

## **MBD4 modulates prostate lineage and tumorigenesis by restricting FOXA1-driven enhancer reprogramming**

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**Background:** Epigenetic reprogramming of lineage-defining transcriptional programs is a central driver of cancer initiation, progression, and therapy resistance. In prostate cancer, androgen receptor (AR) coordinates distinct transcriptional programs associated with normal prostate differentiation or tumorigenesis through utilization of distinct regulatory elements. However, the epigenetic mechanisms that direct these fates remain unclear. Using a system that selectively modulates canonical androgen response element (ARE)-containing regulatory elements, we identified MBD4 as a candidate regulator of divergent AR transcriptional programs. We hypothesized that MBD4 preserves lineage-restricted enhancer states by limiting FOXA1 pioneer-factor engagement.

**Methods:** An epigenetic-focused CRISPR screen was performed in LNCaP activating MACC cells. MBD4 loss or restoration was evaluated in AR-positive prostate cancer models and genetically normal mouse prostate organoids using growth assays, RNA-seq, ATAC-seq, CUT&RUN, whole-genome bisulfite sequencing, RIME, and co-immunoprecipitation. HDAC1 function was interrogated using genetic and pharmacologic perturbation. Multi-omic data were integrated with human prostate single-cell, epigenomic, dependency, and clinical datasets.

**Results:** MBD4 loss enhanced growth of AR-positive prostate cancer cells, shifted AR output toward an oncogenic program, and produced widespread gains in chromatin accessibility enriched for FOXA1 motifs. MBD4 deletion expanded the FOXA1 cistrome, with 46,338 gained FOXA1 sites accompanied by increased AR occupancy, H3K27ac, and accessibility at affected enhancers. Conversely, acute MBD4 induction preferentially reduced FOXA1 binding. MBD4 occupied FOXA1-enriched active regulatory elements and associated with HDAC1/2-containing complexes by RIME and co-immunoprecipitation. Loss of HDAC1, but not HDAC2, shifted cells toward the MBD4-loss transcriptional state. Whole-genome bisulfite sequencing showed no substantial change in global or enhancer DNA methylation after MBD4 loss. In normal prostate organoids, *Mbd4* loss promoted luminal differentiation at the expense of progenitor features and reprogrammed FOXA1-bound enhancers toward disease-relevant regulatory programs. Human datasets further showed that MBD4-associated transcriptional and chromatin states vary across prostate cancer lineages, with distinct MBD4 dependency in AR-positive versus AR-independent models.

**Conclusions:** MBD4 is a lineage-dependent chromatin regulator that restricts FOXA1 enhancer reprogramming and shapes AR transcriptional output. MBD4 associates with HDAC1-linked repression at FOXA1-enriched regulatory elements, while MBD4 loss expands the accessible FOXA1/AR enhancer landscape and promotes luminal oncogenic states, connecting enhancer selection with prostate lineage identity and tumorigenesis.

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