Loss of FOXA1 Results in Genome-wide Epigenetic Reprogramming and activation of Interferon-Response Genes including *CD274*/PD-L1

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Abstract

Forkhead Box A1 (FOXA1) is a pioneer transcription factor (TF) critical in epigenetic regulation of chromatin state and cell fate determination. Reduced FOXA1 is an independent predictor of poor overall survival in bladder cancer (BC) patients. However, the impact of FOXA1 loss on chromatin epigenetics in BC is unknown. Therefore, we determined the impact of *FOXA1* KO on epigenetic modification of chromatin and associated gene expression. Our integrated analysis identifies FOXA1 as an important regulator of chromatin epigenetic state and expression of immune checkpoint (IC) targets in BC.

METHODS: We used CRISPR/Cas9 to delete *FOXA1* in the human luminal BC cells. We performed RNA-seq and Chip-seq for H3K27ac, an epigenetic mark of active enhancers/promoters. Motif and GSEA analysis of RNA/Chip-seq were performed to identify *FOXA1* KO-induced epigenetic differences influencing gene expression. Western blotting (WB)/qPCR confirmed FOXA1-mediated CD274/PD-L1 expression. *In silico* analysis of TCGA data also confirmed relevance to human disease.

RESULTS: We identified 8,230 differentially expressed genes in *FOXA1* KO. Notably, GSEA identified IFNα/ɣ gene signatures as enriched in *FOXA1* KO. As expected, the majority of differences in H3K27ac across genomic areas in *FOXA1* KO were mapped to intergenic (n=6,250 peaks; 42% of total peaks) and intronic (n= 6,490 ; 43%) regions. In addition, differential H3K27ac levels were also mapped to proximal promoters (n= 1,306 ; 9%) as well as within gene bodies (n=931 ; 6%). Integrated analysis of RNA/ChIP-seq shows changes in gene expression are mirrored by differences in H3K27ac. Motif analysis of DNA sequence enriched for H3K27ac identified significant increases in TF binding motifs including the interferon (IFN) sensitive response element (ISRE) and IFN response factors such as IRF1. Moreover, we identified increased H3K27ac of regulatory elements as being associated with several upregulated ISGs in *FOXA1* KO. These include *ISG15*, *IFIT2/3*, *IFI44L* and *CD274*/PD-L1. WB/qPCR confirmed upregulation of several ISGs, including *CD274*/PD-L1 following *FOXA1* KO. Analysis of TCGA data confirmed an inverse relationship between *FOXA1* and *CD274* in BC and other cancers.

CONCLUSIONS: In summary, we provide evidence of widespread epigenetic reprogramming after *FOXA1* KO in BC cells. Additionally, we provide evidence that epigenetic changes contribute to activation of a global IFN-dominant signature, including *CD274*/PD-L1 in a cancer cell-intrinsic manner in *FOXA1* KO.

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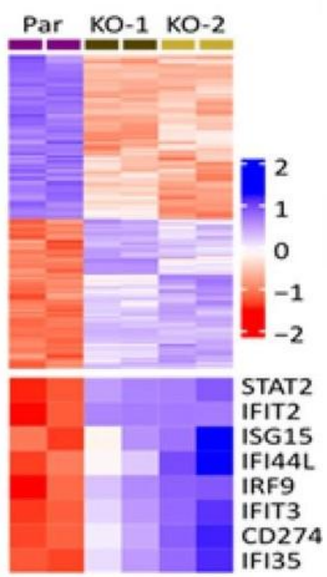
Results



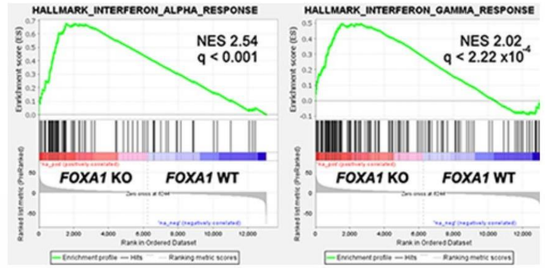
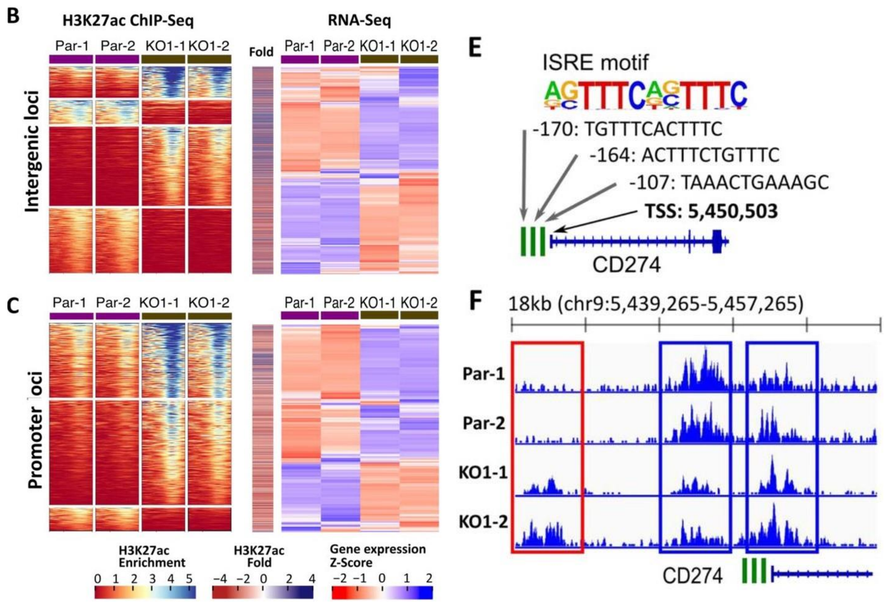
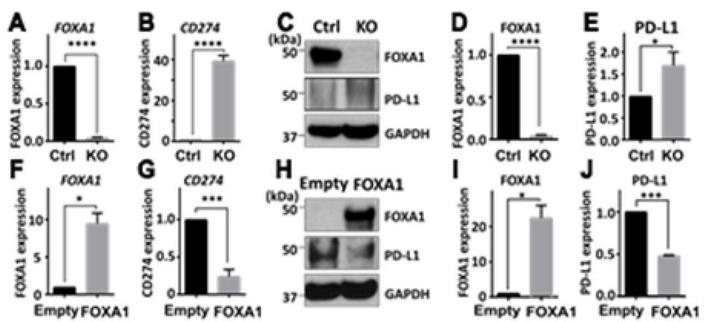


Figure2: *FOXA1* knockout in UMUC1 bladder cancer cells activates a global IFNα/ɣ gene expression program associated with response to immune checkpoint treatment. (**A**) Heat map showing 1,914 differentially expressed genes (FDR q<0.05) following *FOXA1* KO in UMUC1. Two replicates are shown for each sample, with two biologic replicates for UMUC1 *FOXA1* KO. (**B**) Gene Set Enrichment Analysis (GSEA) identified enrichment for genes included in the Hallmark (**A**) IFNα and IFNɣ response in *FOXA1* KO UMUC1 cells (clone 2 shown; see text for NES and q values for clone 1). (**D**) Differentially expressed interferon-sensitive genes (ISGs) are highlighted.





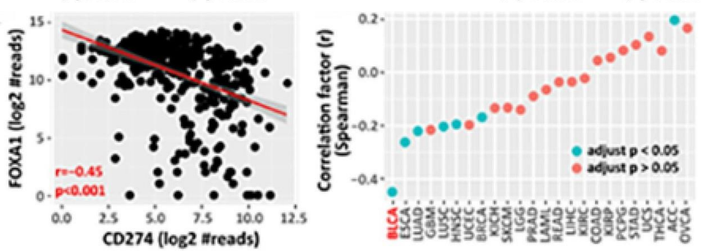


Figure3: *CD274*/PD-L1 is a FOXA1-repressed immune checkpoint target. Q-RT-PCR (**A** and **B**), western blotting (**C**) and densitometry (**D** and **E**) confirms decreased FOXA1 and increased *CD274*/PD-L1 expression in UMUC1 *FOXA1* KO cells. Q-RT-PCR (**F** and **G**), western blotting (**H**) and densitometry (**I** and **J**) confirms increased FOXA1 and decreased *CD274* /PD-L1 expression in UMUC3 *FOXA1* overexpressing cells. (**K**) Inverse correlation between *FOXA1* and *CD274* (encoding PD-L1 expression) in the TCGA bladder data set (r=−.45; p<0.001; Spearman correlation test). (**L**) Spearman correlation of *FOXA1* and *CD274* across cancers identifies a significant correlation between these genes in a number of malignancies.

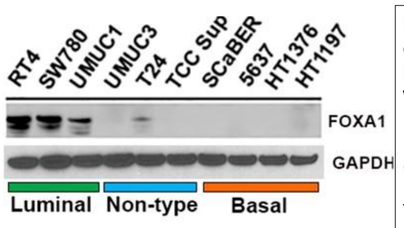


Figure1: FOXA1 is highly expressed in luminal bladder cancer cell lines. Western blot analysis of FOXA1 protein expression in 10 human BC cell lines (Luminal: RT4, SW780, UMUC1/ Non-type: UMUC3, T24, TCCSup/Basal: SCaBER, 5637, HT1376, HT1197).

Figure4: Genetic knockout of *FOXA1* results in widespread enhancer and promoter reprogramming in human cells (**A** and **B**) H3K27ac cluster analysis (left panels) and expression heatmaps (right panels) showing relationship between H3K27ac ChIP-seq and expression of 1,724 associated genes (RNA-seq) at (**A**) intergenic (including enhancers) and (**B**) promoter levels. These data show decreased H3K27ac is associated with reduced gene expression following *FOXA1* KO, while increased H3K27ac is associated with increased gene expression following *FOXA1* KO. (**C**) Illustration identifying the location of several interferon-sensitive response element (ISRE) motifs in the areas of increased acetylation in the *CD274* promoter. (**D**) Increased acetylation of *CD274* regulatory elements including an upstream enhancer (red box) and the proximal promoter region (blue boxes) following *FOXA1* KO.

Acknowledgements

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