A176 · Reconstructing Early Tumor Evolution in BRCA Carriers Using Long Read Single-Cell RNA-sequencing

Presenter: Grace Li — Krantz Family Center for Cancer Research, Mass General Brigham, Boston, MA, USA

Authors: Grace Li, Ping Lu, Allen Lynch, Zuen Ren, Kazi Nazrul Islam, Moshe Sade-Feldman, Ioannis Sanidas, Peter J. Park, Leif W. Ellisen, Vinay Vinswanadham, Doga C. Gulhan

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Germline BRCA1/2 pathogenic variants predispose breast epithelium to cancer, yet only rare lineages undergo malignant transformation. The steps linking haploinsufficiency to malignancy remain poorly characterized because precursor cells are rare and difficult to identify. Distinguishing cancer-relevant precursors from non-progressing clones is critical for early detection and interception. Leveraging field effects within breast ducts, where malignant and precursor cells may coexist, we profiled thousands of cells from matched triple-negative breast cancer and histologically normal tumor-adjacent tissue from a germline BRCA2 carrier using PacBio Kinnex long-read single-cell RNA sequencing. This approach jointly resolved transcriptional states, copy-number alterations (CNAs), and expressed somatic single-nucleotide variants (SNVs). Inferred CNA profiles were concordant with shallow bulk whole-genome sequencing. An aneuploid malignant population shared 106 SNVs absent from cells lacking cancer-associated aneuploidies. Mutational signature analysis of these SNVs identified signatures associated with homologous recombination deficiency (HRD) and APOBEC activity. Hundreds of SNVs were also detected in luminal progenitor cells outside the malignant cluster; however, individual variants were shared by only a few cells, providing no evidence of a large SNV-defined clone. Focal CNAs nevertheless identified small progenitor clones. Despite this limited clonal expansion, low-variant-allele-frequency SNVs in diploid luminal progenitors were enriched for an HRD-associated signature, suggesting that HRD mutagenesis may precede clonal expansion. Targeted long-read mitochondrial sequencing provided complementary lineage information and resolved clones indistinguishable by nuclear alterations alone. Together, these results establish a framework for reconstructing early clonal evolution and distinguishing non-progressing field clones from trajectories linked to malignancy, potentially informing surveillance and preventive intervention.

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Grace Li*[1,†], Ping Lu[1,†], Allen Lynch[2], Zuen Ren[1,3], Kazi Nazrul Islam[1,3], Moshe Sade-Feldman[1,3], Ioannis Sanidas[1,3], Peter J Park[2], Leif W. Ellisen[1,3], Vinay Vinswanadham[2], Doga C. Gulhan[1,3,4]

[1]*Krantz Family Center for Cancer Research, Mass General Brigham, Boston, MA, USA*
[2]*Department of Biomedical Informatics, Harvard Medical School, Boston, MA, USA*
[3]*Department of Medicine, Harvard Medical School, Boston, MA, USA*
[4]*Broad Institute of MIT and Harvard, Cambridge, MA, USA*

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