Abstract #1813

FOXM1 target genes associated with cell cycle regulation predict breast cancer metastatic outcome

C. Yau[1], L. Meyer[2], S. Benz[3], C. Vaske[3], G. Scott[1], B. Egan[4], P. Labhart[4], E. Mitchell[2], N. Pourmand[2], C.C. Benz[1]

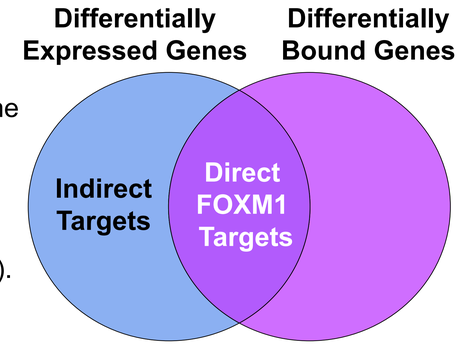
[1] Buck Institute for Research on Aging, Novato, CA; [2] University of California, Santa Cruz, Santa Cruz, CA; [3] Five3 Genomics, Santa Cruz, CA; [4] Active Motif, Carlsbad, CA

Introduction

FOXM1 is a key transcription factor regulating both cell proliferation and DNA damage checkpoint responses; and previous studies have demonstrated that higher breast cancer FOXM1 expression is associated with worse patient survival. Despite its functional and prognostic significance, the FOXM1 cistrome remains largely uncharacterized. Thus, in this study, we utilized chromatin immunoprecipitation sequencing (CHIPseq) and paired-end RNA sequencing (RNAseq) to comprehensively characterize the direct FOXM1 target genes associated with proliferation in ER+ MCF-7 breast cancer cells, and assessed their prognostic value in a pooled set of 683 adjuvant chemotherapy naive breast cancers. As well, we evaluated the context dependency of the FOXM1 cistrome by contrasting these direct targets to FOXM1 bound genes unique to p53 upregulation.

Methods

Proliferating and confluent MCF-7 cells were harvested. RNA-seq and FOXM1 CHIP-seq were performed using UCSC Genome Sequencing Center and Active Motif services, respectively. Genomic regions showing differential FOXM1 binding were identified using MACS (p<1e-7); and regions mapping within 5kb (-4.5kb to +0.5kb) of a transcription start site were assigned to genes. Differentially expressed genes were identified using DESeq (Benjamini-Hochberg (BH) corrected p < 0.05, >1.5 absolute fold change). Enriched functional categories (GO biological processes, KEGG, Reactome or BioCarta pathways) among target genes were identifed using DAVID Bioinformatics Resources (BH-corrected EASE < 0.05). The average expression levels of target genes were computed and their median value used to dichotomize a pooled set of 683 node negative chemotherapy naive breast cancers. Prognostic significance was assessed by log rank test.



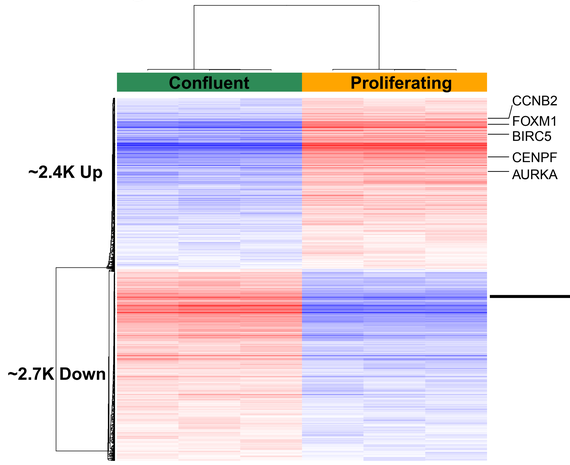
Characterization of FOXM1 Levels

I. Western blot analysis shows that proliferating (Pro) MCF-7 cells has higher FOXM1 levels than confluent (Conf) cells.

Identification of FOXM1 Direct Targets Associated with Proliferation

Transcriptome Profiling

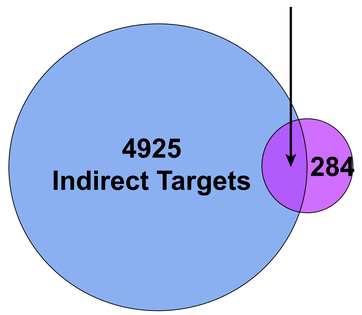
II. ~5.1K unique genes are differentially expressed between proliferating and non-proliferating confluent MCF-7 cells.



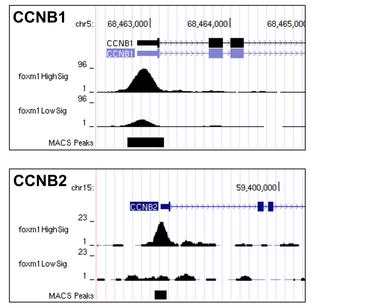
FOXM1 CHIP-sequencing

III. FOXM1 CHIP-sequencing identified ~2.5K differentially bound FOXM1 sites.

IV. Only 429 genes have a differentially bound FOXM1 sites within 5kb of their transcription start site.



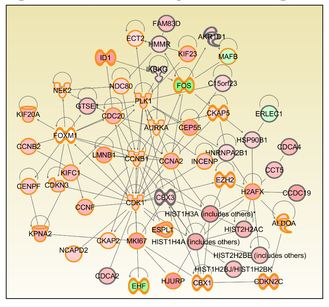
Representative Differentially Bound FOXM1 Sites



Note: FOXM1 itself is an indirect target

Functional Analysis

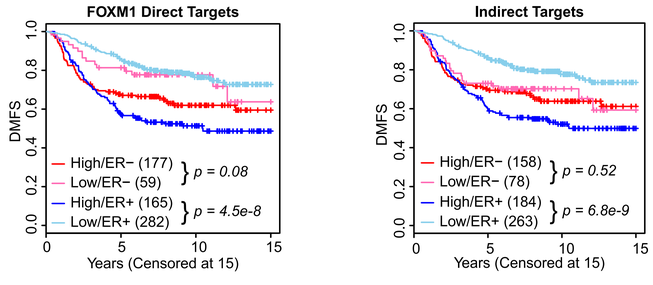
V. FOXM1 direct targets were enriched in 43 functional categories, mostly relating to cell cycle and chromatin assembly.



Ingenuity Pathway Analysis Connecting Direct Targets to FOXM1. Genes involved in cell cycle or cellular assembly and organization are highlighted in orange.

Prognostic Significance

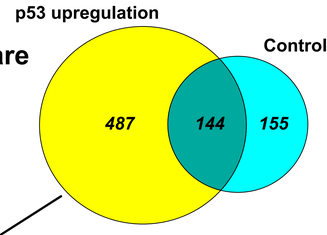
VI. FOXM1 direct targets are prognostic in ER+ breast cancers.

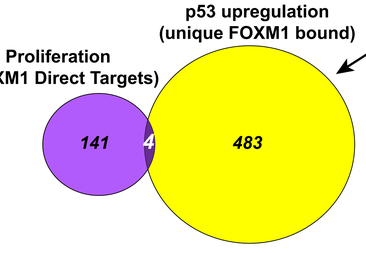


VII. They appear better prognostic markers than indirect targets in ER- breast cancers.

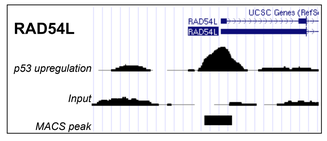
Context Dependent FOXM Target Genes

VIII. 487 FOXM1 bound genes are unique to the p53 upregulation condition when compared to control MCF-7 cells.





Cellular response to stress is the top enriched functional category among these 487 FOXM1 bound genes.



XI. Only 4 genes are potentially shared FOXM1 direct targets between proliferating and p53 up-regulated MCF-7 cells.

Conclusion

Our findings demonstrate that following induction of breast cancer cell proliferation, FOXM1 direct target genes are primarily associated with cell cycle regulation and appear to be better biomarkers of breast cancer metastatic outcome than proliferation associated indirect gene targets not associated with FOXM1 binding changes. Interestingly, these direct target genes appear context dependent, and have minimal overlap with potential FOXM1 direct targets associated with p53 upregulation.