Age-Dependent Mammary Tumorigenesis in Mice Driven by the PIK3CA H1047R Oncogene

Thangarajeswari Mohan[1], Jacob Mackay[1], Morgana McLaughlin[1], Yue Li[2], Alia Nazarullah[3], Lu-Zhe Sun[1]*

[1]Department of Cell Systems & Anatomy; [2]Department of Microbiology, Immunology & Molecular Genetics; [3]Department of Pathology and Laboratory Medicine, UT Health San Antonio, San Antonio, TX 78229

UT San Antonio™ The University of Texas at San Antonio

Background

Breast cancer incidence increases with age, with the HR+/HER2 Negative subtype (often carrying the PIK3CA H1047R mutation) being the most prevalent in older women

Aim

Our objective is to understand how the PIK3CA H1047R mutant interacts with the aging microenvironment to determine age-related susceptibility

Methods

In this study, we examined age-dependent susceptibility to PIK3CA H1047R–driven tumorigenesis by delivering PIK3CA H1047R lentivirus intraductally into female FVB/NJ mouse mammary gland across three age groups (young: 2 mo, n=10; middle-aged: 14 mo, n=8; aged: 22 mo, n=7). Mice were monitored for 6 months, and time to palpable tumor formation was used to generate tumor-free survival curves. Analyses included qPCR, whole-mount imaging, H&E staining, and high-dimensional flow cytometry (t-SNE, FlowSOM, Cluster Explorer) using BD FACSDiscover S8 Sorter. Statistical tests were performed in GraphPad Prism 10.6.1.

Results

Figure1: Generation of the murine intraductal PIK3CA H1047R oncogene model

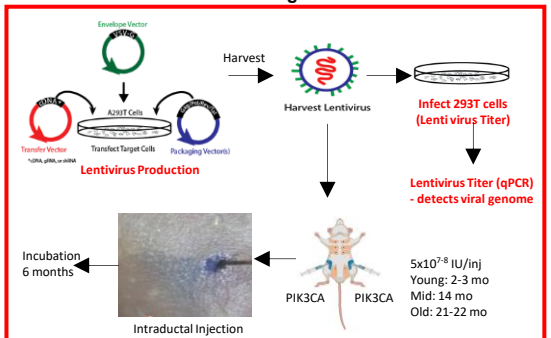


Figure 1: Workflow of Murine Intraductal (MIND) Model. Lentivirus was generated using the Puromycin-excised PIK3CA H1047R plasmid (Addgene ID: #116500) and concentrated using Lenti-X Concentrator. Infectious particle titer was determined by Lenti-X qRT-PCR Titration Kit using 293T cells. Lentiviral particles were injected into the inguinal mammary gland of mice across all age groups.

Figure 2: Mid-aged and aged mice exhibit more palpable tumor and lesion burden

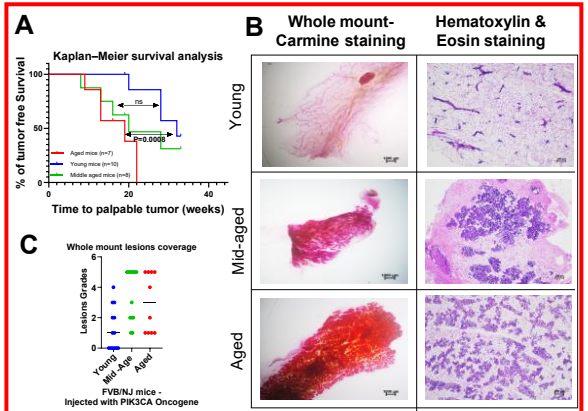


Figure 2: Age correlates with tumor progression and lesion burden. (A) Kaplan–Meier survival plot; p-values determined by Log-rank (Mantel-Cox) test. (B) Representative images of Whole Mount and Hematoxylin & Eosin (H&E) staining (4X) across all age groups. (C) Whole Mount Lesion Coverage Score (Grade 1: minimal lesions; Grade 5: 100% lesion coverage).

Figure 3: PIK3CA H1047R genomic integration and mRNA transcript levels increase with age

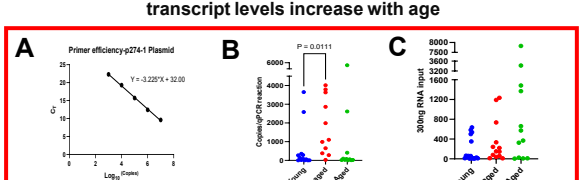


Figure 3: Absolute quantification of mutant PIK3CA integration and expression. (A) Standard curve generated using the PIK3CA H1047R plasmid. (B and C) qPCR data showing absolute quantification of mutant PIK3CA genomic integration and mRNA transcript levels. Statistical analysis: One-way ANOVA (non-parametric), Kruskal-Wallis test.

Conclusion

Together, these findings indicate that aging is not merely a risk factor but actively modifies PIK3CA-driven oncogenic signaling. These age-associated alterations in mammary epithelial cell populations may inform development of targeted therapies for older patients with HR+/HER2− breast cancer.

Figure 4: Unsupervised clustering identifies mammary epithelial populations across all age groups of mice

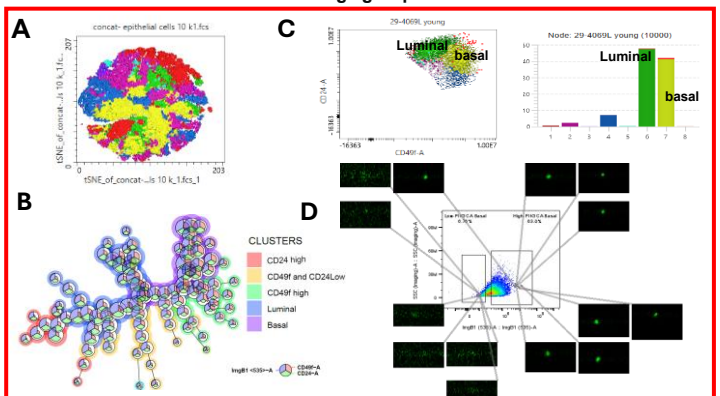


Figure 4: Intracellular flow cytometry with unsupervised clustering of mammary epithelial cells. After surface staining, cells were fixed and stained for PIK3CA-FITC, and doublets, dead cells, Lin$^+$, and stromal cells were excluded. (A) Concatenated t-SNE plot from 10,000 events pooling basal and luminal cells across age. (B) FlowSOM tree showing self-organizing map (SOM) clusters derived from the t-SNE map.(C) Cluster identification using marker expression *via* Cluster Explorer, defining Basal and Luminal populations. (D) Representative CellView images showing low and high PIK3CA-expressing cells.

Figure 5: Mid-age microenvironment enables enhanced PIK3CA signal intensity and phenotypic changes

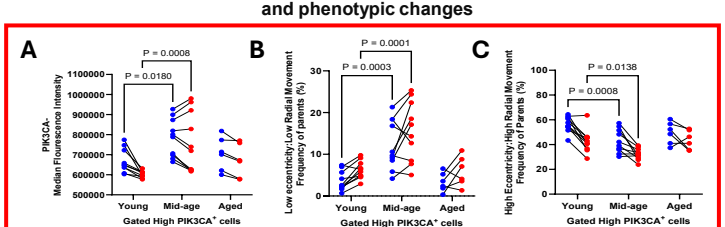


Figure 5: Morphometric analysis of PIK3CA intensity and phenotype by age. (A) Max Intensity (MFI): PIK3CA concentration in Basal and Luminal cells across age groups. (B) Low Eccentricity / Low Radial Moment: Cells displaying a proliferative/epithelial-like phenotype with high PIK3CA expression. (C) High Eccentricity / High Radial Moment: Cells displaying a motile/mesenchymal-like phenotype with high PIK3CA expression. Statistical analysis: Two-way ANOVA followed by Tukey's HSD *post hoc* test. Each dot represents data from one mouse.

Acknowledgement

National Institutes of Health (NIH)-U01Project (5U01CA263684) (PI: Lu-Zhe Sun)