Unveiling Inter- and Intra-Tumor Heterogeneity in Ductal Carcinoma *in Situ* (DCIS) and Invasive Ductal Carcinoma (IDC) through Integration of Unified Single-Cell Copy Number and RNA Expression Data

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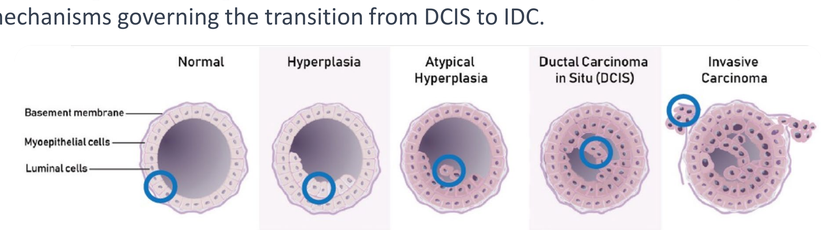
Introduction

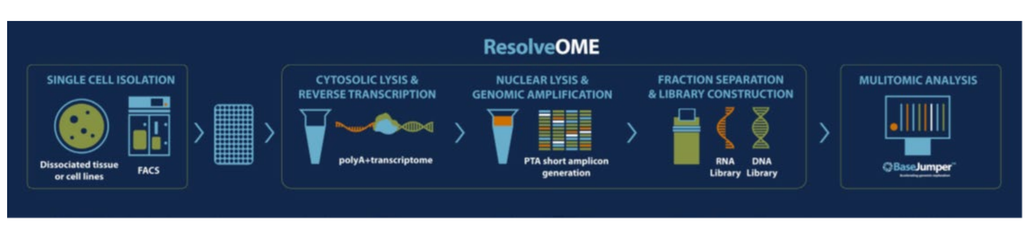
In the field of breast cancer research, Ductal Carcinoma in situ (DCIS) assumes a pivotal role, marking the initial stage preceding the progression to the more invasive and potentially life-threatening Invasive Ductal Carcinoma (IDC). The intricate heterogeneity characteristic of DCIS, wherein aberrant cells are confined within the breast's milk ducts, arises from the complex interplay of genomic alterations and the intricacies of the tumor microenvironment. Understanding this intricate interplay between genetic changes and the microenvironment is of paramount importance for gaining insights into the fundamental mechanisms governing the transition from DCIS to IDC.



Within the context of this comprehensive study, our central objective is to delve deeply into the complexities of breast cancer, with a specific focus on patients with a history of DCIS. To achieve this, we employ the **ResolveOME™** unified single-cell amplification approach, seamlessly integrating DNA copy number and RNA expression analyses. This approach allows us to unravel the enigmatic world of multi-omic breast cancer, bringing forth a comprehensive understanding of this complex disease.

Methods

Sample Collection: Ductal Carcinoma in situ (DCIS) and Invasive Ductal Carcinoma (IDC) samples were obtained from a cohort of 11 patients following mastectomy.
Cell Isolation: Approximately 100 cells were isolated from each sample, were isolated from the tissue specimens, with fluorescence-activated cell sorting (FACS).
DNA and RNA Amplification: To comprehensively profile genomic and transcriptomic aspects of these single cells, we performed a unified whole genome and transcriptome amplification with **ResolveOME™.**
DNA Library Preparation: DNA libraries were enriched for exome with xGen v2 IDT, followed by sequencing with Illumina NextSeq1000 or NovaSeq6000.
Analysis: BaseJumper™ multi-omics analysis software was utilized to process and interpret the massive amount of data generated. Any additional analyses were completed in R.



Results

Table 1: Patient Clinical Breast Tumor Type Diagnosis
An overview of patient demographics with clinical breast tumor type diagnoses providing insights into the diverse breast cancer subtypes analyzed.

Patient	Sample #	Clinical Diagnosis
A	1	IDC; DCIS Present
A	2	Normal
B	3	Invasive Ductal Adenocarcinoma (ER+/PR+/HER2-)
C	4	IDC
C	5	Normal
D	6	IDC
E	7	IDC; DCIS Present (neg for extensive component)
F	8	Invasive Ductal Adenocarcinoma (ER+/PR+/HER2-)
G	9	Invasive Ductal Adenocarcinoma (ER+/PR+/HER2-)
H	10	Invasive Ductal Adenocarcinoma
I	11	ER+/PR+/HER2-
J	12	DCIS
J	13	Normal
K	14	DCIS

Figure 1: Transcriptional Signatures Unveil Cell Type Diversity Across Patient Samples
(a)UMAP clusters of all patients, exposing inter-sample cell type diversity. Notable features include distinct clusters for major cell types, with a particular emphasis on two clusters for epithelial cells.
(b) emphasizes expression patterns within the UMAPs of typical breast cancer marker genes, highlighting luminal, proliferating, and immune markers.

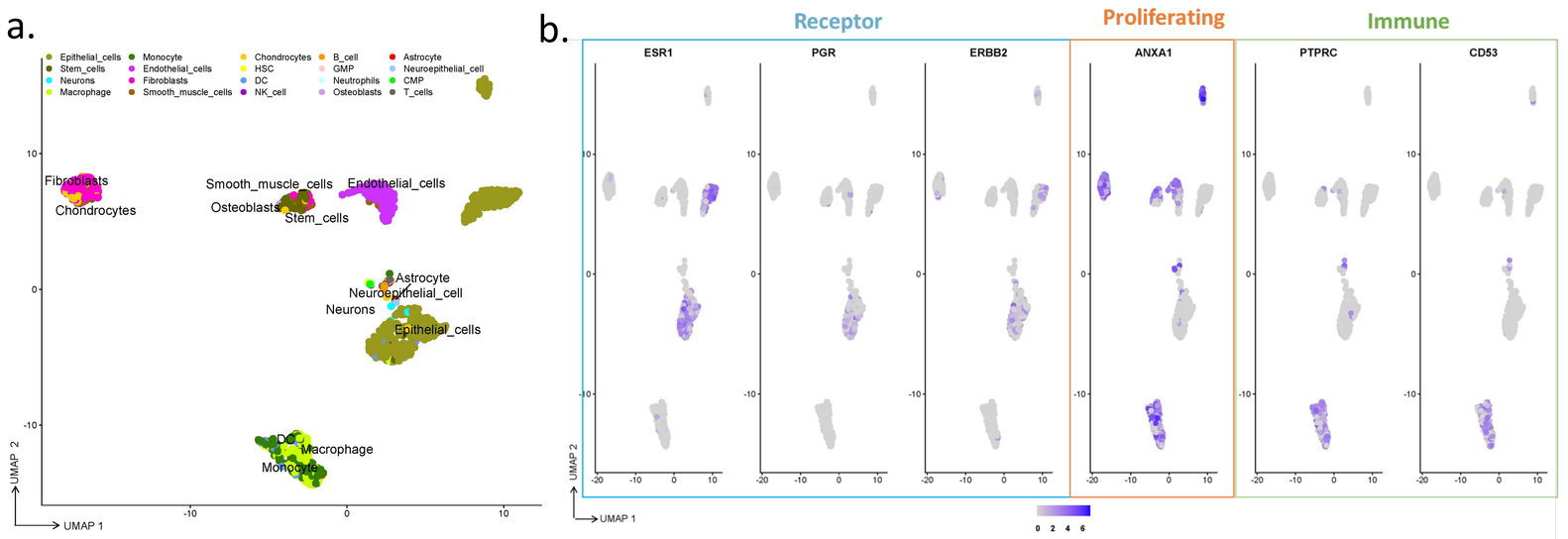
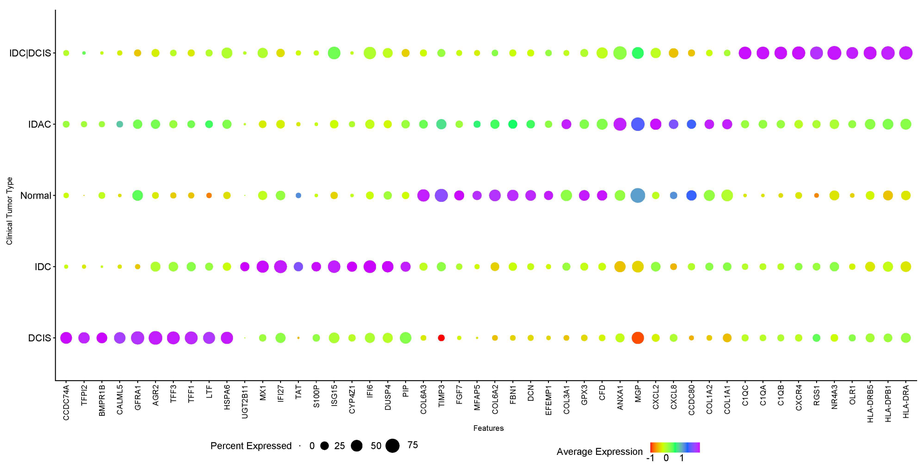


Figure 2: Identification of Gene Features Distinguishing Clinical Tumor Types
This dot plot reveals the top 10 gene features distinguishing clinical tumor types. Differentiated gene classes associated with each tumor type include genes involved in cell migration, cancer progression, immune response modulation, and the tumor microenvironment. This differentiation is valuable for improving diagnosis, prognosis, and treatment strategies. It enables more precise and tailored approaches, enhancing patient care and outcomes.



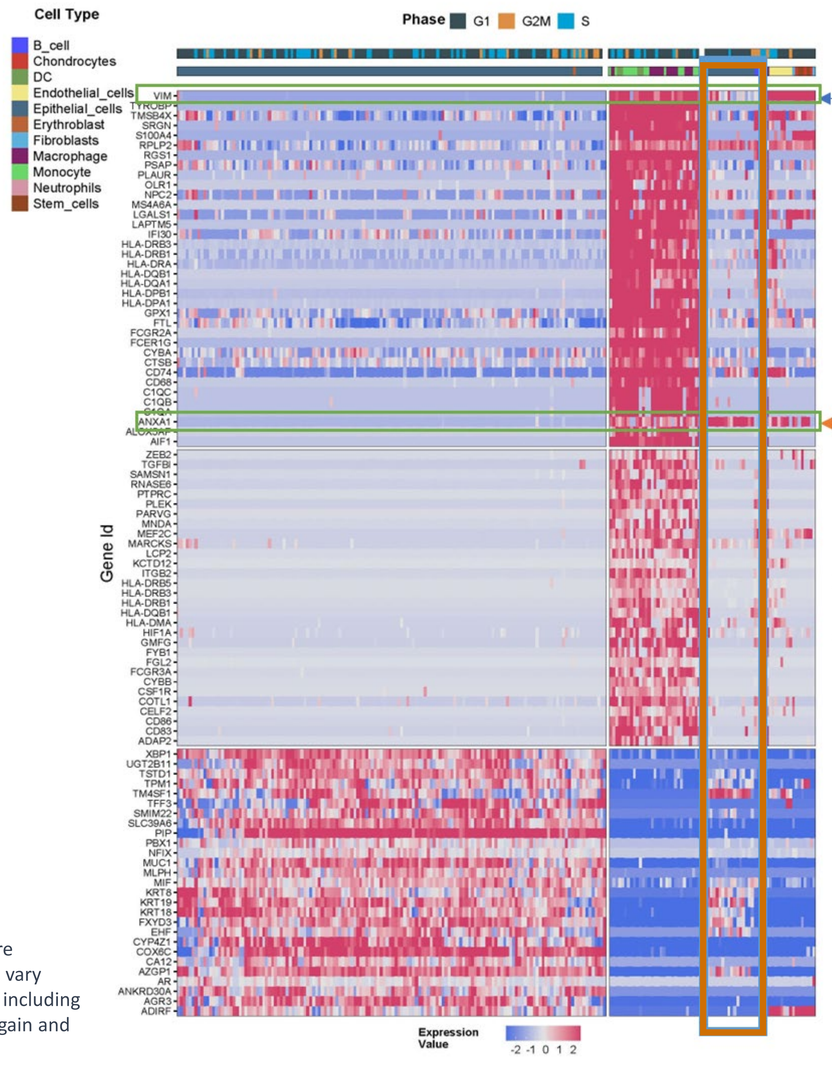


Figure 4: Cellular Diversity Present in Distinct Cell Subpopulations (Patient Use-Case). The heatmap reveals the diversity of cell types present from a single patient. Subpopulations of epithelial cells within the context of intratumoral heterogeneity in DCIS and IDC breast cancer can be seen. These subpopulations of epithelial cells cluster with stem cells, hinting at their potential involvement in tumor development. Additionally, they exhibit elevated expression of EMT genes, including VIM (Vimentin), suggesting their role in tumor invasiveness. Furthermore, these cells demonstrate increased expression of proliferative genes, such as ANXA1 (Annexin A1), indicating their potential contribution to tumor growth.

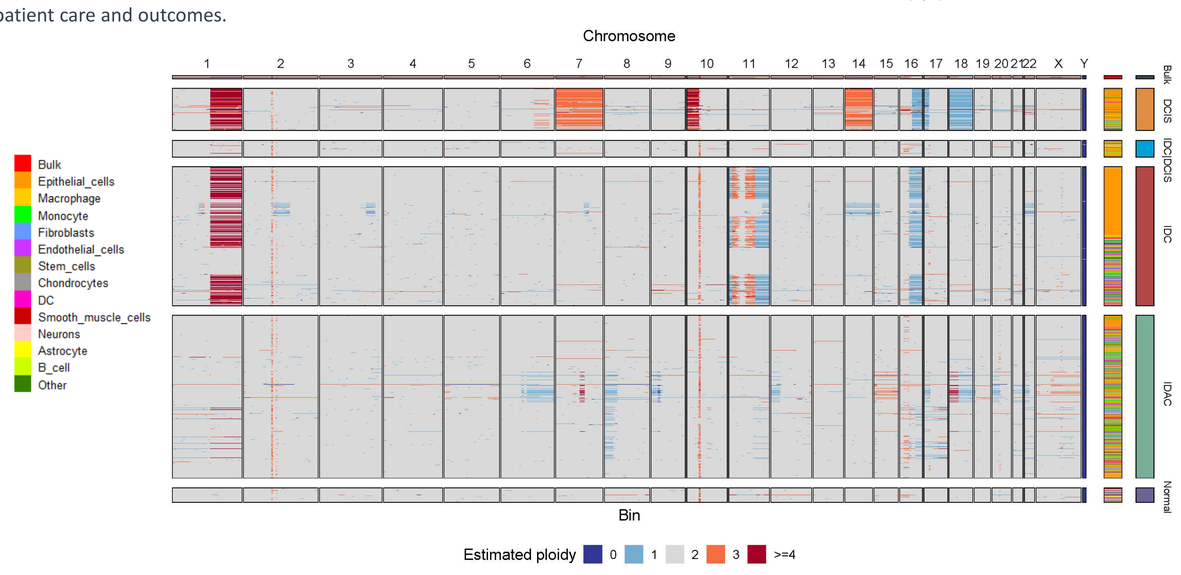


Figure 3: Insights from Inter-Tumoral Copy Number Variation (CNV) Landscape. The CNV landscape unveils unique gains and losses that are discernible only at a single-cell resolution when using low-pass DNA data. This analysis identifies distinct sub-chromosomal alterations that vary among patients, along with shared gains and losses. In this cohort, multiple CNV regions have been linked to DCIS/IDC in collective studies, including 1q amplification, 16q loss, and 18 loss. Bulk CNV analysis predominantly reveals alterations present in single cells of the biopsy, such as 1q gain and 11 gain. However, it fails to uncover various losses (16, 17, 18) that are exclusively disclosed through single-cell resolution analysis.

Figure 5: Copy Number Alterations and Associated Differentially Expressed Genes in a Patient Sample. Copy number changes were aligned with differentially expressed genes in a selected patient sample. Columns are cells in the same order as Figure 5. Copy number gains and losses correlated with genes involved in immune signaling and response, tumor suppression, cell growth and proliferation, and cell migration.

Conclusions

DCIS and IDC patient samples exhibit diverse copy number and expression profiles, underscoring the presence of genomic heterogeneity.
Crucial genes play pivotal roles in driving breast cancer progression, offering promising therapeutic avenues.
ResolveOME's single-cell amplification reveals profiles omitted in bulk analysis, enriching our understanding of cancer biology. This illumination contributes to unraveling the potential unified genomics mechanisms involved in the transition from DCIS to IDC in breast cancer.

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