Selection for a Preferred Threshold Level of PI3K Activation in Myc-driven Mammary Carcinogenesis

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Introduction: Although *Myc* overexpression is a recurring driver event in breast cancer, treatments targeting *Myc* remain elusive. Efforts to identify druggable oncogenic pathways that cooperate with oncogenic *Myc* show recurring selection for activating mutations in *Kras*, but such mutations rarely occur in human breast cancer. Through a transposon-based genetics screen, we discovered that Myc cooperates with a Ras-driven pathway, PI3K (phosphoinositide 3-kinase) signaling. Human breast cancers frequently activate PI3K signaling through mutant *PIK3CA*, a clinically validated target. **Methods and Results:** To verify whether Myc cooperates with oncogenic PI3K signaling, we generated mice that express doxycycline (Dox)-inducible and mammary-specific *Myc* and *PIK3CA*^{H1047R} transgenes (iMYC/iPIK). Littermates with singular transgene were used as genetic controls. Using Dox-impregnated chow, we discovered that a "high" level of transgene induction (2000 mg/kg) resulted in global mammary overgrowths and lethality in iMYC/iPIK mice within 5-12 days of treatment but not in "single oncogene" controls. Whole-mount and histological analyses on #4 mammary glands were conducted on Carmine alum- and hematoxylin/eosin-stained tissues, respectively. The invasiveness of overgrowths was confirmed by tissue explantation and limiting dilution assays in syngeneic hosts. To generate stochastic *Myc/PIK3CA*^{H1047R}-driven tumors, "moderate" (50 mg/kg) and "low" (10 mg/kg) levels of Dox were administered. Moderate levels of transgene expression consistently generated numerous focal tumors in iMYC/iPIK mice but not in low Dox and Dox-naïve controls. Sanger sequencing analysis of these tumors reveals no cooperating *Kras*, *Hras*, and *Nras* mutations. Rare tumors in low Dox and Dox-naïve iMYC/iPIK mice expressed transgenes through gene-switch mutations. To evaluate whether iPIK transgene expression, in isolation, can impact *Myc/PIK3CA*^{H1047R}-driven tumorigenesis, we generated mice that express constitutive *Myc* and inducible *PIK3CA*^{H1047R} transgenes (cMYC/iPIK). The same studies were performed in cMYC/iPIK mice, which showed tumorigenesis and genetic results similar to those observed in iMYC/iPIK. To simulate targeted therapy against mutant *PIK3CA*, Dox-withdrawal studies were conducted on tumor-bearing cMYC/iPIK animals. Notably, nearly all tumors regressed following *PIK3CA*^{H1047R}-deinduction despite continued *Myc* expression. Relapsed tumors evolved to restore *PIK3CA*^{H1047R} expression through gene-switch mutations. **Conclusions and Future Directions:** The expression of *PIK3CA*^{H1047R}-encoded iPIK strongly enhanced *Myc*-driven mammary tumorigenesis and relieved the selective pressure to acquire cooperating *Kras* mutations. A threshold level of PI3K activation is crucial for enhancing *Myc*-driven mammary tumor onset and for tumor maintenance. Ongoing work explores the mechanism underlying *Myc/PIK3CA*^{H1047R} cooperation and the effect of alpelisib (PI3K^{mut} inhibitor) on this interaction.