downstream of a luciferase gene, and luciferase reporter assays were performed to verify the direct binding of miR-99a to mTOR transcripts. The mRNA and protein expression level of mTOR were measured by Q-PCR and Western blot, respectively. Cell viability of miR-99a transfected cells was detected by WST-1 and colony formation assays. Inhibition of mTOR complex 1 and 2 (mTORC1 and mTORC2) signaling was monitored by detecting the phosphorylation of S6K and Akt using Western blot. Induction of autophagy was accessed by the expression of LC3-II marker protein.
Results: Transfection of miR-99a expressing vector elevated the expression level of miR-99a up to 4.5-fold in cells compared to vector-only control. The function of matured miR-99a was confirmed by luciferase reporter assays. The level of mTOR RNA and protein were decreased in miR-99a transfected cells. Dual inhibition of mTORC1 and mTORC2 was confirmed by immunoprecipitation (IP) of mTOR associated Rictor and Raptor, and the decreased phosphorylation of S6K and Akt in miR-99a transfected cells. The LC3-II protein was accumulated in miR-99a transfected cells compared to monk transfected control, suggesting that inhibition of mTOR by miR-99a induces autophagy in bladder cancer cells.
Conclusions: This is the first study showed that miR-99a markedly inhibits bladder cancer cell growth via dual inhibition of mTORC1 and mTORC2. miR-99a treatment also induces autophagy through mTOR inhibition. However, the role of miR-99a induces autophagy remains further investigation.

MP4-4.
BLUE-LIGHT EXPOSURE ACCELERATES PHOTO-OXIDATIVE DISRUPTION OF LYSOSOMAL MEMBRANES AND APOPTOSIS IN ACRIDINE ORANGE-LOADED CHLOROQUINE-TREATED HUMAN BLADDER CANCER CELLS

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Introduction: We previously showed that human bladder cancer cells exhibited high basal level of autophagic activity. Administration of chloroquine (CQ) or hydroxychloroquine (HCQ) inhibits autophagy and causes apoptotic cell death in bladder cancer cells. Acridione orange (AO) is commonly used to identify acidic vescular organelles (AVOs) in autophagic cells. The uncharged state of AO in cells was characterized by green fluorescence; while protonated form of AO accumulates in acidic compartments and forms aggregates that characterized by red fluorescence. An incidentally found disruption of the lysosome under treatment of CQ and blue light was observed. An experiment was conducted to verify the phenomenon is meaningful.
Methods: Inhibition of basal autophagy was achieved using CQ inT24 human bladder cancer cell lines by detecting LC3-II formation. Acridine orange relocalization was performed in T24 cells with or without CQ treatment for 2 hours. Immunofluorescence and Western blot were used for detection of cathepsin B and D release from lysosome. Cell viability and induction of cell death were detected using ApoTox-Glo Triplex Assay kit from Promega.
Results: CQ inhibited basal autophagy and decreased cell viability in T24 cells. AO relocalization was detected only in CQ-treated T24 cells. CQ-treated T24 cells that exposed for a short period of time to AO and under ordinary culture condition, accumulate the AO within AVOs, giving rise to a mainly red, granular fluorescence upon excitation with blue light. When AO-loaded CQ-treated cells are irradiated with intense blue light, AO soon starts to leak from lysosomes to nuclear and cytosol diffusely. Severe lysosomal damage that causing necrosis and apoptosis was only detected in AO-loaded CQ-treated T24 cells. This photo-oxidative disruption of lysosome was responsible for triggering cell death in bladder cancer cells. Necrotic and apoptotic death were both detected in cell treated with CQ up to 50%.
Conclusions: Photo-oxidative disruption of lysosomal membrane with AO and CQ may be an effective cancer therapy in human bladder cancer.

MP4-5.
THE APPLICATION OF SPIES IN THE DIAGNOSIS AND TREATMENT OF BLADDER UROTHELIAL CARCINOMA-THE PRELIMINARY REPORT IN TAIPEI CITY HOSPITAL

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Purpose: To report our preliminary experience regarding the application of SPICE technology in the diagnosis and treatment of bladder urothelial carcinoma.
Patients and methods: A prospective randomized trial will be conducted from January 2015 to May 2015. A total of 30 patients with bladder urothelial carcinoma will be enrolled into this study. All patients received standard cystoscopy followed by SPICE technology cystoscopy. The number of tumor was counted under direct vision by two experienced urologists. All patients received transurethral resection of bladder tumor. All patients received standard treatment protocol and followed every 3 months using standard cystoscopy.
Results: The number of tumor diagnosed under standard cystoscopy or SPICE technology cystoscopy will be compared in this study. The incidence of tumor recurrence and metastasis will be calculated in this study.
Conclusion: Cystoscopy empowered with SPICE technology will be a viable option to assist the diagnosis and treatment of bladder urothelial carcinoma.

MP4-6.
TOLL-LIKE RECEPTOR 6 AND CONNECTIVE TISSUE GROWTH FACTOR ARE SIGNIFICANTLY UPREGULATED IN MITOMYCIN-C-TREATED UROTHELIAL CARCINOMA CELLS UNDER HYDROSTATIC PRESSURE STIMULATION

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Purpose: Urothelial carcinoma (UC) is the most common histologic subtype of bladder cancer. The administration of mitomycin C (MMC) into bladder after transurethral resection of the bladder tumor (TURBT) is a common treatment strategy for preventing recurrence after surgery. We previously applied hydrostatic pressure combined with MMC in UC cells and found that hydrostatic pressure synergistically enhanced MMC-induced UC cell apoptosis via the Fas/FasL pathways.
Materials and methods: To understand the alteration of gene expressions in UC cells caused by hydrostatic pressure and MMC, oligonucleotide microarray was used to explore all of the differentially expressed genes.
Results: After bioinformatics analysis and gene annotation, toll-like receptor 6 (TLR6) and connective tissue growth factor (CTGF) showed significant up-regulation among altered genes, and their gene and protein expressions with each treatment of UC cells were validated by quantitative real-time PCR and immunoblotting.
Conclusion: Under treatment with MMC and hydrostatic pressure, UC cells showed increasing apoptosis via extrinsic pathways through upregulation of TLR6 and CTGF.

MP4-7.
CLINICAL OUTCOMES IN CASTRATION RESISTANT PROSTATE CANCER PATIENTS TREATING WITH CABAZITAXEL

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Purpose: The aim of this study was to assess the clinical outcomes of Cabazitaxel in treating castration resistant prostate cancer patients in Taichung Veterans General Hospital.
Materials and methods: From Aug 2011 to Dec 2014, thirty-two patients with castration resistant prostate cancer were treated with Cabazitaxel 20-