Abstract 6929: Inter- and intratumoral *PIK3CA* subclonal diversity in breast cancer contextualized by single-cell multiomics

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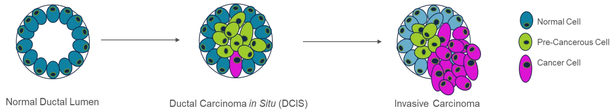
Introduction

The transition of Ductal Carcinoma In Situ (DCIS), a non-obligate precursor, to Invasive Ductal Carcinoma (IDC)

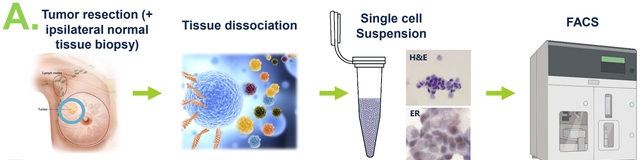


The diversity of molecular determinants of the transition from the premalignant state of DCIS to invasive cancer are multi-faceted and will require single-cell, multimodal analysis across the Central Dogma of Molecular Biology

- 55,720 new cases of DCIS in 2023 [1]
- 20-50% of DCIS progress to invasive breast cancer [2]
- Virtually all patients with DCIS have surgery; almost a third of patients have unilateral or bilateral mastectomy [3]
- Identifying markers of progression could reduce overtreatment of low-risk lesions

Is it possible to "Treat the biology; not the diagnosis"?

Methods



ResolveOME™ single-cell workflow to obtain unified genomic, transcriptomic, and targeted protein data.
A. Sample singulation workflow upstream of ResolveOME™[4] reactions. **B.** ResolveOME™ workflow steps and time to completion (top) and performance metrics for each analyte type (bottom). **C.** Inclusion of BioLegend oligo-conjugated antibodies in ResolveOME™ workflow to report surface protein expression in conjunction with genomic/transcriptomic tiers.

DNA Performance Characteristics	Observed Values
Accuracy	99.99%
Sensitivity	96.04%
Specificity	99.99%
Precision	98.75%
Allelic balance	93.46%
Genome coverage	97.59%

RNA Performance Characteristics	Observed Values
Protein coding genes detected	3,451 ± 731.71*
Proportion exonic	0.77
Average concordance	0.97
Reproducibility (CV)	32.9%

Protein Performance Characteristics	Observed Values
Surface proteins detected	~ 165
Antibody-derived tag (barcode) signal (1M reads)	10^3 - 10^4
Isotype control signal (1M reads)	< 10^2

Results

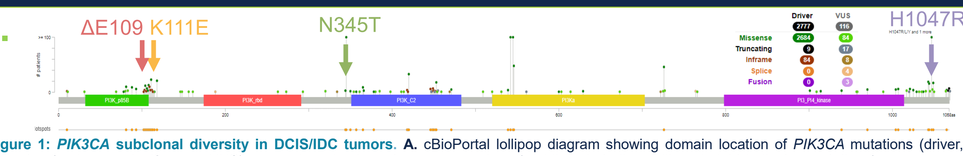
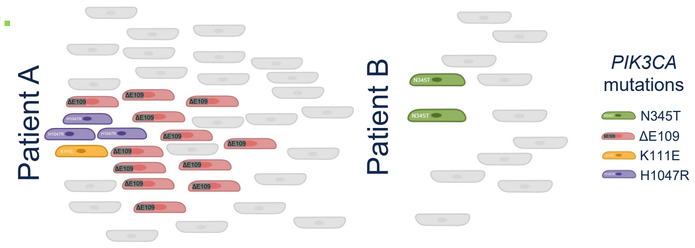


Figure 1: *PIK3CA* subclonal diversity in DCIS/IDC tumors. **A.** cBioPortal lollipop diagram showing domain location of *PIK3CA* mutations (driver, or variants of unknown significance, VUS) among invasive breast cancer samples of published studies; lollipop height indicates mutation frequency among cohorts. Highlighted are domain locations of mutations found in single cells from Patient A (ΔE109, K111E, H1047R) and Patient B (N345T) of our study.



B. Schematic of *PIK3CA* mutations found in single cells from Patient A (Invasive Ductal Adenocarcinoma, IDAC) or Patient B (DCIS & IDC) tumors, and their relative abundance, derived from exome enrichment of ResolveOME™ amplified cells. Gray icons indicate cells sequenced that lack any *PIK3CA* variants. **C.** Copy number alteration plots from low coverage WGS (2M total reads, 1 Mb window size) from the cells harboring the indicated *PIK3CA* mutation. Distinct CNV classes are associated with specific *PIK3CA* mutations. Several of these copy number alterations are prototypical for DCIS/IDC[5].

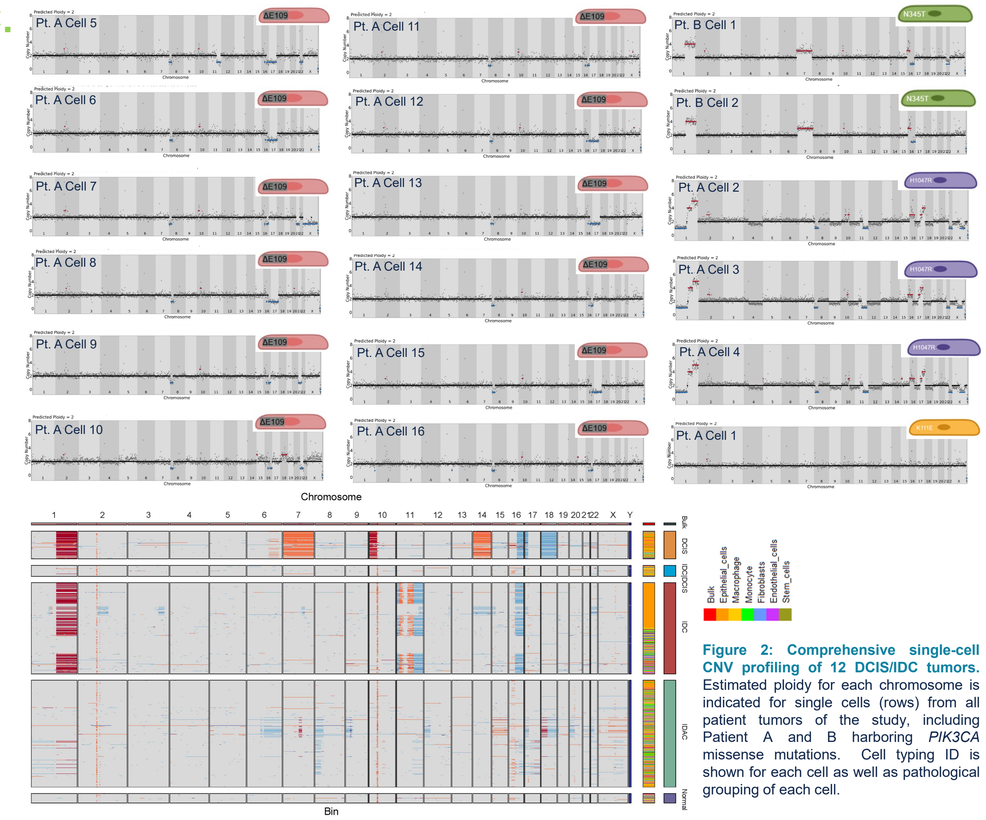


Figure 2: Comprehensive single-cell CNV profiling of 12 DCIS/IDC tumors. Estimated ploidy for each chromosome is indicated for single cells (rows) from all patient tumors of the study, including Patient A and B harboring *PIK3CA* missense mutations. Cell typing ID is shown for each cell as well as pathological grouping of each cell.

Patient #	Cell #	*PIK3CA* mutation	TCGA tissue	BaseJumper cell type	HPCA cell type	Cell cycle stage
A	1	K111E	breast	epithelial	epithelial	S
A	2	H1047R	breast	epithelial	epithelial	G1
A	3	H1047R	breast	epithelial	epithelial	S
A	4	H1047R	breast	epithelial	epithelial	G1
A	5	ΔE109	breast	epithelial	epithelial	G1
A	6	ΔE109	breast	epithelial	epithelial	S
A	7	ΔE109	thyroid	epithelial	epithelial	G2M
A	8	ΔE109	breast	endothelial	endothelial	S
A	9	ΔE109	breast	hepatocyte	epithelial	S
A	10	ΔE109	breast	epithelial	epithelial	G1
A	11	ΔE109	breast	epithelial	epithelial	S
A	12	ΔE109	breast	epithelial	epithelial	G1
A	13	ΔE109	breast	epithelial	epithelial	G2M
A	14	ΔE109	breast	epithelial	epithelial	G1
A	15	ΔE109	breast	epithelial	epithelial	G1
A	16	ΔE109	breast	epithelial	epithelial	G2M
B	1	N345T	lung	macrophage	epithelial	G1
B	2	N345T	-	-	-	-

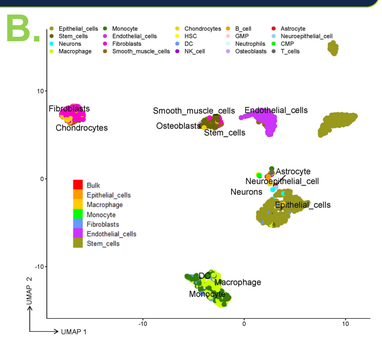


Figure 3: Transcriptomic analysis of cells harboring mutant *PIK3CA*. **A.** TCGA tissue designation and BaseJumper® and Human Primary Cell Atlas (HPCA) cell type identification for Patient A and B *PIK3CA*-mutant tumor cells. Cell cycle staging from RNA-level data is shown in the right-most column. Cell 2 from Patient tumor B lacked sufficient expressed transcripts for accurate cell ID calling. **B.** UMAP analysis depicting diversity of cell types across 12 DCIS/IDC tumors transcriptionally ascertained by ResolveOME™.

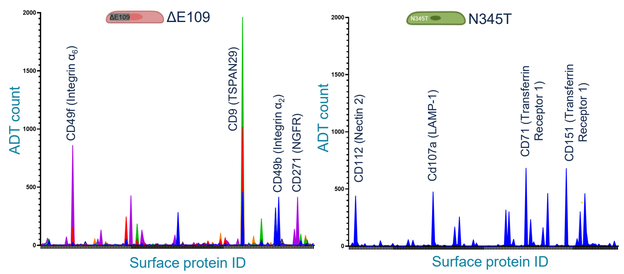


Figure 4: Surface protein expression in cells harboring *PIK3CA* ΔE109 in Patient tumor A or *PIK3CA* N345T in Patient tumor B.
Left: Single-cell protein expression from Patient A ΔE109 cells is indicated by antibody derived tag (ADT) counts from RNA arm of ResolveOME™. Colors indicate relative abundance between individual cells. **Right:** Dominant protein signals from N345T Cell 1 from Patient B tumor. The signature is indicative of highly-proliferative stem-like and drug-resistant cellular states.

Conclusions

- A *PIK3CA* mutational spectrum, displaying inter- and intra-tumoral variation, is defined within single cells from a subset of patients in a DCIS/IDC cohort
- Distinct copy number alteration profiles are associated with specific *PIK3CA* mutations, suggestive of a cooperativity between the two in clonal evolution and in invasiveness capacity
- RNA and surface protein-level data contextualize the genomic lesions of *PIK3CA* mutations and copy number alterations by defining cell identity and cell state

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Acknowledgements

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