Chromatin accessibility landscape in primary human invasive lobular and ductal breast carcinomas

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Abstract

Chromatin accessibility patterns of Invasive lobular carcinoma (ILC) and Invasive ductal carcinoma (IDC) tumors remain largely unexplored. The epigenetic differences at the level of chromatin accessibility, potentially linked to distinct differentiation states, might reveal transcriptional vulnerabilities associated with ILC and IDC. We characterized tumor intrinsic chromatin accessibility using primary tumors from The Cancer Genome Atlas (TCGA) breast cancer Assay for Transposases-Accessible Chromatin with sequencing (ATAC-seq) dataset. We inferred patient-specific transcription factor (TF) activities, revealing regulatory differences between and within IDC and ILC tumors based on the ATAC-seq data. Next, we generated new single cell ATAC-seq (scATAC-seq) data and profile chromatin accessibility in independent patient-derived organoids of ILC (n=2) and IDC (n=2) and validated the chromatin accessibility from the TCGA data.

Data and methods

- TCGA BRCA ATAC-seq data were downloaded from Genomic Data Commons (GDC, https://portal.gdc.cancer.gov): **IDC (n=52) and ILC (n=15)**
- We used the CREMA (Cis-Regulatory Element Motif Activities) tool to analyze genome-wide DNA-accessibility and predict TF activities.
- Raw BCL files of scATAC-seq data in breast cancer organoids were demultiplexed and mapped to the GRCh38 using the Cell Ranger – atac v.2.0 (10x Genomics)
- We used ArchR, a full-featured R package for processing and analyzing single-cell ATAC-seq data.

Table 1. Pathological report of 4 BRCA organoids

		Organoid 43	Organoid 56	Organoid 53 *	Organoid 55 *
Clinical subtype	ER status	Positive	Positive	Positive	Positive
	PR status	Positive	Positive	Negative	Negative
	HER2 status	Negative	Negative	Negative	Negative
	Invasive carcinoma	IDC	IDC	ILC	ILC
	Metastasis	One lymph node Negative	Intramammary lymph node Positive	Bone, left femoral head	Bone tumor tibia proximal

* Organoid 53 and 55 are derived from the same patient

Results

Table 2. Summary of 4 BRCA organoids scATAC-seq data

		Organoid 43	Organoid 56	Organoid 53	Organoid 55
Single-cell summary	Number of cells	4,171	4,453	4,193	2,542
	Median high-quality fragments per cell	11,364	13,475	33,015	31,569

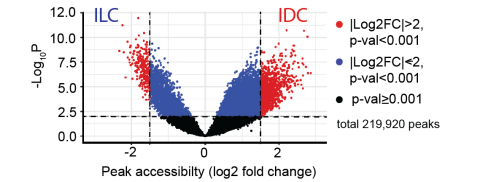


Figure 1. Volcano plot for peak accessibility differences in TCGA BRCA ATAC-seq data between ILC and IDC.

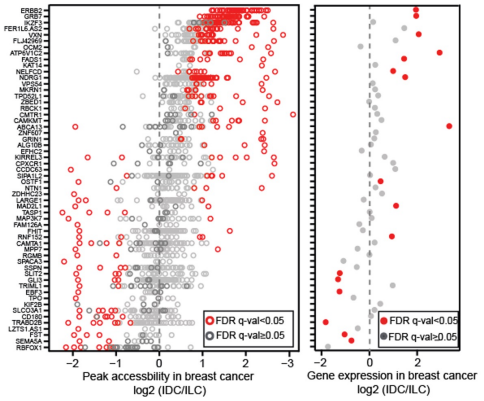


Figure 2. Differential peak accessibility in TCGA BRCA ATAC-seq and differential gene expression in RNA-seq between ILC and IDC

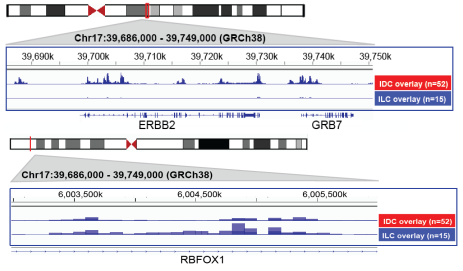


Figure 3. Genome browser tracks of differential peak accessibility for ERBB2, GRB7 and RBFOX1 between ILC and IDC.

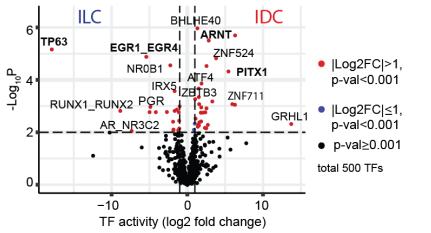


Figure 4. Differential TF motif activities in TCGA BRCA ATAC-seq between ILC and IDC.

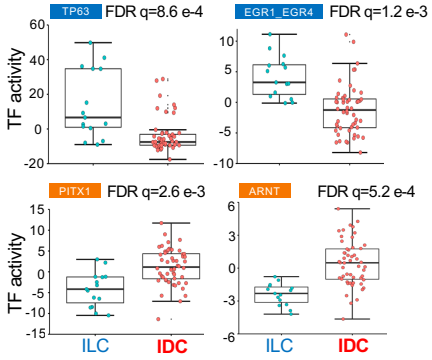
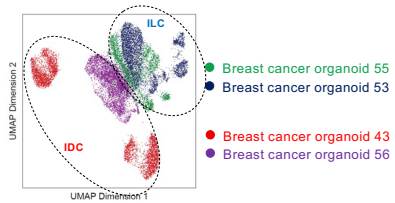


Figure 5. Box plots with differentially inferred TF activities of TP63, EGR1_EGR4, PITX1, and ARNT.



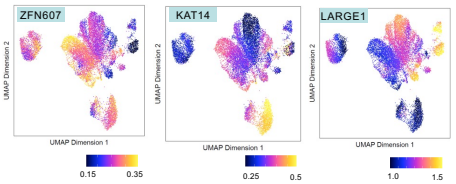


Figure 6. Uniform Manifold Approximation and Projection (UMAP) colored by breast cancer organoid scATAC-seq samples or gene scores.

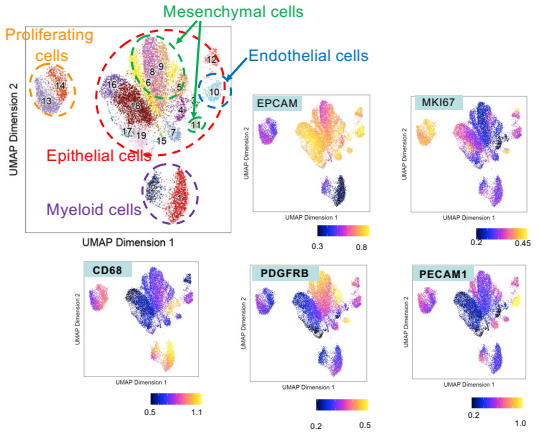


Figure 7. UMAP visualization of 16,335 cells across 4 breast cancer organoids. Clusters were annotated by gene score of markers for epithelial cells (EPCAM), proliferating cells (MKI67), myeloid cells (CD68), endothelial cells (PECAM1) and mesenchymal cells (PDGFRB).

Conclusion

- ❑ TP63, RUNX1, and EGR1, which are associated with tumor growth and proliferation, had significantly higher inferred TF activities in ILC than IDC (Figure 4 and 5).
- ❑ Single cells of breast cancer organoids are clustered by IDC and ILC (Figure 6). Cell clusters were annotated for their cell types using canonical markers; epithelial (EPCAM), proliferating (MKI67), myeloid (CD68), mesenchymal (PDGFRB), and endothelial (PECAM1) cells.

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References

- *M. R. Corces, J. M. Granja, S. Shams, B. H. Louie, J. A. Seoane, W. Zhou, et al. The chromatin accessibility landscape of primary human cancers. Science 2018 Vol. 362 Issue 6413*
- *T. Du, L. Zhu, K.M. Levine, N. Tasdemir, A. Lee, and S. Oesterreich. Invasive lobular and ductal breast carcinoma differ in immune response, protein translation efficiency and metabolism. Scientific Reports 2018 Vol. 8 Issue 7205*
- *M. R. Corces, J. M. Granja, S. Pierce, T. Bagdatl, H. Choudhry, H.Y. Chang, and W.J. Greenleaf.* **ArchR** *is a scalable software package for integrative single-cell* **chromatin** *accessibility analysis. Nature Genetics 2021 Vol. 53*

➢ ***Lab Homepage:*** *https://www.osmanbeyoglulab.com*