Decreases in NKX2-5 expression during prostate carcinogenesis induce malignant prostate features

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Background: Prostate cancer (CaP) causes more than 33,000 American cancer deaths annually because therapies for metastatic CaP fail. Novel targets are needed for functionally diverse treatments. We explored whether cancer-initiating events rather than drivers of CaP progression can provide such alternatives. We determined the functional consequences of NKX2-5 CpG island promoter hypermethylation, a recurrent epigenetic alteration that marks the transition from benign to malignant prostate and is maintained in metastatic treatment-resistant CaP. NKX2-5 is a cardiac transcription factor whose role in prostate (cancer) is unknown.
Methods: LNCaP and C4-2 CaP cells, the transformed prostate epithelial cell lines RWPE1 and RWPE2, and non-targeting control siRNAs and NKX2-5 targeting siRNAs were used in rhodamine phalloidin staining, immunofluorescence staining, cell proliferation, cell migration and cell-matrix adhesion assays, ATAC-Seq and RNA-Seq followed by GSEA and Homer analyses, and ChIP and CoIP studies.
Results: Silencing of NKX2-5 in both benign and malignant prostate cells increased cell size, number of cellular extensions, and nuclear size but decreased nuclear heterochromatin content -- all morphological features on which pathologists rely for a CaP diagnosis. These changes were accompanied by decreased CaP cell-matrix adhesion, an initiating step in cancer metastasis. Integrated ATAC-Seq and RNA-Seq assays confirmed a preferential association with metastasis and revealed that loss of NKX2-5 increased accessible chromatin regions, which were significantly enriched for binding sites for TEAD1. TEAD1 is a transcription factor that executes Hippo signaling, which is frequently altered in human malignancies, linked to aggressive cancer morphology and progression, but not yet implicated in CaP. ChIP and CoIP studies confirmed TEAD1 regulation of genes whose expression was induced by loss of NKX2-5 and verified formation of functional TEAD1 transcriptional complexes upon NKX2-5 silencing.
Conclusions: NKX2-5 promoter hypermethylation, a biomarker of prostate carcinogenesis, induces aggressive CaP morphology and behavior that is mediated by activation of Hippo signaling, whose pharmacological inhibition may serve as alternative CaP treatment.