**PIK3CA and cMYC Promote the Expansion of Distinct Ras-initiated, Long-lived Premalignant Clones in a Multistage Murine Breast Cancer Model**

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**Introduction:** A remote carcinogen exposure can predispose to a clinical presentation of breast cancer decades later. Standard multi-stage carcinogenesis models posit that carcinogen-induced mutations generate long-lived premalignant clones, which acquire additional oncogenic mutations as they evolve toward invasive cancers. However, the biological features of the earliest clones “initiated” on the path to cancer by carcinogen-induced mutations remain obscure, hindering the rational design of chemoprevention strategies. In prior work, we showed that a one-time exposure to carcinogen 7,12-dimethylbenzanthracene (DMBA) generates initiated mammary epithelial cell (iMEC) clones bearing signature \( \text{Hras}^{Q61L} \) mutations, which remain subclinical indefinitely until inducible activation of oncogenic Wnt signaling triggers their rapid clonal expansion into malignant outgrowths. **Methods:** Here, we adapted this multistage model by replacing Wnt pathway activation with Doxycycline-inducible expression of either \( \text{c-MYC} \) (iMYC mice) or \( \text{PIK3CA}^{H1047R} \) (iPIK mice), recapitulating two of the most prevalent oncogenic events in human breast cancer. Additional cohorts of adult mice also underwent one or two full-term pregnancies to test parity protection against premalignant clones. Mice were monitored for tumor onset, and tumors were subjected to DNA sequence and histological analyses to uncover their mutational and microscopic features. **Results:** Despite using a DMBA exposure identical to that used in our Wnt work, neither inducible \( \text{c-MYC} \) (iMYC) expression nor inducible \( \text{PIK3CA}^{H1047R} \) (iPIK) expression efficiently selected for the outgrowth of \( \text{Hras}^{Q61L} \) iMEC clones. Instead, iMYC and iPIK expression selected for the outgrowth of iMECs bearing activating mutations in distinct Ras family genes, with iMYC expression promoting \( \text{Kras}^{\text{mut}} \) and \( \text{Nras}^{\text{mut}} \) tumors and iPIK expression prompting the development of \( \text{Kras}^{\text{mut}} \) tumors. Selection for these preferred Ras family mutations occurred whether oncogene expression was induced within days of DMBA exposure or months later. Similar to our Wnt work, parity failed to diminish tumor onset in iPIK and iMYC mice. However, our parity-induced protection schemes decreased the frequency of
Kras\textsuperscript{mut} iMEC clones in iPIK and iMYC mice without affecting Nras\textsuperscript{mut} iMEC clones in iMYC mice. **Conclusions:** Together, our findings demonstrate that oncogenes PIK3CA and cMYC select for the expansion of long-lived, premalignant clones carrying distinct Ras mutations and sensitivity to parity protection. Further investigation of the cellular and molecular mechanisms underlying the differential selection of these premalignant clones may uncover targets for breast cancer chemoprevention.