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Epigenomic analysis of cancer stem cell (CSC)-enriched triple-negative breast cancer (TNBC) populations reveals gene regulatory circuitry and novel tumor cell vulnerabilities

Matthew G. Guenther[1], Arthur W. Lambert[2], Christopher Fiore[1] , Yogesh Chutake[1], Mei Wei Chen[1], David Orlando[1], Matthew Eaton[1], Brian Bierie[2], Robert Weinberg[2], Christian C. Fritz[1], Eric Olson[1]

[1]Syros Pharmaceuticals, 620 Memorial Drive, Cambridge, MA 02139; [2]Whitehead Institute for Biomedical Research and Massachusetts Institute of Technology, Nine Cambridge Center, Cambridge, MA 02142

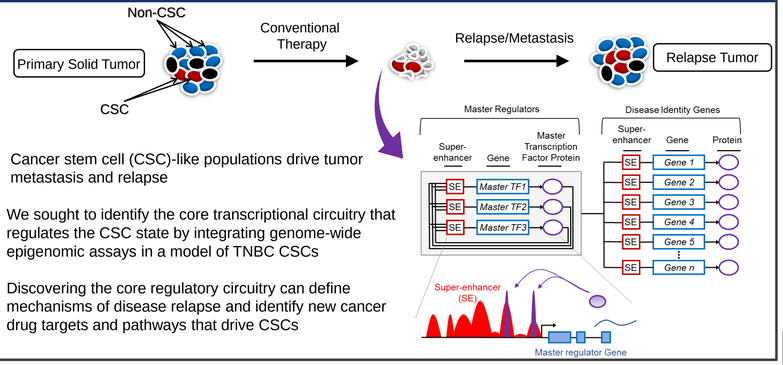
Abstract

Tumor-initiating cells (TICs), also termed cancer stem cells (CSCs) are involved in breast cancer chemoresistance, metastasis and disease relapse. To pinpoint tumor cell vulnerabilities and transcriptional drivers of therapeutic relevance, we have characterized the triple negative breast cancer (TNBC) CSC transcriptional landscape using epigenome mapping and nucleosome occupancy determination. We identify a set of transcriptional regulators and signaling mediators that enforce the cancer stem cell state and instruct potential therapeutic strategies.

The basal epithelial marker, integrin-β4 (ITGB4), can be used to stratify mesenchymal-like triple-negative breast cancer (TNBC) cells into populations of low and high tumor-initiating ability *in vivo*. We used ChIP-seq to measure H3K27ac occupancy and map the transcriptional enhancers in SUM159 cells segregated into isogenic ITGB4^{hi} (High tumor initiating ability) and ITGB4^{lo} (Low tumor initiating ability) populations. Gene-enhancer linking and comparative analysis of enhancer usage revealed an epigenomically defined set of genes that are candidate drivers of the CSC cell state, including signaling proteins, DNA-binding transcription factors and cellular adhesion proteins. To further define the chromatin architecture and transcriptional regulatory circuitry that underlies CSC state, we deployed ATAC-seq (Assay for Transposase-Accessible Chromatin with high throughput sequencing) within ITGB4^{hi} and ITGB4^{lo} populations. By pairing nucleosome occupancy and transcription factor kinetics, we created enhancer-linked transcriptional regulatory circuitry of these tumor-initiating cells.

Together, the isolation of partially mesenchymal ITGB4^{hi} CSCs, coupled with enhancer mapping and distillation of transcriptional regulatory circuitry from these cells enable the identification of cancer vulnerabilities and therapeutic opportunities for high-risk patients with TNBC.

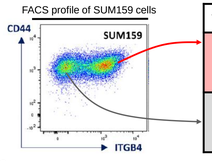
Transcriptional Regulatory Circuitry of CSCs



- Cancer stem cell (CSC)-like populations drive tumor metastasis and relapse
- We sought to identify the core transcriptional circuitry that regulates the CSC state by integrating genome-wide epigenomic assays in a model of TNBC CSCs
- Discovering the core regulatory circuitry can define mechanisms of disease relapse and identify new cancer drug targets and pathways that drive CSCs

SUM159 Subpopulations as Model of CSCs

- Integrin-β4 (ITGB4), can be used to enable stratification of mesenchymal like triple-negative breast cancer (TNBC) cells that are genetically identical but differ from one another in their relative tumorigenic (Tumor Initiating Cell, TIC) abilities
- ITGB4+ cancer stem cell (CSC)-enriched mesenchymal cells (SUM159 ITGB4^{hi}) reside in an intermediate epithelial/mesenchymal phenotypic state



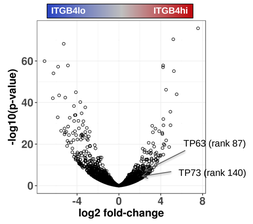
TIC limiting dilution assay			TIC Frequency
ITGB4^{hi}	5.0x10^4	5/5	1/14,679
	2.5x10^4	4/5	
	1.25x10^3	3/5	
ITGB4^{lo}	5.0x10^4	1/5	1/199,348
	2.5x10^4	1/5	
	1.25x10^3	0/5	

Bierie et al., **PNAS**, 2017

Identification of CSC Drivers through Transcriptional Circuitry

RNA-seq

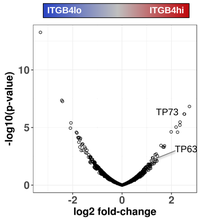
Define steady-state transcriptional output



Differential expression by RNA-seq between ITGB4^{hi} and ITGB4^{lo} populations

Super-enhancer

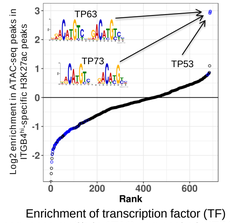
Define Epigenomic state



Differential super-enhancer scores by H3K27ac between ITGB4^{hi} and ITGB4^{lo} populations

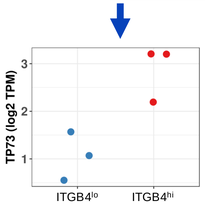
ATAC-seq

Define open chromatin and TF binding

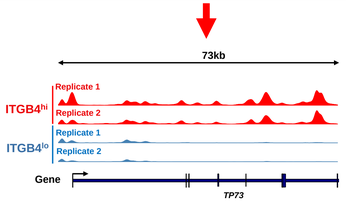


Enrichment of transcription factor (TF) sequence motifs in ATAC-seq peaks in ITBG4^{hi} specific regions

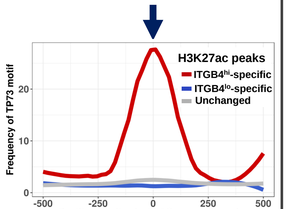
TP73 Identified as a CSC Driver



TP73 is more highly expressed in ITGB4^{hi} than ITGB4^{lo} (FDR<10^{-4}, Wald test)

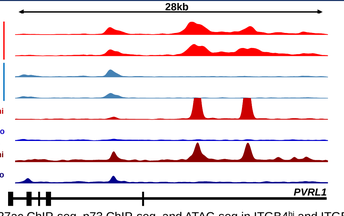


The super-enhancer (SE) at the TP73 locus is stronger in ITGB4^{hi} vs. ITGB4^{lo} (FDR<10^{-3}, Wald test)



p73 motif is enriched in ATAC-seq peaks in H3K27ac peaks stronger in ITGB4^{hi} (FDR<10^{-20}, Fisher's exact test)

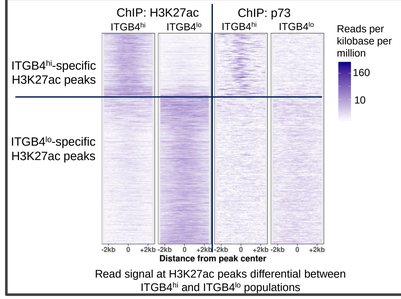
p73 Localizes to ITGB4^{hi}-specific Enhancers



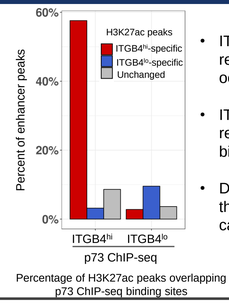
Reads from H3K27ac ChIP-seq, p73 ChIP-seq, and ATAC-seq in ITGB4^{hi} and ITGB4^{lo} populations at SE-linked locus

- p73 is a SAM/Pointed p53-like domain DNA-binding transcription factor
- p73 binds at regions of ITGB4^{hi}-specific H3K27ac
- p73 binds at regions with ATAC-seq signal (Open chromatin).
- Super-enhancer derived transcriptional circuitry validated by empirical binding data

p73 Localizes to Regions of Differential H3K27ac



Read signal at H3K27ac peaks differential between ITGB4^{hi} and ITGB4^{lo} populations

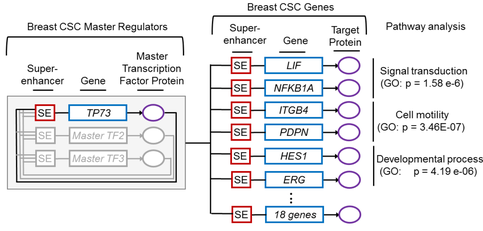


Percentage of H3K27ac peaks overlapping p73 ChIP-seq binding sites

- ITGB4^{hi} specific enhancer regions are preferentially occupied by p73
- ITGB4^{lo} specific enhancer regions are absent of p73 binding
- Data indicate p73 regulates the enhancer landscape of cancer stem cells

Transcriptional Circuitry of ITGB4^{hi} Cancer Stem Cells

- TP73 is a Super-enhancer-linked core driver of ITGB4^{hi} cancer stem cells with predicted autoregulatory function
- p73 target genes are enriched for signal transduction, cell migration and developmental transcription factor genes
- Circuitry enables focused exploration of p73 target genes for therapeutic benefit and drug discovery in the TNBC metastatic setting



Conclusions

- We have built an initial TNBC cancer stem cell transcriptional circuitry to define mechanisms of relapse and to discover new targets against metastasis
- TP73 is a core driver of CSC transcriptional circuitry and controls Super-enhancer linked genes including genes involved in cell migration, signal transduction and developmental processes
- We have identified a set of core transcriptional circuitry-driven genes that drive cancer stem cell state and improve our understanding of cancer relapse and metastasis, therefore leading to new starting points for cancer drug discovery and eventually, therapeutic benefit

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