Exome, IcWGS-based copy number assessment, complete transcriptome & surface protein expression from the same individual cell with ResolveOME®

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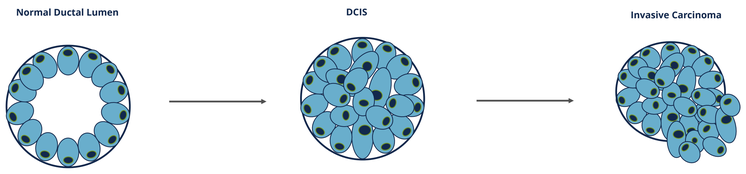
Introduction

Ductal carcinoma *in situ* (DCIS) may progress to invasive ductal carcinoma in 20-50% of cases[1]. In 2023, there were an estimated 55,720 new cases of DCIS[2] , virtually all of whom have surgery, and whom up to 1/3 of which have a full or partial mastectomy[3]. Unfortunately, well defined markers of progression have thus far failed to be elucidated, likely due to the degree of 'normal' tissue in DCIS lesions.



What lesions truly drive ductal carcinoma in situ (DCIS) to progression towards invasive ductal carcinoma (IDC) ?

Single cell multi-omic analysis that is **unified** at the single cell level, may allow translational scientists to identify actionable, functional targets offering better prognostic evaluations. Importantly, such workflows must be sufficiently cost effective in order to be scientifically and clinically useful.

In this study, we assess greater than 900 cells across 12 primary tumor samples, harvested by the Surgery Department and Duke University at the single cell level. Uni**fied multi-omic findings highlight tumor heterogeneity within and between samples at the genomic, transcriptomic and proteomic levels.**

Methods

Primary cells harvested from mastectomy samples (IRB # PRO00034242, Shelley Hwang) were incubated with BioLegend oligo-conjugated antibody cocktail prior to FACS staining, allowing for a direct NGS read out of protein counts. Plated single cells then proceeded through the ResolveOME® workflow. Briefly, cytosolic lysis & reverse transcription is performed followed by genomic amplification via primary template-directed amplification**.** Workflow finishes with fraction separation, library construction & sequencing. Exome enrichment was performed using IDT xGen v2 kit. Exomes were sequenced on Illumina instrumentation with data analysis using BaseJumper™.

Results

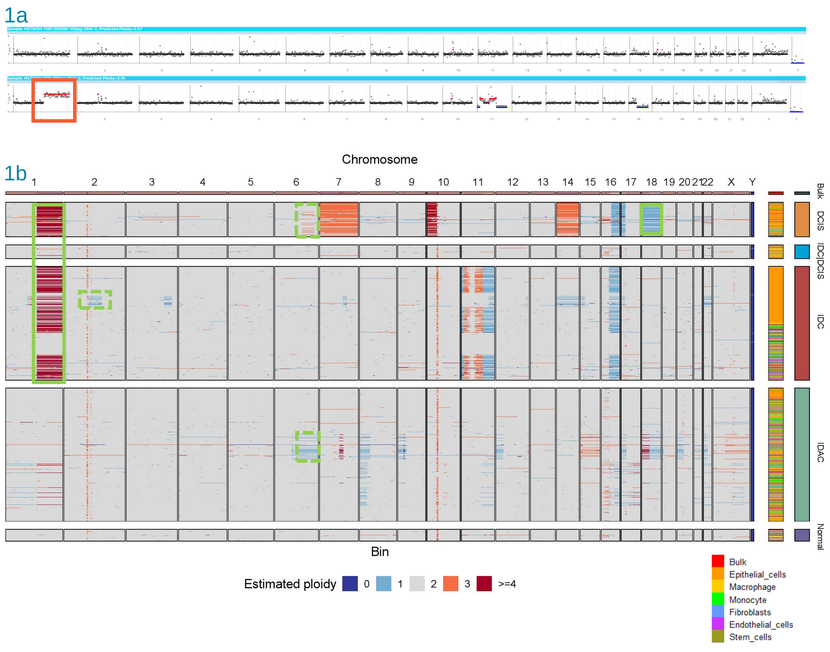


Figure 1: Copy number profiles in breast tumors of multiple patients demonstrate marked heterogeneity that cannot be captured by bulk sequencing alone – A) Copy number profiles of representative bulk tumors, showing common recurrent gains (chromosome 1, red box)[4]. **B)** Single cell copy number profiles across multiple patient tumors with copy number **gains** in **orange**/**red** colors and copy number **losses** in **blue**. Recurrent copy number alterations commonly seen in DCIS/IDC are also readily apparent including gains (chromosome 1-q arm) and losses (chromosome 18) (green boxes)[4]. Discrete sub-chromosomal alterations can be seen within cases (green dashed box). Multiple recurrent clonal aberrations are seen with distinct CNV profiles.

Results Continued

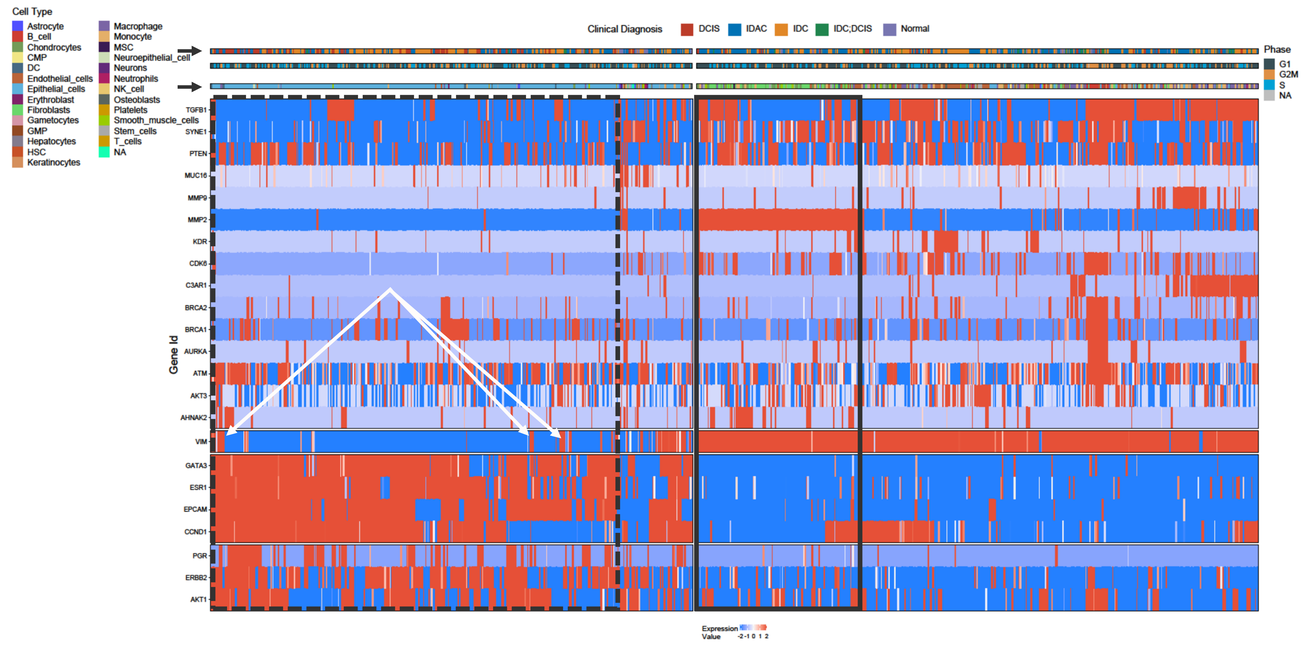


Figure 2: Single cell expression signatures across all patient samples highlight heterogeneity – Epcam positive tumor cells (dashed box) demonstrate highly heterogenous expression profiles including some cells in active EMT transition (white arrows)

Signatures within the un-biased tumor micro-environment demonstrate tissue remodeling by fibroblasts (demonstrated by unique clusters, MMP2/MMP9 expression), in both inflammatory and non-inflammatory subsets (black box)

Across all diagnoses, (black arrow, top) there are a multitude of cell types (black arrow bottom) at various phases of the cell cycle (middle)

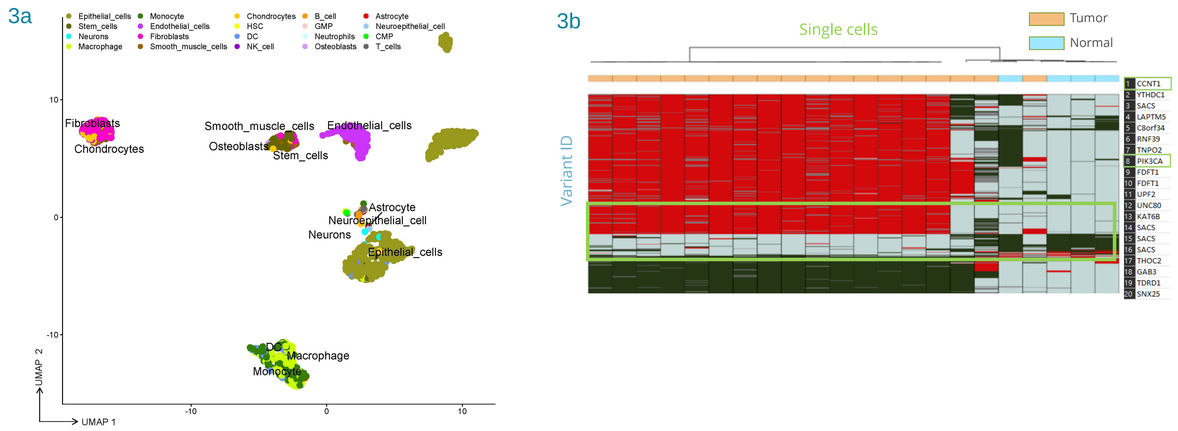
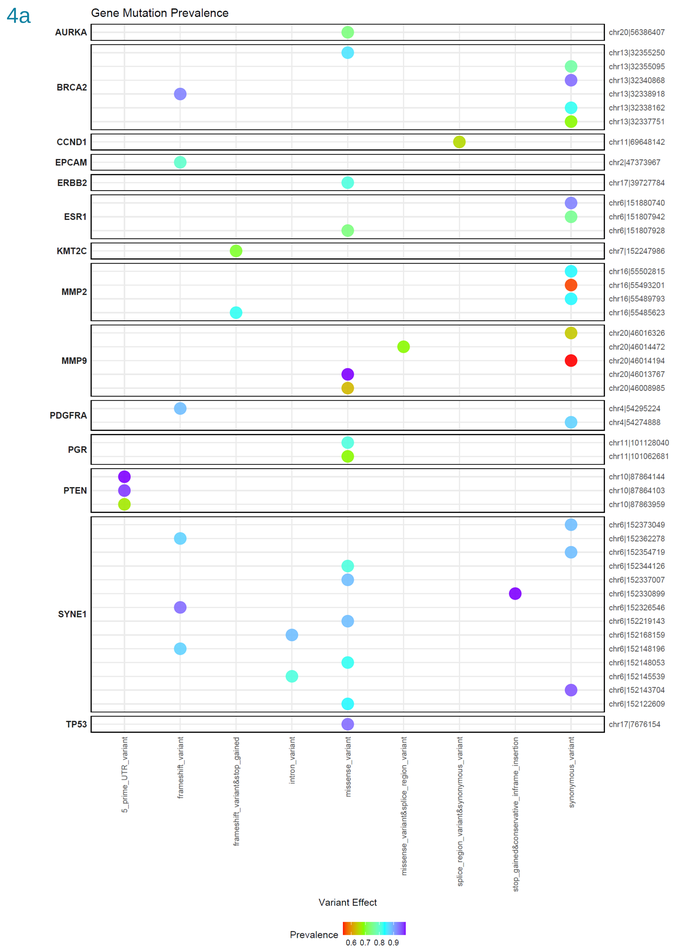


Figure 3: Expression profiles resolve cell type, and when unified with SNV profiles identify targetable oncogenic drivers – A) UMAP cluster of all single cells across all patients. Resolution of cell type is demonstrated by unique clusters, including multiple distinct clusters of epithelial cells. **B)** SNV profiles reveal oncogenic signatures in tumor vs. normal cells.



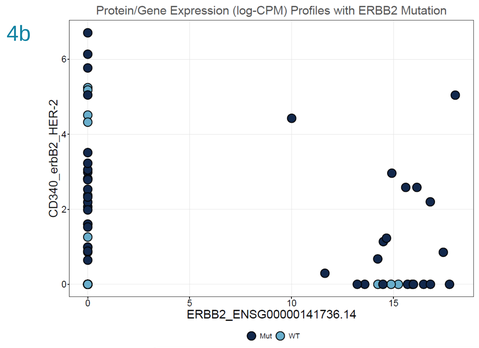


Figure 4: Unified genomic, proteomic and transcriptomic profiles show variable, conserved genomic alterations linked with tumor aggression – A) Prevalence of genomic alterations of significantly differentially expressed genes in a single patient tumor highlighting DNA damage repair mutations (*BRCA2*), and *HER2*. **B)** Paired expression (X-axis), protein levels (y-axis) and *HER-2* mutational status (dot color) demonstrating diverse expression profiles. **Note: among cells with high *Her-2* expression, ONLY cells with mutation have concurrent high protein levels.** This suggests the mutation is likely acting with a second (yet to be identified mutation or up-regulation gene)

Conclusions

- Single cell heterogeneity in breast tumors is missed at the bulk level and may drive researchers and translational scientists to 'red-flag' targets that ultimately do not fully address patient needs.
- Unified, multi-omic workflows allow basic researchers and translational scientists to focus on actionable, functional targets more quickly than non-unified data.

References

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