Abstract 5933: Trimodal Molecular Analysis in Single Cells of a Primary Breast Cancer Cohort with ResolveOME Amplification

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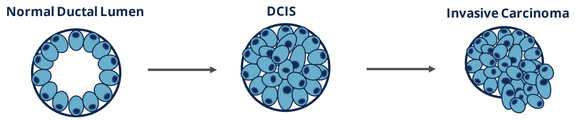
Introduction

Ductal carcinoma *in situ* (DCIS) is a non-obligate precursor to invasive ductal carcinoma (IDC). While there will be an estimated 55,720 new cases of DCIS in 2023[1], only 20-50% of DCIS cases progress to IDC[2]. Virtually all patients with DCIS have surgery; almost a third of patients have unilateral or bilateral mastectomy[3]. Identifying markers of progression from DCIS to IDC could reduce overtreatment of low-risk lesions.



What are the genomic, transcriptomic, and proteomic lesions driving the ductal carcinoma in situ (DCIS) to invasive ductal carcinoma (IDC) progression?

The ResolveOME™ single-cell amplification solution unites whole-genome and full-length transcriptome information from the same cell[4], providing critical insight between these layers not possible when analyzed in isolation. We expand here, in primary breast cancer, upon the core layers of ResolveOME with the incorporation of BioLegend TotalSeq™ oligo conjugated antibodies to evaluate the expression of a panel of extracellular proteins in conjunction with genomic and transcriptomic data.

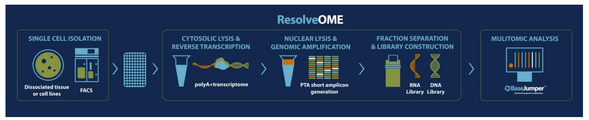
Methods

Isolation of 960 Single Cells from 12 Patients with a History of DCIS

IDC samples from 12 female patients with a history of DCIS were collected following mastectomy under IRB PRO00034242 "Biologic Characterization of the Breast Cancer Tumor Microenvironment" (PI: Hwang). Cells were singulated and either underwent fluorescence assisted cell sorting (FACS) or were dispensed as single cells, relying on the full transcriptome output to report cell identity. 80 cells per sample were examined, resulting in 960 total cells analyzed with unified genomic, transcriptomic, and targeted extracellular protein analysis.

Integrated Trimodal Molecular Analysis with ResolveOME and BaseJumper

Cells underwent the ResolveOME whole genome and transcriptome amplification workflow (below), which was coupled with TotalSeq (BioLegend) oligo conjugated antibodies that allow for next-generation sequencing (NGS)-mediated detection of 165 extracellular proteins. DNA libraries were enriched for exomes with xGen v2 (IDT) and sequenced using an Illumina NovaSeq6000 or NextSeq1000. Data analysis was performed using the bioinformatics platform, BaseJumper (BioSkryb Genomics).



Results

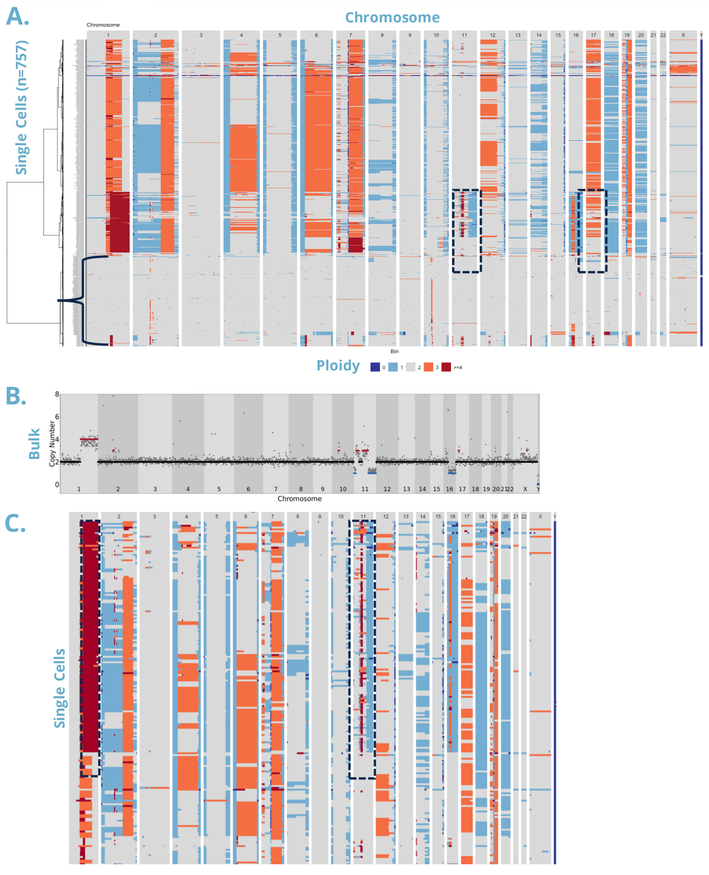


Figure 1: The copy number variation (CNV) landscape reveals gains and losses only visible at a single-cell resolution. **A.** Discrete sub-chromosomal alterations are observed between patients (dashed boxes) in addition to common gains and losses. A block of primarily diploid cells (bracket) encompasses diverse non-epithelial cell types and cells from matched normal control biopsies. Multiple CNV regions seen in this cohort have been associated with DCIS/IDC in aggregate studies including 1q amplification, 11q loss and 18 loss[5]. **B/C.** In a representative sample, bulk CNV analysis (B) reveals predominant alterations present in single cells of the biopsy (1q gain, 11 gain and loss) but fails to reveal multiple gains (4, 6, 7) and losses (18) exposed exclusively by single cell resolution (C).

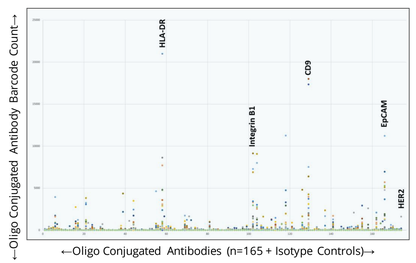


Figure 3: Surface protein expression varies between cells of DCIS/IDC biopsy. TotalSeq barcode counts (y axis) reporting relative surface protein expression levels are shown for 165 oligo conjugated antibodies (x axis) among single cells of diverse type identity (colored circles) of a representative patient sample. 5 oligo conjugated antibodies with high relative expression levels or epithelial relevance are highlighted with black text.

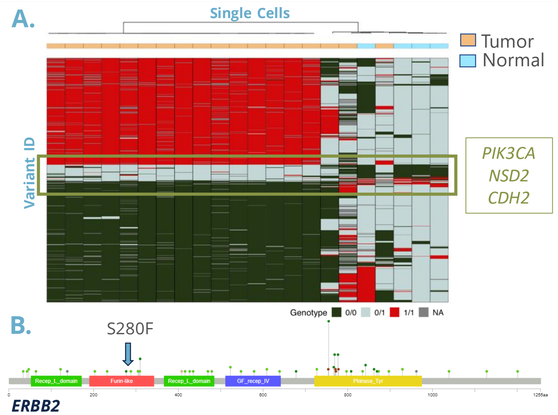


Figure 2: Candidate oncogenic mutations exist in individual cells. **A.** Single nucleotide genotypic variation with differential prevalence between tumor and normal cells of one individual of the cohort. The green box highlights heterozygous genotypes present in tumor cells with corresponding homozygous reference genotypes in normal biopsy cells. Within this clade, *PIK3CA* (missense), *NSD2* (frameshift), and *CDH2* (missense) mutations were identified **B.** A rare *ERBB2* S280F mutant clone found in 2/59 cells from a different individual of the cohort. *ERBB2* lollipop diagram highlights a missense mutation spectrum from a publicly available invasive carcinoma dataset.

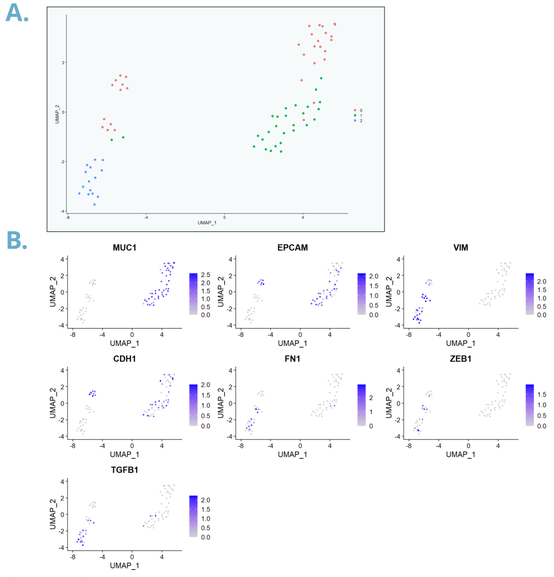


Figure 4: Transcriptional signatures in single cells reveal cell type diversity in tumor samples **A.** UMAP clustering of a representative individual sample highlighting intra-sample cell type diversity. **B.** Transcripts contributing to cluster identity; high *MUC1, CDH1, EPCAM* and low *VIM* expression define, in part, epithelial versus mesenchymal identity emerging in this tumor sample.

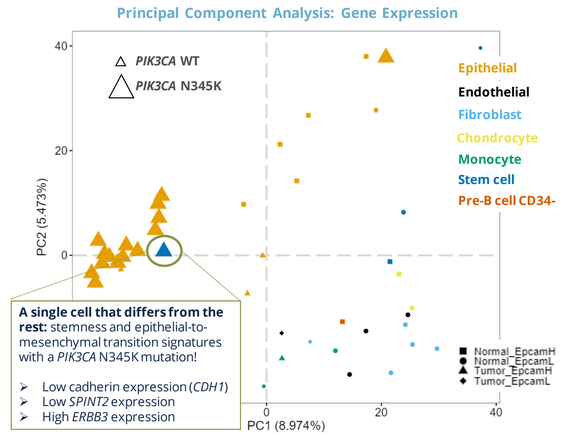


Figure 5: Multiomics uncovers physiological cell states. Single cells (individual points) of one DCIS/IDC sample separated by gene expression by principal component analysis (PCA). The size of the icon indicates *PIK3CA* N345K oncogenic mutation status and the color of the icon indicates cell ID as determined by Seurat v4 (Satija Lab) from transcriptome data. This multiomic analysis shows the cell type diversity present. A single cell harboring oncogenic *PIK3CA* types as a cell with stemness identity and harbors gene signature elements indicative of epithelial-to-mesenchymal transition (EMT).

Conclusions

- ResolveOME unites complete genome, transcriptome, and targeted proteome data with BioLegend oligo conjugated antibodies to expose insights unattainable in isolation
- Single-cell multiomic analysis in a breast cancer cohort defines heterogeneous candidate oncogenic drivers and exposes cell identity and cell state variation, allowing for the interpretation of genotype in context

Acknowledgements

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References

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