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TITLE: Function and Clinical Utility of the HOXB13 Cofactors MEIS1 and MEIS2 in Prostate Cancer Progression

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14. ABSTRACT Prostate cancer continues to be a significant and incurable health problem that will only become more prevalent as life expectancy increases. Thus, there is a dire need for alternative approaches to prevent cancer initiation, discern indolent from aggressive tumors, and treat metastatic disease. Recent evidence of germline HOXB13 mutations within a subset of familial prostate cancers supports a key role for HOX regulation pathways in prostate initiation and progression. Moreover, the majority of HOXB13 mutations are located within the MEIS-interacting domain and thus emphasizes the importance of MEIS-HOX protein interactions in prostate tumor biology. This proposal builds upon significant published and unpublished work demonstrating a key role for the MEIS proteins as critical transcription factors and HOX protein co-factors in suppressing prostate tumor progression, blocking cell proliferation, and promoting anti-metastatic gene expression. However, there remain significant shortcomings in our ability to translate our pathologic and mechanistic discoveries into patient benefit. The work proposed here has the high potential to identify new therapeutic directions for targeting prostate cancer cells and achieving more efficacious approaches to preventing, staging, and treating prostate cancer.					
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1. Introduction

Prostate cancer continues to be a significant and incurable health problem that will only become more prevalent as life expectancy increases. Thus, there is a dire need for alternative approaches to prevent cancer initiation, discern indolent from aggressive tumors, and treat metastatic disease. Recent evidence of germline HOXB13 mutations within a subset of familial prostate cancers supports a key role for HOX regulation pathways in prostate initiation and progression. Moreover, the majority of HOXB13 mutations are located within the MEIS-interacting domain and thus emphasizes the importance of MEIS-HOX protein interactions in prostate tumor biology. This proposal builds upon significant published and unpublished work demonstrating a key role for the MEIS proteins as critical transcription factors and HOX protein co-factors in suppressing prostate tumor progression, blocking cell proliferation, and promoting anti-metastatic gene expression. However, there remain significant shortcomings in our ability to translate our pathologic and mechanistic discoveries into patient benefit.

The goal of this study is to translate our pathologic and mechanistic discoveries of MEIS-associated tumor suppression into patient benefit. The **objective** of this proposal is to define how MEIS disrupts oncogenic AR-HOXB13 interactions, develop robust and reliable reagents to detect MEIS expression in tumor specimens, and to further develop compounds that can be used clinically to increase MEIS expression. Our **central hypothesis** is that MEIS protein expression confers an indolent tumor phenotype, MEIS inhibits AR-activity by disrupting HOXB13 interactions, and drugs that increase MEIS expression will also disrupt AR-HOXB13 interactions and block cell proliferation. Completion of this work will represent a significant leap forward in our understanding of MEIS, AR, and HOX protein function in prostate cells, and has the high potential to lead to new reagents for cancer staging and therapeutic intervention. We will accomplish our objectives via the following three Aims:

Specific Aim 1: To define the functional and phenotypic impact of MEIS expression on AR signaling in prostate cancer cells

Specific Aim 2: To develop robust MEIS detection reagents for formalin-fixed, paraffin-embedded (FFPE) prostate tissues.

Specific Aim 3: To determine if epigenetic restoration of MEIS expression can suppress cell growth and inhibit formation of AR-HOXB13 complexes.

2. Keywords

Prostate Cancer; MEIS1, MEIS2, HOXB13, Androgen Receptor (AR)

3. Accomplishments

Research accomplishments are based upon the outlined Statement of Work. These are as follows:

Major Task 1: Determine the ability of MEIS expression to alter responses to AR agonists and antagonists in vitro.

Subtask 1: Perform growth, death, invasion, and differentiation assays of MEIS-expressing cells in the presence or absence of AR agonist and/or antagonist.

Progress: We have engineered and characterized MEIS-expressing CWR22Rv1 and LAPC4 cells; MEIS-expression results in decreased in vitro growth and invasion. Further, MEIS expression results in variable AR protein expression. We are thus characterizing how these changes in AR protein expression modify the expression of AR-target genes in response to AR antagonists and agonists. Data is shown in **Figure 1**.

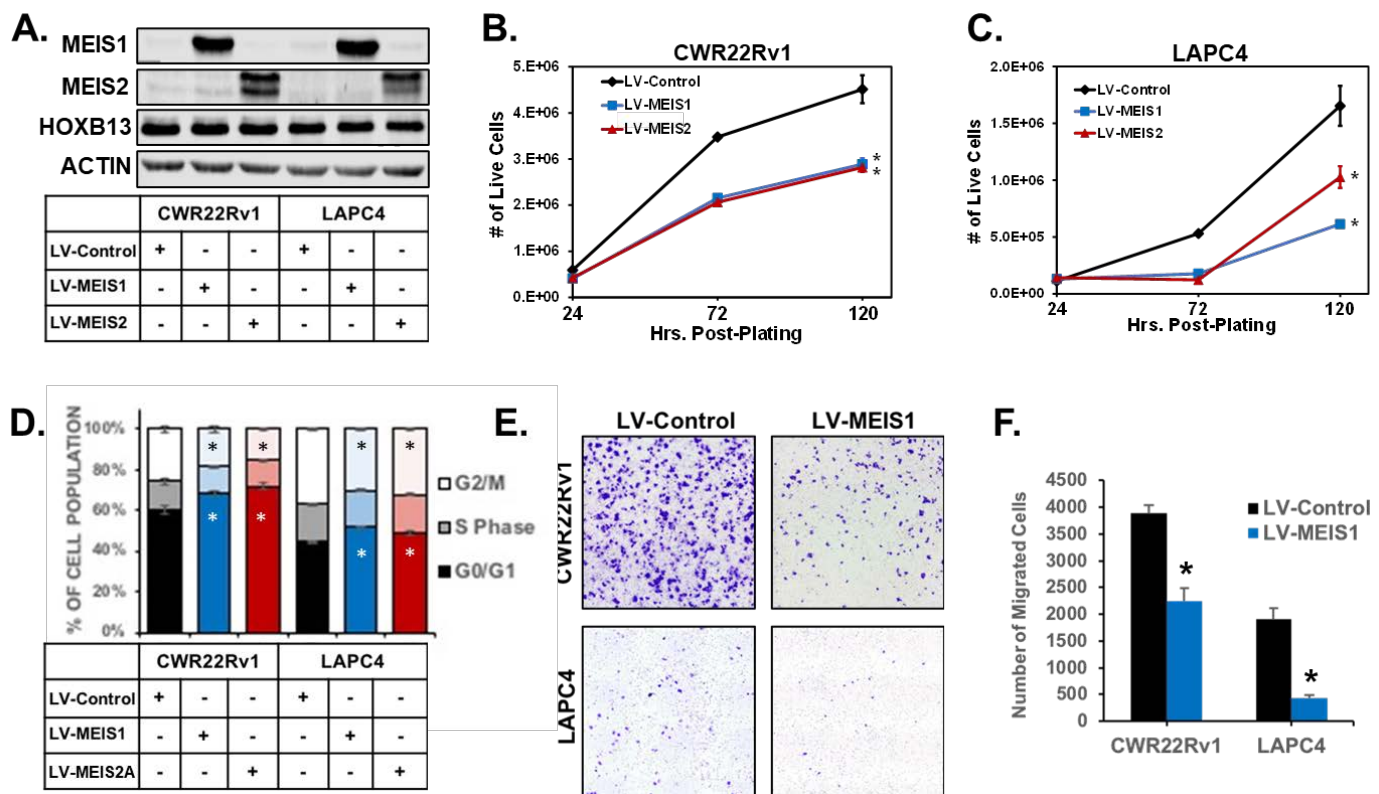


Figure 1: Expression of MEIS1 or MEIS2 in PrCa cell lines is sufficient to decrease growth and invasion *in vitro*. (A) Western blot confirmation of lentiviral overexpression of MEIS1 or MEIS2 in CWR22Rv1 and LAPC4 cell lines. LV-Control encodes an expression plasmid for constitutive Cas9 expression. Endogenous HOXB13 expression was also assessed in all lines. Actin was used as a loading control. (B and C) Proliferation of CWR22Rv1 and LAPC4 with exogenous expression of MEIS1 (blue), MEIS2A (red), or control (black). Cell number over time was assessed by manual counting of live cells on a hemocytometer. Data represent mean count and SEM at each time point (technical replicates, n=3). Data for LV-Control and LV-MEIS2A is the same as in Figure S2D-E (D) Cell cycle analysis determined by propidium iodide (PI) fluorescence intensity in CWR22Rv1 and LAPC4 cells with exogenous expression of MEIS1 (blue), MEIS2A (red), or control (black). Data represent mean (technical replicates, n=3) and SEM. (E) Representative images of transwell migration assays for CWR22Rv1 (top) and LAPC4 (bottom) of cells with exogenous expression of control (left) or MEIS1 (right). (F) Quantification of transwell migrations performed in Figure 1E. Data represent mean (technical replicates, n=4) and SEM. * indicates P<0.05.

Major Task 2: Determine the ability of MEIS expression to alter responses to host castration *in vivo*.

Subtask 1: Obtain IACUC and ACURO regulatory approval

Progress: We obtained IACUC and ACURO regulatory approval.

Subtask 2: Tumor xenograft growth and response to host castration.

Progress: We have completed the proposed animal experiment using both CWR22Rv1 and LAPC4 cell lines. These data demonstrated a clear and statistically-significant tumor suppression by MEIS1 in both cell lines between both intact and castrate conditions. These tumor tissues are now under analyses for AR-target gene expression, immunohistochemical analyses of tumor markers of proliferation and apoptosis, and AR interactions with MEIS1 and HOXB13. Kaplan-Meier data of host survival is shown in Figure 2.

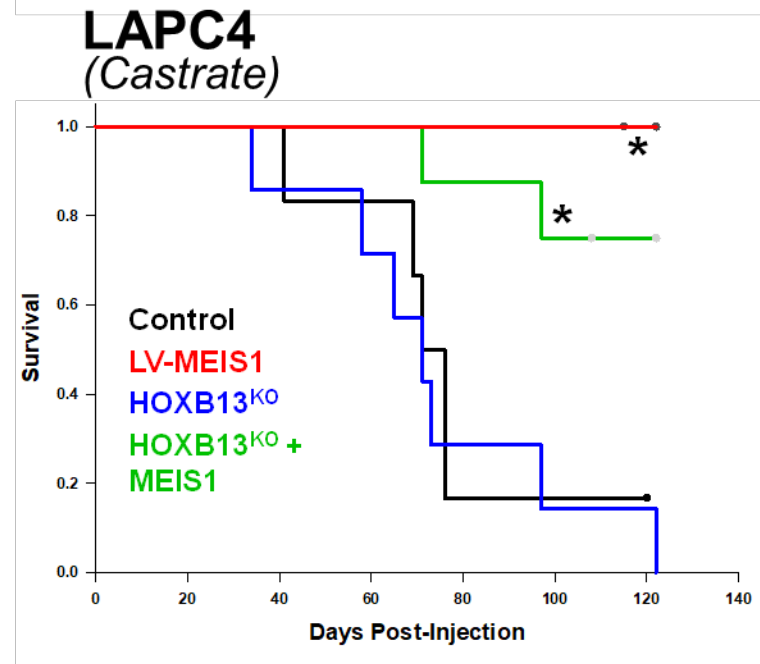
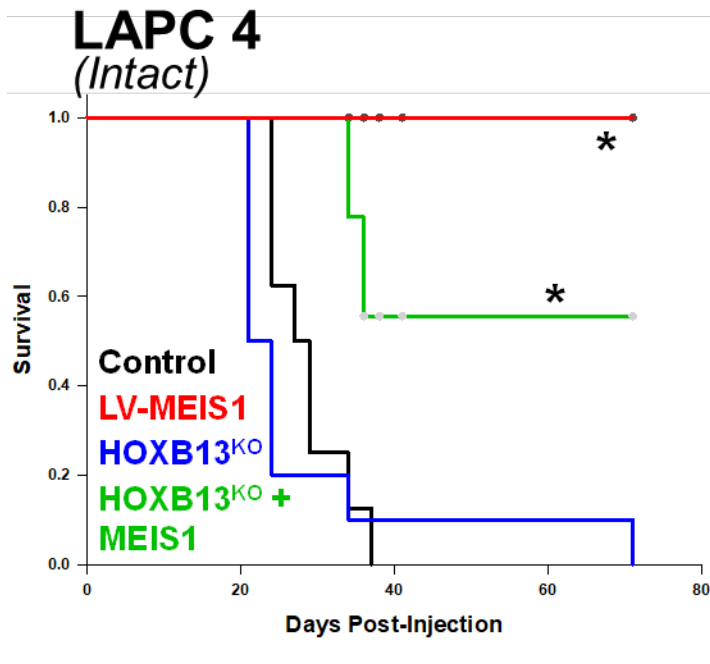
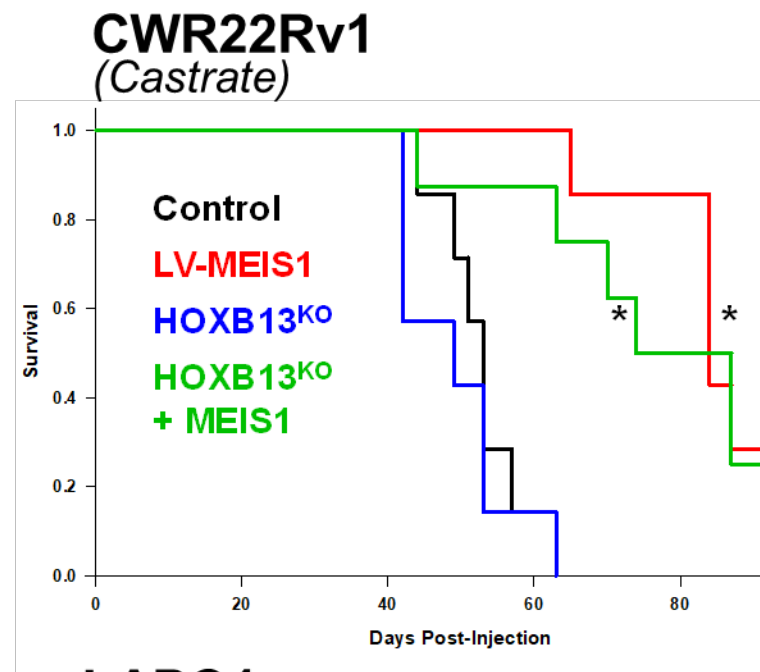
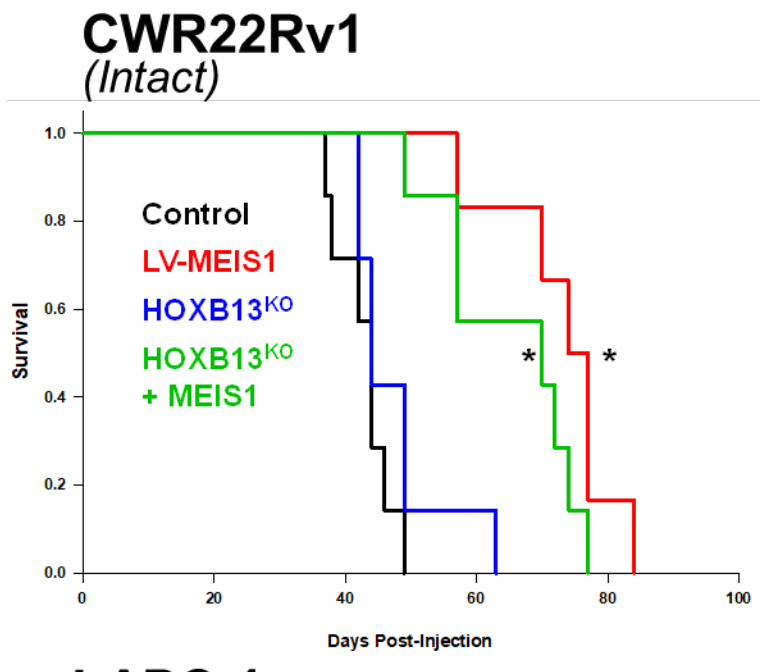


Figure 2: In Vivo Tumor Xenograft Growth of MEIS1-Expressing CWR22Rv1 and LAPC4 Prostate Cancer Cells in Hormonally Intact vs. Castrate Conditions. Stable MEIS1 expression in prostate cancer cells (CWR22Rv1 and LAPC4) was tested for its ability to modulate tumor growth and AR-mediated tumor growth between hormonally-intact vs. castrated conditions. In addition, CRISPR-mediated deletion of HOXB13, a major MEIS1 transcriptional co-factor, was also evaluated. These data show significant suppression of tumor growth when MEIS1 is expressed, regardless of HOXB13 expression. * indicates $P < 0.05$.

Major Task 3: Identify differential AR transcriptional targeting and gene regulation in the presence of MEIS1.

Subtask 1: Treat MEIS1-expressing CWR22Rv1 and LAPC4 cells with either AR agonist or antagonist. Collect RNA at various time points post treatment and perform RNA-seq.

Subtask 2: Treat MEIS1-expressing CWR22Rv1 and LAPC4 cells with either AR agonist or vehicle. Perform AR ChIP-seq.

Progress on both Subtasks: RNA and protein from the CWR22Rv1 and LAPC4 tumor xenografts are being utilized to conduct RNA-seq (Subtask 1), and AR ChIP-Seq (Subtask 2). RNA-seq libraries have been

prepared and are undergoing final QC for sequencing. The AR ChIP workflow is optimized, and we are currently conducting AR ChIP to prepare and sequencing additional libraries. Data QC is described in **Figure 3**.



Figure 3: Quality Scores of 48 RNA-Sequencing Libraries derived from the Tumor Xenografts in Figure 2. Three distinct CWR22Rv1 and LAPC4 tumor xenografts (Control, LV-MEIS1, HOXB13^{KO}, and HOXB13^{KO}+LV-MEIS1) were prepped for RNA extraction and RNA-Seq library creation. This resulted in 48 total RNA-Sequencing libraries (CWR22Rv1 and LAPC4; Control, LV-MEIS1, HOXB13^{KO}, and HOXB13^{KO}+LV-MEIS1 lines; intact vs. castrate conditions; 3 libraries per condition for a total of 48 libraries). Graphs depict satisfactory QC and per Sequence QC scores for all 48 libraries. Bioinformatic analyses is currently underway.

4. Impact

Data from a few of our initial experiments were incorporated into our recent manuscript, entitled: “*MEIS-mediated suppression of human prostate cancer growth and metastasis through HOXB13-dependent regulation of proteoglycans.*” This was published in eLife in June 2020.

5. Changes/Problems

We have encounter no problems or hurdles which require modifications to our Statement of Work. Further, we were fortunate to have initiated our animal experiments prior to COVID-related shutdowns, and were able to maintain these experiments during lab closures.

6. Products

Nothing to report.

7. Participants & Other Collaborating Organizations

Nothing to report.

8. Special Reporting Requirements

Nothing to report.

9. Appendices

VanOpstall C, Perike S, Brechka H, Gillard M, Lamperis S, Zhu B, Brown R, Bhanvadia R, and **Vander Griend DJ**. *MEIS-Mediated Suppression of Prostate Cancer Growth and Metastasis Through HOXB13-Dependent Regulation of Proteoglycans*. *eLife*; June 18, 2020; 9:e53600. doi: 10.7554/eLife.53600.

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