**MP68-10**

HOXB13 EXPRESSION AND ITS ROLE IN PROSTATE CANCER PROGRESSION AND NEUROENDOCRINE DIFFERENTIATION

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INTRODUCTION AND OBJECTIVES: HOXB13 expression is involved in normal prostate development, is a known regulator of androgen receptor (AR)-mediated transcriptomes in prostate tissue, and is also maintained in the formation of prostatic adenocarcinoma (PCa). In breast and other hormone-sensitive gynecological cancers, deregulated expression of HOXB13 correlates with aggressive tumor phenotypes and poor response to hormonal therapies. The predictive role of HOXB13 expression within prostate tumor progression and its associated outcomes remains unexplored.

METHODS: We utilized HOXB13 RNA transcriptome expression from several datasets of PCa radical prostatectomy (RP) genome-wide expression profiles from the Decipher GRID registry and public cohorts (n=6,679). We compared levels of HOXB13 expression by tumor progression and by histology (adenocarcinoma, neuroendocrine/small cell). We assessed the association of HOXB13 expression with pathologic/oncologic outcomes and risk of metastasis based on genomic signatures (Decipher score). Finally, we analyzed gene expression profiling of canonical AR and AR-V7 targets to investigate the association of HOXB13 with AR signaling.

RESULTS: There was a stepwise increase in the expression of HOXB13 from benign tissue, to primary tumor, to metastatic PCa (p=0.01). Increased levels of HOXB13 expression were associated with higher pathologic grade group (p=0.001), metastasis (p=0.001), and higher Decipher scores (p<0.001). In two retrospective cohorts (Hopkins, Mayo), higher HOXB13 expression was associated with decreased metastasis-free survival (p=0.02 and p=0.003, respectively). HOXB13 expression was also highly correlated to AR/AR-V7 target genes, NKX3-1 (r=0.73), and FOXA1 (r=0.67). Lower HOXB13 was associated with neuroendocrine biomarkers NCAM1 and ASXL3. Furthermore, HOXB13 expression was decreased in metastatic castrate-resistant PCa and neuroendocrine and small cell prostatic carcinoma (p<0.001).

CONCLUSIONS: In primary PCa, HOXB13 expression increases with progression and is associated with higher AR signaling and adverse pathologic and oncologic outcomes. Though, its expression is lowered when PCa becomes castrate-resistant and when these cancers develop neuroendocrine differentiation. These data support the hypothesis that increased levels of HOXB13 confer a more aggressive PCa phenotype with metastatic potential. Future research can explore the role of HOXB13 not only as a biomarker of aggressive disease but also as a therapeutic target in PCa.

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**MP68-11**

IDENTIFICATION OF INTERMEDIATE CELL POPULATIONS DURING TREATMENT INDUCED LINEAGE CONVERSION TO NEUROENDOCRINE PROSTATE CANCER.

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**MP68-12**

IMPACT OF SPOP MUTATION ON PROSTATE CANCER PROGNOSIS

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INTRODUCTION AND OBJECTIVES: SPOP mutation defines one of the dominant prostate cancer subclasses, present in ~10% of cases, or 16,000 newly diagnosed men in 2018. The independent impact of SPOP mutation on prostate cancer prognosis is unknown. Here, we define the impact of SPOP mutation on prostate cancer pathologic and clinical outcomes and determine whether delineation of this subclass impacts prostate cancer prognostic models.

METHODS: Molecularly profiled samples obtained as part of a multi-institutional cohort, the Decipher retrospective cohort, Decipher prospective cohort, and The Cancer Genome Atlas were utilized. SPOP mutation was defined either by DNA sequencing, or according to a highly accurate subclass predictor based on transcriptional data developed by our group. We assessed the relationship between SPOP mutation and clinical outcomes, and evaluated the utility of adding SPOP mutational status to prognostic models using distinct training and validation cohorts.

RESULTS: Incorporating results from multivariable proportional hazards models from all four cohorts using a fixed effects model demonstrated that SPOP mutation was associated with favorable pathology independent of preoperative prostate specific antigen (PSA), age, and pathologic Gleason score, OR 0.57, 95% confidence interval (CI) 0.34 to 0.93. SPOP mutation was not associated with biochemical recurrence, the development of metastases, or cancer specific mortality. The addition of SPOP status did not meaningfully alter prognostic models for adverse pathology, with a probability of difference in the area under the curve with and without addition of SPOP status of 0.93. Models incorporating SPOP status did not alter the positive and negative predictive ability of models for adverse pathology in a clinically meaningful fashion when tested in a validation cohort.

CONCLUSIONS: The presence of SPOP mutation is an independent predictor of favorable pathology at prostatectomy. Delineation of this subtype was not clinically useful in prognostic models. This finding underscores some of the challenges associated with incorporating genomics into prostate cancer prognostic models, and highlights the need for understanding the impact of progression events as they relate to genomic subclass.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>N</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Decipher Retrospective</td>
<td>1,421</td>
<td>0.64 (0.41 to 0.98)</td>
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<tr>
<td>Decipher Prospective</td>
<td>3,632</td>
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<td>The Cancer Genome Atlas</td>
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<td>Multi-Institutional</td>
<td>281</td>
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<td>Overall</td>
<td>5,811</td>
<td>0.57 (0.34 to 0.93)</td>
<td>0.026</td>
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</table>

Source of Funding: None
DDX3-MEDIATED TRANSLATIONAL REGULATION OF ANDROGEN RECEPTOR IN PROSTATE CANCER PROGRESSION

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INTRODUCTION AND OBJECTIVES: Prostate cancer (CaP) driven by androgen receptor (AR) can be targeted therapeutically by androgen deprivation therapy (ADT); however, in 10-20% of cases, ADT fails, allowing disease recurrence. AR heterogeneity is a negative prognostic marker, and AR negative cell growth has recently been implicated as a mechanism of therapy resistance. DDX3, an RNA helicase, can both aid and prevent translation of target mRNAs depending on localization. While DDX3 is implicated in cancer, its role as a translational regulator in CaP remains unstudied. Our objective was to investigate DDX3-mediated translational regulation of AR, and its contribution to the development of castration-resistant prostate cancer (CRPC).

METHODS: Using human tissue microarrays, we quantified expression of AR and DDX3 in CaP progression. To assess functional implications, we utilized the BCaP and LNCaP-C4 models of CaP progression to CRPC. To determine the RNA binding capacity of DDX3, we utilized 1) RNA immunoprecipitation (RIP) and 2) RNAscope. Effects of loss-of-function (siRNA, small molecule inhibitor) and gain-of-function (overexpression, ADT) experiments were assessed with qPCR, Western blot, and IF.

RESULTS: In patient samples and cell line models, DDX3 protein expression increased through progression, concurrent with localization to cytoplasmic puncta. RIP experiments identified AR as an mRNA target of DDX3 in the metastatic and CRPC cell lines. To determine the implications of DDX3 binding to AR mRNA, AR expression was investigated; while AR mRNA expression increased through progression, AR protein expression decreased. Loss-of-function experiments 1) reduced DDX3 punctate localization, 2) increased AR protein, and 3) increased PSA. Conversely, with DDX3 gain-of-function we saw DDX3 localized to puncta, reduction in AR protein, and reduction in PSA. These data suggest DDX3 acts as a translational repressor of AR in metastasis and CRPC; therefore, co-treatment of DDX3 inhibitors with ADT may prevent AR negative cell growth underlying recurrence. in vitro co-treatment with DDX3 inhibitors and antiandrogens showed decreased proliferation and increased apoptosis compared to either treatment alone, suggesting increased efficacy of antiandrogens with DDX3 inhibition.

CONCLUSIONS: DDX3 as a repressor of AR translation could have clinical implications as a mechanism of resistance to ADT. Based on preliminary data, DDX3 could be contributing to the regulation of AR translationally, and targeting DDX3 could reduce resistance and disease recurrence.

Source of Funding: T32 CA009135 (JEV) 1U54DK104310-01 (WAR)

MOLECULAR DISSECTION OF ANDROGEN RECEPTOR SIGNALING IN PROSTATIC ENDOTHELIAL, EPITHELIAL, AND STROMAL CELLS

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INTRODUCTION AND OBJECTIVES: Androgen receptor (AR) is involved in development prostate cancer (CaP), and is a primary target for treatment of CaP. Androgen deprivation therapy (ADT) inhibits AR signaling by reducing AR ligands and/or blocking AR-ligand binding. ADT induces apoptosis of both endothelial cells and epithelial cells of human prostate, with apoptosis of endothelial cells preceding the apoptosis of epithelial cells. Further, the AR response to stimulation by androgen also differs between the 2 cell types. The current understanding of AR signaling was gained predominantly using CaP cell lines and samples prepared from whole tissue specimens. The present study sought to delineate AR signaling specifically in endothelial, epithelial, and stromal cells isolated from fresh clinical prostate tissue specimens and primary xenografts of human prostate tissue. Our results reveal the potentially different roles of AR signaling in different human prostate cell compartments, how AR regulates normal differentiated cell functions, and the biological consequences of ADT.

METHODS: Prostate tissue remnants were transplanted to male nude mice to establish tissue xenografts. Endothelial and epithelial cells of both fresh prostate tissue and prostate tissue xenografts were isolated sequentially using magnetic beads conjugated with an antibody specific to epithelial cell or endothelial cell surface markers; the remaining cell fraction was defined as stromal cells. Transcriptomes were obtained using RNASeq, and were analyzed for differential expression of AR-regulated genes, AR co-regulators, and androgen metabolism enzymes.

RESULTS: Antibody-mediated cell type-specific enrichment isolated effectively the 3 cell types from the prostate from 16 patients. Among 1263 AR-regulated genes, 399, 313, and 223 were predominantly expressed in epithelial, endothelial or stromal cells, respectively. Among 179 AR co-regulators, 26, 26, and 18 were expressed predominantly in epithelial, endothelial or stromal cells, respectively. Among 1263 AR-regulated genes, 399, 313, and 223 were predominantly expressed in epithelial, endothelial or stromal cells, respectively. Among 128 potential androgen metabolic enzyme genes, 42, 15, and 12 were expressed predominantly in epithelial, endothelial or stromal cells, respectively.

CONCLUSIONS: Differences in expression of genes associated with AR-mediated trans-regulation were apparent between the different cell types of prostate, whereas, the difference in expression of androgen metabolism genes was less striking. Therefore, the organ level outcome of ADT can only be understood by determining the response of the different cell types in CaP tissue.

Source of Funding: 1R01CA193829