

accelerates bladder cancer progression via inducing EMT by activating miR-1305/Tgf-β2/smad3 pathway. The research implies that circRIP2 might be a potential biomarker and therapeutic target for bladder cancer patients.

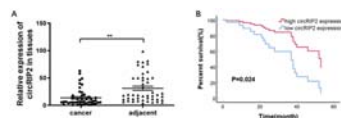


Figure 1: higher circRIP2 expression associates with better patients outcome in bladder cancer
 A. the expression of circRIP2 in 45 paired bladder cancer tissues were measured by qPCR. B. overall survival of 43 bladder cancer patients in following was analyzed based on the level of circRIP2.

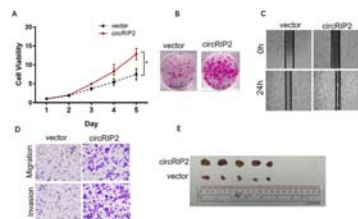


Figure 2: circRIP2 promote bladder cancer growth and invasion
 The effect of circRIP2 on bladder cancer growth was determined by A. CCK8 assay and B. clone forming assay, on migration and invasion was determined by C. wound healing and D. trans-well assay. E. in vivo effect of circRIP2 was evaluated by subcutaneous mice model.

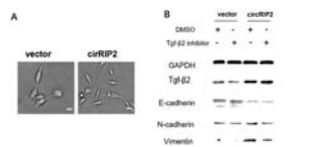


Figure 3: circRIP2 promotes epithelial-mesenchymal transition in bladder cancer
 A. the phenotype of bladder cancer was observed under light microscope. B. the levels of different endothelial and mesenchymal markers were determined by western blot.



Figure 4: circRIP2 sponges miR-1305 to elevate Tgf-β2 in bladder cancer cell
 A. miRNA expression was determined by mRNA-seq after circRIP2 over-expressed. B. Western blot verified the up-regulation of Tgf-β2 caused by circRIP2 over-expression.

modulation, and interleukin signaling. Associated cellular functions included cell death, cell-cycle regulation, cell proliferation, and cellular movement. Merged analysis identified eight molecules (*TNF*, *ERBB2*, *IL1B*, *APP*, *EGFR*, *FYN*, *VEGFA*, *ABL1*) in the top 90 %ile with associated monoclonal antibodies or small molecule inhibitors (top tox pathway $p \leq 3.84E-10$); several of these have not been tested in bladder cancer. Another nine molecules that are variously influenced by chemotherapeutics were also identified.

CONCLUSIONS: This study describes a metagenomic approach using prior prognostic biomarkers that may be used to identify druggable targets in bladder cancer. This paradigm may be employed in the setting of an individual patient to flag molecules that play putative central roles in modulating the tumor's molecular circuitry, and may therefore be a consensus target and potentially minimize the probability of tumor escape.

Source of Funding: None

**MP17-08
 HLA-E EXPRESSION PREDICTS PROGRESSION, THERAPEUTIC RESPONSE AND OVERALL SURVIVAL IN PATIENTS WITH BLADDER CANCER**

Jorge Daza*, New York, NY; Dan Fu Ruan, Berengere Salome, New York, NY; Andrew Charap, Peter Wiklund, Matthew Galsky, New York, NY; Petros Grivas, Seattle, WA; Reza Mehrazin, John Sfakianos, New York, NY; Amir Horowitz, New York, NY

INTRODUCTION AND OBJECTIVE: Binding of HLA-E to CD94/NKG2A expressed on NK and CD8 T cells is strongly associated with impaired anti-tumor immunity. We aimed to demonstrate that HLA-E expression is a major determinant of tumor evasion in bladder cancer (BCa).

METHODS: Germline DNA and tumor RNA sequencing data were analyzed from the TCGA bladder cancer cohort. Genotypes of *HLA-A* and *-B* in 407 muscle invasive bladder cancer (MIBC) patients were analyzed to assess the amount of leader peptide provided for HLA-E binding to infer HLA-E expression. The prognostic impact of inferred HLA-E expression, Stroma-related gene expression, and NK cell gene expression was assessed. An independent cohort of 22 patients with non-muscle invasive bladder cancer (NMIBC) and MIBC were profiled for gene expression using HTG EdgeSeq *in situ* hybridization. Imaging mass cytometry (IMC) was performed on tumor tissue from patients before PD1 blockade therapy.

RESULTS: Inferred HLA-E expression, stromal-gene expression, and NK cell gene expression were independently associated with overall survival (OS) in the TCGA bladder cancer cohort (Fig 1). Gene expression analysis from tumor and adjacent tissue from 22 BCa patients by HTG showed upregulation of HLA genes including HLA-E predominantly in MIBC (Fig 2). IMC analysis showed a significantly higher expression of HLA-E in a patient with no response to anti PD1 therapy (Fig 3). Conversely, a patient with complete response showed low HLA-E expression and infiltration of NKG2A+ NK cells and CD8+ T cells in the TME.

CONCLUSIONS: HLA-E expression in BCa is a major determinant of OS, progression and therapeutic response. These findings strongly suggest an increased risk of adverse outcomes driven by evasion of NK anti-tumoral response mediated by HLA-E.

Source of Funding: No

**MP17-07
 IDENTIFYING NOVEL THERAPEUTIC TARGETS BASED ON PERTURBATION OF BLADDER CANCER CELLULAR CIRCUITRY USING METAGENOMICS**

Anirban P Mitra*, Los Angeles, CA

INTRODUCTION AND OBJECTIVE: Several novel therapeutics have been approved for bladder cancer treatment based on empiric observations of overexpression in tumors, without much significance attributed to their overall role in altering the circuitry within cancer cells. This study employed metagenomics to interrogate previously described prognostic biomarkers to identify molecules that represent the confluence of cellular networks affected during bladder carcinogenesis and progression. The hypothesis is that central molecules may be ideal therapeutic targets regardless of differential expression as they coordinate several cellular circuits crucial to the tumor's survival.

METHODS: Prior profiling studies were reviewed to identify protein-coding genes associated with bladder cancer prognosis. After excluding non-mappable genes, curated pathway analyses were used to enumerate direct and indirect interactions between mRNAs within the context of merged biological networks. Top ranking molecules, defined by the highest %ile of interactions, were evaluated for their value as therapeutic targets.

RESULTS: 825 genes were used to construct two interactomes. 8,776 direct and indirect molecular interactions were noted. Identified genes were %ile-ranked based on number of interactions with neighboring genes within curated pathways. 78 molecules contributed to the top 90 %ile of all interactions. 27%, 14%, 19% and 28% of these molecules are typically located in the extracellular, plasma membrane, cytoplasmic and nuclear compartments, respectively. Top canonical pathways included intranuclear and death receptor signaling, T cell

Figure 1. Inferred HLA-E combined with gene expression signatures for NK cell abundance and EMT phenotype reveal strong effects on OS in BCa.

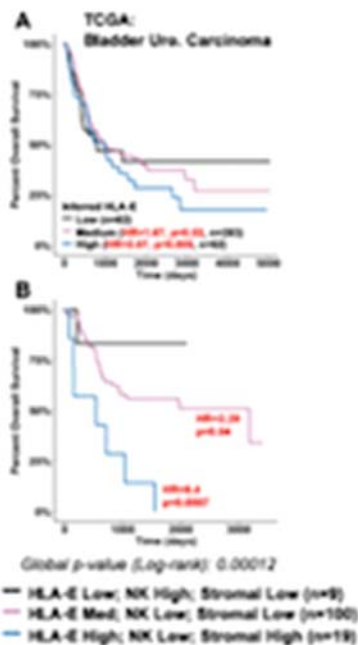


Figure 2. Gene expression analysis evaluated by HTG EdgeSeq in situ hybridization on HLA genes showing upregulation of HLA expression predominantly in MIBC

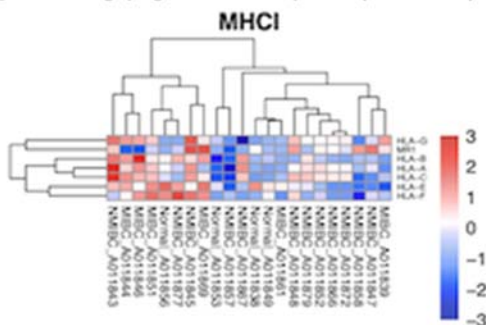
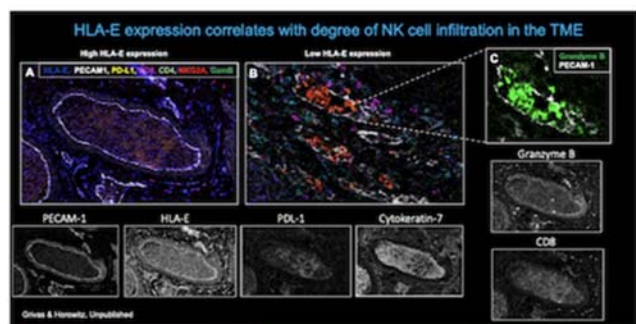


Figure 3. IHC on pathology sections from a A) MIBC patient with poor and B) adequate response to Anti-PD1 therapy. C) Granzyme B expression in NK cells infiltrating tumorigenic lesions



Source of Funding: None

MP17-09 THROMBOSPONDIN-4 PROMOTES BLADDER CANCER CELL MIGRATION AND INVASION BY UPREGULATING MMP9 EXPRESSION

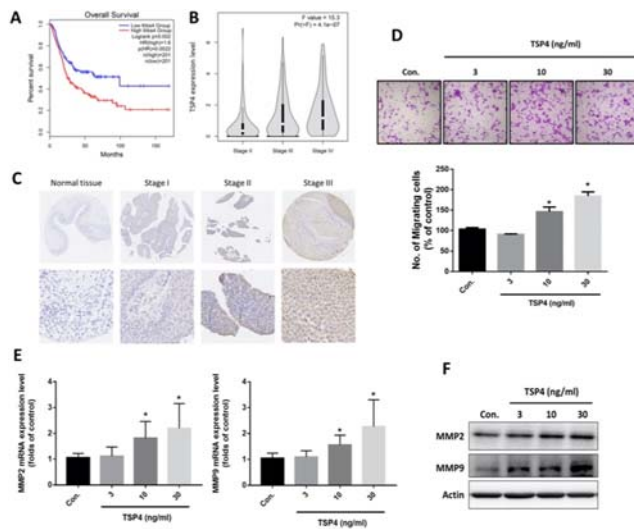
Yi-Chia Lin*, Po-Chun Chen, Shan-Che Yang, Te-Fu Tsai, Hung-En Chen, Kuang-Yu Chou, Thomas I. Hwang, Taipei, Taiwan

INTRODUCTION AND OBJECTIVE: Patients with non-muscle-invasive bladder cancer (NMIBC) are at risk of recurrence to progress into muscle-invasive bladder cancer (MIBC), and the prognosis of patients with MIBC is determined by the presence of metastasis. Thrombospondin-4 (THBS4/TSP4), a secreted matricellular glycoprotein participates cell-to-extracellular matrix attachment and regulates various physiological and pathological processes. However, little is known about the prognostic role of TSP4 and its role in bladder cancer progression.

METHODS: The expression level of TSP4 and its prognostic value in bladder cancer (BC) specimens was evaluated in online database GEPIA. Moreover, the protein expression level of TSP4 in BC and normal tissue was investigated in the tumor tissue array by the immunohistochemistry. The pro-invasive role of TSP4 in BC was examined by *in vitro* cell migration and invasion assay. Finally, the molecular mechanism of TSP4 on BC metastasis was defined by using Western blot and QPCR analyses.

RESULTS: Based on the results of meta-analysis in GEPIA database and immunohistochemistry, we found that expression level of TSP4 was in accordance with the progression of BC, especially in MIBC. Interestingly, its expression is positively correlated with the matrix metalloproteinase-9 (MMP-9) expression, which are two well-characterized invasive markers in BC. Incubation with recombinant TSP4 dramatically promoted BC cell migration, invasion and MMP-9 expression.

CONCLUSIONS: Conclusively, our present study first describes the prognostic role of TSP4 in BC and defines its pro-invasive role in BC via promoting MMP-9 expression. TSP-2 may represent a novel promising new biomarker and therapeutic target for treating BC.



Source of Funding: none

MP17-10 N⁶-METHYLADENOSINE MODIFICATION OF CIRCANKS1A ACCELERATES CYTOPLASMIC EXPORT AND STABILIZES RACGAP1 TO PROMOTE BLADDER CANCER CELL PROLIFERATION AND METASTASIS

Qiangqiang Ge*, Yang Xun, Junlin Lu, Shaogang Wang, Wuhan, China, People's Republic of

INTRODUCTION AND OBJECTIVE: Circular RNAs (circRNAs) have been identified to serve as significant regulators in cancer