Podium

Podium-1
Oncology

PD1-1:
A NOVEL BIOMARKER FOR PROSTATE CANCER DETECTION IN PATIENT WITH GRAY ZONE PSA LEVEL

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Purpose: Prostate cancer screening with PSA is plagued by high rate of unnecessary prostate biopsies, especially in the “gray zone” (4.0 ng/mL – 10.0 ng/mL). We introduce a new circulating-tumor-cell (CTC) biomarker for detection of prostate cancer in patients in the PSA “gray zone” level, with the clinically verified potential to substantially decrease the number of unnecessary prostate biopsies.

Materials and Methods: A total of 97 patients underwent routine prostate screening including PSA testing and DRE. One tube of blood was drawn for each patient and sent for CTC analysis in a double blinded study. A subset screening including PSA testing and DRE. One tube of blood was drawn for each patient and sent for CTC analysis in a double blinded study. A subset of 23 patients with PSA in the 4.0 ng/mL – 10.0 ng/mL range was selected with consent to undergo prostate biopsy for comparison with blinded CTC test results. The CTC test utilized a microfluidic platform with EpCAM as capture antibody. Suspected CTCs were eluted to a membrane chip and immunofluorescently stained with CK18, PSMA, and CD45 antibodies to confirm. Positive CTCs are defined as CK18+ or PSMA+ and CD45-.

Results: Prostate cancer was confirmed by biopsy in 60 out of 97 patients. CTC assay reported 83% of the cancer cases, demonstrating prostate cancer detection ability of the assay. In the subset category of 23 patients (PSA in the 4.0 ng/mL – 10.0 ng/mL range, and prostate biopsy), the CTC assay was able to detect cancer in 100% of the prostate cancer cases.

Conclusion: This CTC-based blood test is a valuable new tool in effective screening for prostate cancer. We have demonstrated that this new CTC biomarker is able to reduce unnecessary invasive prostate biopsies in the PSA “gray zone” by over 60%, with the potential to reduce cost to the system and reduce complication rates due to prostate biopsies, thus improving patient outcomes.

PD1-2:
OVEREXPRESSION OF PTP4A3 IS ASSOCIATED WITH METASTASIS AND UNFAVORABLE PROGNOSIS IN UROTHELIAL CARCINOMA

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Purpose: Urothelial carcinoma (UC) is the most common malignancy involving urinary bladder (UB) and upper urinary tract (UT) for which the therapeutic markers remain under-investigated. Increasing evidence has shown that protein tyrosine phosphatases (PTPs) play dominant roles in setting the levels of tyrosine phosphorylation and promote oncogenic processes. However, their expression has not been systemically investigated in UC, and our aim is to examine their potential impact on UC.

Materials and Methods: We performed data mining from a published transcriptome of UBCs (GSE32894), and PTP type IVA member 3 (PTP4A3) was identified as the most significantly upregulated gene among those related to prenylated PTP activity (GO: 0004727). The importance of PTP4A3 was initially analyzed in paired normal urothelium, non-invasive UC, invasive UC, and nodal metastatic tissue. PTP4A3 transcript level was assessed in snap-frozen UC samples by laser capture microdissection and real-time RT-PCR. PTP4A3 protein expression was determined by immunohistochemistry in 295 UBCs and 340 UTUCs, respectively. The association of PTP4A3 expression with clinicopathological features, disease-specific survival (DSS), and metastasis-free survival (MeFS) was further evaluated.

Results: For both UBUC and UTUC, the level of PTP4A3 significantly increased from normal urothelium, non-invasive UC, invasive UC, to nodal metastatic tissue (both p < 0.001). The PTP4A3 transcript level was also markedly upregulated in high stage UC (UBUC, p = 0.001; UTUC, p = 0.002). Overexpression of PTP4A3 protein was significantly associated with advanced pT status, nodal metastasis, and lymphovascular invasion (all p < 0.001). PTP4A3 overexpression not only predicted worse DSS and MeFS on univariate analysis (all p < 0.001), but also implicated in inferior DSS (UBUC, p = 0.001; UTUC, p = 0.001) and MeFS (UBUC, p = 0.003; UTUC, p = 0.001) on multivariate analysis.

Conclusion: PTP4A3 overexpression independently predicted metastasis and outcome of both UBUC and UTUC, suggesting its potential theranostic value in UC.
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 ($P < 0.001$). In subgroup analysis, we found the prognostic significance of RDW for OS was limited in organ-confined disease ($\leq$T2 without pT2). 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

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**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 cells line under blue-light exposure.

**Materials and Methods:** To evaluate phototoxicities 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 relocation in treated-BC cells was recorded using a fluorescence microscopy equipped with a color CCD camera in 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 cells line (5637 and T24) treated with indicated concentration of AO with or without blue light exposure.

**Results:** The AO relocation was clearly monitored by fluorescence microscopy with decreased red fluorescent intensity over exposure duration within 5-15 seconds in BC cells. Treatment of AO 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

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**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 herina (control) and bladder cancer patients.

**Materials and Methods:** The urine specimens of bladder cancer patients and age-matched herina 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 herina. TACSTD2 concentrations measured by LC–MRM/MS were 6.5-fold higher in bladder cancer urinary microparticles than in herina 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

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**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 shut- tle, 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 immunofluorescence. 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 cancer than in hernia urinary microparticles, and thus represents a potential biomarker for noninvasive screening for bladder cancer.