

## **CIT kinase and HSPB8 as Novel Biomarkers for Identification of Metastatic Prostate Cancers**

Jiqiang Yao<sup>1\*</sup>, Duy T. Nguyen<sup>2\*</sup>, Arun Renganathan<sup>2</sup>, Alexey M. Eroshkin<sup>3</sup> Youngchul Kim<sup>1</sup>, Eric Kim<sup>2</sup>, Gerald Andriole<sup>2</sup>, and **Kiran Mahajan**<sup>2#</sup>

<sup>1</sup>Department of Biostatistics and Bioinformatics, H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Drive, Tampa, FL. <sup>2</sup>#Department of Surgery, Washington University in St. Louis, MO, USA. <sup>3</sup>Bioinformatics Core, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, [CA.#kiranm@wustl.edu](mailto:CA.#kiranm@wustl.edu)

**Background** Epigenetic deregulation of tissue specific and developmentally regulated transcription factors in normal cells, leads to chromatin remodeling, reactivation of gene expression programs associated with pluripotency and inhibition of cell differentiation thereby promoting neoplastic transformation, oncogene addiction and self-renewal of cancer cells. The homeobox gene HOXB13, a PC risk gene, has emerged as an important therapeutic target in high-risk PCs due to its association with early dissemination, lymph node positivity, recurrence after radical prostatectomy and overexpression in a majority of prostate adenocarcinomas. We recently demonstrated that in metastatic Castrate Resistant Prostate Cancers (mCRPCs) HOXB13 is epigenetically regulated by the BET bromodomain protein BRD4. While HOXB13 expression is observed in early disseminated prostate tumor cells, the HOXB13 effectors promoting metastasis were largely unknown.

**Methods** To identify HOXB13 transcriptional targets in metastatic CRPCs, we performed integrative bioinformatics analysis of differentially expressed genes (DEGs) in the proximity of the human prostate tumor-specific AR binding sites. Descriptive analysis was conducted for all the variables interested (age, Gleason score, clinical tumor stage, lymph node involvement, and margin status, PSA at diagnosis and gene expression data for selected genes). Fisher's exact test was used to compare distribution of clinical variables between patients with primary cancer and those with metastatic cancer. Kaplan-Meier (KM) survival curves were plotted with R 3.3.2. Heat maps and Venn diagrams were produced with Partek (Partek, Inc.) and GENE-E software from Broad Institute (<https://software.broadinstitute.org/GENE-E/>). Analysis of overlaps from the lists of differentially expressed genes was performed using custom Perl scripts.

**Results** This analysis revealed for the first time a role for HOXB13 in regulating the expression of a small 22kDa heat shock protein HSPB8. Total Cancer Genome Atlas Analysis (TCGA) revealed a striking progressive decrease in HSPB8 in PCs with increase in Gleason scores. Consistently, we reported for the first time that HSPB8 expression is suppressed in a majority of metastatic PCs. Importantly, treatment with the AR antagonist Enzalutamide was ineffective in inducing HSPB8 protein expression indicating its inefficacy in blocking metastasis. In contrast, exogenous HSPB8 overexpression or treatment with microtubule inhibitor Colchicine induced HSPB8 mRNA and protein expression to prevent PC cell migration. The expression of a two-gene set, *CIT* and *HSPB8* with an overall balanced accuracy of 98.8% and a threshold value of 0.2347, was sufficient to classify metastasis. HSPB8 mRNA levels was significantly increased following HOXB13 depletion in multiple metastatic CRPC models.

**Conclusions** Collectively our studies highlight a role for HSPB8 as a critical suppressor of prostate cancer progression and inducers of HSPB8 as potentially important therapeutics for the treatment of high-risk PCs.

**Conflict of Interest** None

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