTS WITH UROTHELIAL CARCINOMA

Kai-Fu Yang [1], Chien-Feng Li [2], Hsiang-Ying Lee [1,3], Ching-Chia Li [1,3], Wei-Ming Li [1,4], Hung-Lung Ke [1], Hsin-Chih Yeh [1,3], Chun-Nung Huang [1], Wen-Jeng Wu [1,5]. [1] *Department of Urology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan;* [2] *Department of Pathology, Chi Mei Medical Center, Tainan, Taiwan;* [3] *Department of Urology, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung, Taiwan;* [4] *Department of Urology, Ministry of Health and Welfare Pingtung Hospital, Pingtung, Taiwan;* [5] *Department of Urology, Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung, Taiwan*

Purpose: Urothelial carcinoma (UC) originating from bladder (UBUC) and upper urinary tract (UTUC) is the most common tumor type of urinary tract. However, its pathogenesis remains obscured. Through data mining from a published transcriptomic database of UBUCs (GSE31684), we identified *SULF1* as the most significant gene showing upregulation from early tumor development to progression among those associated with growth factor activity (GO:0030201). Sulfatase-1 (SULF1), encoded by *SULF1*, is an extracellular 6-O-endosulfatase, which selectively removed 6-O-Sulfate from heparan sulfate proteoglycans (HSPGs), involving in multiple biologic processes including tumorigenesis and cancer progression proven in various cancers. However, the significance of SULF1 expression has never been evaluated in UC. We therefore analyze its significance in our well-characterized cohort of UC.
Materials and Methods: Quantigene assay was used to detect *SULF1* messenger RNA (mRNA) level in 36 UTUCs and 30 UBUCs. Immunohistochemistry evaluated by H-score was used to determine SULF1 protein expression in 296 UBUCs and 340 UTUCs. We then correlated protein expression status with clinicopathological features and evaluated the prognostic significance of SULF1 protein expression for disease-specific survival (DSS) and metastasis-free survival (MeFS).
Results: Increments of *SULF1* transcript level were associated with higher pT status in both UTUC and UBUC (all $P < 0.05$). SULF1 protein overexpression was significantly associated with advanced primary tumor and nodal statuses, and presence of vascular invasion in both groups of UC. SULF1 overexpression not only predicted worse DSS and MeFS by univariate analysis, but implied inferior DSS (UTUC, $P < 0.001$; UBUC, $P = 0.011$) and MeFS (UTUC, $P < 0.001$; UBUC, $P = 0.021$) in multivariate analysis.
Conclusion: SULF1 overexpression is associated with advanced tumor status and implicated adverse clinical outcome for both patients of UTUC and UBUC. Our study unveiled the important role of SULF1 expression in tumor progression in UC and may serve as a potential prognostic biomarker and a novel therapeutic target of UC.

IPD27:
CHEMOKINE CXCL14 (BRAK) EXPRESSION AND POTENTIAL ROLE IN HUMAN RENAL CELL CARCINOMA

Yuan-Hung Pong [4,5], Fei-Tsuei Liu [1], Tzyh-Chyuan Hour [1], Wang-Yi Kang [3], Hong-Jeng Yu [4], Chao-Yuan Huang [4], Bao-Wen Pan [1], Wen-Jeng Wu [2], Chun-Hsiung Huang [2], Yeong-Shiau Pu [4]. [1] *Institution of Biochemistry, Taiwan;* [2] *Department of Urology, Kaohsiung Medical University College of Medicine, Kaohsiung, Taiwan;* [3] *Department of Pathology, Kuo General Hospital, Tainan, Taiwan;* [4] *Department of Urology,*