cell line: PC3 and human breast cancer cell line: MCF-7 were used in this study. Inhibition of basal autophagy was achieved using CQ, HCQ and Bafilomycin A1 (Baf A1) in multiple human bladder cancer cell lines. Cell viability was assessed by WST-1 assay. Western blot detection of LC3-II was performed to monitor autophagy, while detection of caspase 3/7 activities and DNA fragmentation were conducted to investigate apoptotic induction in treated cells. The disruption of mitochondria membrane potential (MMP), the generation of reactive oxygen species (ROS) and lysosome permeability were assessed by JC-1, H2DCFDA staining and IF detection of cathepsin D and E, respectively, in CQ- and HCQ-treated cells.

**Results:** Changes in LC3 flux, monitored by Western blot and IF, indicated inhibition of autophagy at the level of the autophagosome by CQ and HCQ. Both two autophagy inhibitors induced cytotoxicity in multiple human bladder cancer cell lines in time- and dose-dependent manner especially in advanced cancer cell lines. CQ and HCQ also significantly impacted the clonogenic formation of bladder cancer cells. However, the inhibition of cell viability was only observed in bladder cancer cell lines but not in SV-Huc-1, PC3 and MCF-7 cells that reported to be with low basal autophagy activity. Induction of apoptosis was found in cells treated with CQ and HCQ. We cannot detected the disruption of mitochondria membrane potential nor the generation of reactive oxygen species in CQ- or HCQ-treated cells. Translocation of cathepsin B in CQ-treated cells suggesting the change of lysosome permeability that leads to the blockage of autophagy and increasing apoptotic cell death by these agents.

**Conclusion:** Targeting autophagy with CQ or HCQ may be an effective cancer therapy in human bladder cancer.

**PD7-5:**

**INHIBITION OF AUTOGRAPH POTENTIALATES APOPTOSIS IN BITC-TREATED HUMAN BLADDER CANCER CELLS**

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**Purpose:** Benzyl isothiocyanate (BITC) is contained in cruciferous plants which are part of the human diet, and has been shown to induce apoptosis in various cancer cell lines including human bladder cancer cells. In our previous work, we showed that BITC induces protective autophagy via inhibition of mTOR signaling pathway in prostate cancer cells. And the induced apoptosis and autophagy in human prostate cancer cells were mediated by BITC-induced ROS generation. Here we investigated weather inhibition of autophagy enhances apoptosis in human bladder cancer cells as a novel therapeutic strategy.

**Materials and Methods:** Human bladder cancer cell lines including grade II, 5637, and grade III, T24, were used in this study. We also included mouse bladder cancer cell line, MBT2, for the development of orthotopic mice bladder cancer model for further study. Bafilomycin A1 (Baf A1) and chloroquine (CQ) were used as autophagy inhibitors. Cell viability in BITC-treated cells with or without the pretreatment of autophagy inhibitors were measured by WST-1 reagent. Detection of autophagy was conducted by measuring the LC3-II processing and the accumulation of p62 by Western blotting and immunofluorescent staining of these marker protein. Detection of apoptosis was performed by the monitored the caspase 3/7 activity, Western blot of cleavage caspase3 and cell flowcymetry of DNA fragmentation in treated cells. Detection of apoptosis was conducted by measuring the LC3-II processing and the accumulation of p62.

**Results:** Exposure of 5637, T24 and MBT2 cells to pharmacological concentrations of BITC resulted in the decrease of cell viability and autophagy induction. Although inhibition of basal autophagy by Baf A1 or CQ alone caused cell viability loss in these bladder cancer cell lines, pre-treatment of Baf A1 or CQ to inhibit BITC-induced autophagy further enhanced the inhibition of cell growth. Enhanced apoptosis judged by the increased caspase 3/7 activity, increased amount of cleaved caspase 3 and elevated level of DNA fragmentation in BITC-treated cell with Baf A1 or CQ pretreatment suggesting inhibition of autophagy significantly potentiates the anti-cancer effect of BITC in human bladder cancer cells.

**Conclusion:** This is the first study showed that inhibition of autophagy induced by BITC markedly enhance apoptosis in human and mouse bladder cancer cells. We are currently using orthotopic mice bladder cancer model to translate our results from in vitro to in vivo studies. Our data may be beneficial for further development of novel therapeutic strategies against bladder cancer.

**PD7-6:**

**THROMBOMODULIN EXPRESSION REGULATES TUMORIGENESIS IN BLADDER CANCER**

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**Purpose:** The identification of potential tumor markers will help improve therapeutic planning and patient management. Thrombomodulin (TM) is a sensitive urothelial marker. TM was reported to be one of the endogenous anti-metastatic factors and has diagnostic and prognostic values for the progression of carcinoma. In the present study, we examine the role of TM in bladder cancer.

**Materials and Methods:** We studied the role of TM in tumor behavior and related signaling pathways in vitro using the human bladder cancer cell lines HT1376, HT1197, J82 and T24, and in vivo using animal models. We also selected clinical specimens from 100 patients with bladder cancer for immunohistochemical staining to evaluate the predictive capacity of TM in tumor invasiveness.

**Results:** The data revealed that positive immunoreactivity for TM was inversely correlated with clinical stage and DNA methyltransferase 1 immunoreactivity. Decreased TM expression could predict the aggressive tumor growth and advanced clinical stage in bladder cancer. When TM was inhibited, tumor growth rate and invasion ability were augmented in vitro and in vivo. Moreover, inhibition of NF-kB activation significantly increased TM expression and attenuated tumor aggressiveness in bladder cancer.

**Conclusion:** TM plays an important role in bladder cancer tumor aggressiveness in vitro and in vivo and is a clinically significant predictor that may represent a suitable therapeutic target for bladder cancer.

**Podium-8 Oncology**

**PD8-1:**

**URETERAL INVOLVEMENT AND DIABETES INCREASE THE RISK OF SUBSEQUENT BLADDER RECURRENCE AFTER NEPHROURETERECTOMY FOR UPPER URINARY TRACT UROTHELIAL CARCINOMA**

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**Purpose:** To investigate the prognostic factors for bladder recurrence after radical nephroureterectomy (RNU) in patients with upper urinary tract urothelial carcinoma (UUT-UC)

**Materials and Methods:** From 1994 to 2012, 695 patients with UUT-UC treated with RNU were enrolled in National Taiwan University Medical Center. Among them, 532 patients with no prior bladder UC histories were recruited for analysis. We assessed the impact of potentially prognostic factors on bladder recurrence after RNU.

**Results:** The median follow-up period was 47.8 months. In the Cox model, ureteral involvement and diabetes mellitus (DM) were significantly associated with a higher bladder recurrence rate in the multivariate analysis (hazard ratio [HR]: 1.838; p = 0.003 and HR: 1.821; p = 0.010, respectively). In the Kaplan-Meier analysis, DM patients with concomitant ureteral UC...