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TITLE: HOXB13-Dependent Metastasis Suppression of Prostate Cancer by Proteoglycan Signaling

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14. ABSTRACT

HOXB13 is the predominant HOX gene expressed in prostate epithelial cells, and germline mutations in HOXB13 are strongly associated with increased prostate cancer incidence. Significant research has focused on the role of HOXB13 as it relates to Androgen Receptor (AR) signaling and cancer cell responses to AR-targeted therapies. Our laboratory recently determined that the HOX co-factors MEIS1 and MEIS2 are novel tumor suppressor genes in prostate cancer, are the predominant HOXB13 binding partner in non-malignant prostate epithelial cells, and interfere with oncogenic AR/HOXB13 transcription. MEIS proteins function as critical transcriptional co-factors during development and within adult tissues to bind HOX proteins and specify HOX gene targeting. In fact, the majority of germline *HOXB13* mutations are located within the MEIS-interacting domain, emphasizing the importance of MEIS/HOX interactions in prostate tumor biology. Our data supports a critical tumor-suppressive and anti-androgen role for MEIS proteins in prostate cancer, and while HOXB13 mutations are uncommon, we have shown that MEIS proteins are frequently down-regulated in prostate tumors. However, there remain significant gaps in our understanding of the mechanistic function of MEIS proteins to block metastatic progression, and the impact of HOXB13 mutations on MEIS binding and gene regulation; filling such knowledge gaps has a high potential to functionally implicate new biomarkers to predict cancer progression and new targets to therapeutically block prostate cancer metastasis.

15. SUBJECT TERMS
Prostate Cancer, MEIS proteins, HOXB13, Decorin, metastasis, Androgen Receptor

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The goal of this project is to understand how MEIS proteins suppress prostate tumor metastasis in order to develop functional biomarkers and pharmacologic strategies to prevent and treat prostate cancer progression. The objective of this proposal is to define how MEIS proteins suppress metastasis, how germline *HOXB13* mutations impact MEIS-mediated tumor suppression, and whether MEIS re-expression can block AR-V7 function in Enzalutamide-resistant prostate cancer cells. Our **central hypothesis** is that MEIS expression drives prostate tumor indolence via proteoglycan-mediated inhibition of cell proliferation and migration, blocks AR-V7 binding to HOXB13, and that *HOXB13* mutations reduce MEIS-mediated tumor suppression.
Specific Aim 1: To determine the role of MEIS-mediated regulation of proteoglycans in suppressing prostate cancer growth and metastasis.
Specific Aim 2: To elucidate the functional impact of *HOXB13* mutations on MEIS-mediated tumor suppression.
Specific Aim 3: To determine the role of MEIS expression on DCN expression and activity using patient-derived xenograft (PDX) models.

2. Keywords

Prostate Cancer; MEIS1, MEIS2, HOXB13, Decorin, Patient-Derived Xenograft, Androgen Receptor (AR)

3. Accomplishments

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

Major Task 1: Determine the necessity of DCN expression for MEIS-mediated metastasis suppression.
Subtask 1: Obtain IACUC and ACURO regulatory approval for animal work.
Progress: We obtained IACUC and ACURO regulatory approval.
Subtask 2: CRISPR Targeting of DCN and MEIS-re-expression in CWR22Rv1 and LAPC4 cells.
Progress: We designed targeting guide RNAs against DCN, and have targeted the DCN gene CWR22Rv1 and LAPC4 cells using CRISPR-Cas9 editing approaches. We are currently screening clonal lines via western blotting for loss of DCN expression.

Major Task 3: Determine the impact of *HOXB13* mutations on MEIS-mediated growth suppression.
Subtask 1: Creation and validation of HOXB13 mutants using CRISPR-Cas9 targeting.

Progress: We have successfully acquired multiple CWR22Rv1 cells harboring the G84E HOXB13 mutation. This includes a wild-type isogenic clone (**Figure 1A**). These cells were analyzed for HOXB13 protein expression as well (**Figure 1B**). We are currently ectopically-expressing lentiviral MEIS1 in these lines, and plan to evaluate DCN, among other targets.

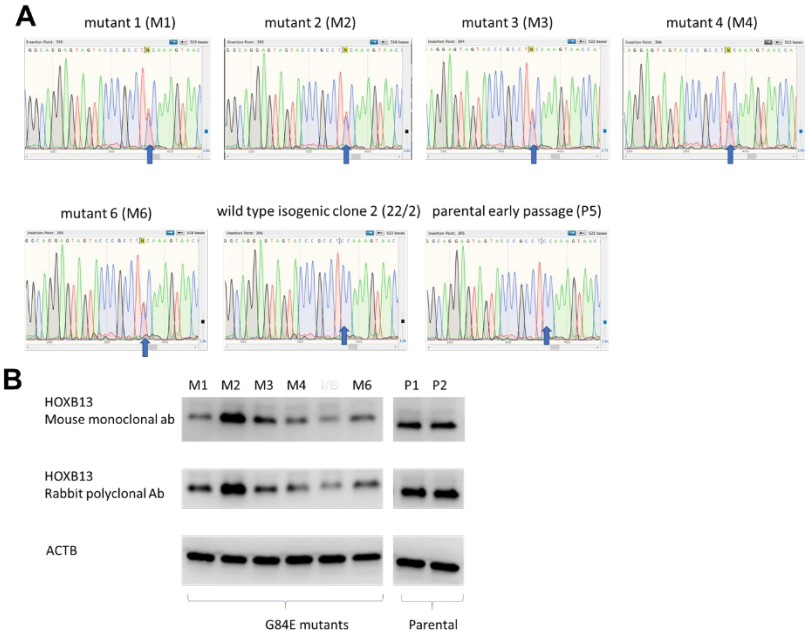

Figure 1: CRISPR gene targeting to create heterozygous HOXB13 (G84E) mutant prostate cancer cell lines. A) Sequencing data demonstrating heterozygous HOXB13 (G84E) mutations in one allele of CWR22Rv1 cell lines. An isogenic wild-type control was also acquired. B) Western blotting demonstrating HOXB13 protein expression of the G84E clones.

Major Task 5: Elucidate the expression of DCN target pathways within $MEIS^{High}$ vs. $MEIS^{Low}$ PDX tumors.
Subtask 1: Complete MTA, obtain, and expand LuCaP PDX tumor xenograft models.
Progress: We have completed the MTA for the PDX tumor models, have received them from our collaborator, and have successfully established them within our own animal facility. These tumors are currently growing and being expanded into additional mice for cryopreservation, analyses, and viral infection.

4. Impact

The majority of the preliminary data utilized for the grant was published in 2020:
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.

DCN knockout prostate cancer cell lines will have value to the prostate cancer research community.

The implementation of PDX models in our lab represent new model systems for our team and research community.

The HOXB13 mutant lines will have value to the prostate cancer research community.

5. Changes/Problems

We have encountered no problems or hurdles which require modifications to our Statement of Work. Further, we were fortunate to have initiated our animal and cell-based experiments during COVID-related slowdowns and the need for decreased personnel in the lab.

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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