Modeling Interplay Between Cell-of-Origin and Oncogenes in BRCA-Mutated Breast Cancers

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Breast cancer is a leading cause of mortality in women. The American Cancer Society has estimated that 276,480 new cases of invasive breast will be diagnosed in 2020, and that 42,170 women will die. The physiological tissue of the breast is highly heterogenous, including basal/stem cells, luminal progenitor cells, and differentiated luminal cells. Research from the Nakshatri group, which is currently under review, has identified the importance of cell-of-origin and oncogene interactions in determining phenotype, differentiation, metastasis properties and therapeutic response of breast cancers. Among the most significant genes associated with breast cancer risk are the two Breast Cancer genes (BRCA) 1 and 2, which together are responsible for most cases of familial breast cancer. These genes are important for DNA repair, transcription, and cell cycle and control, therefore it is hypothesized that BRCA1 and BRCA2 mutations influence the interplay between normal breast cell-of-origin and oncogenes, which could be responsible for early onset of breast cancer in BRCA mutation carriers. The goal of this project is to develop a model system to test this hypothesis. An immortalized cell-line carrying a BRCA2 mutation was transformed with H-Ras^{G12V}, H-Ras^{G12V} + SV40-T/t, and PI3KCA^{H1047R} + SV40-T/t - oncogenes. Successful overexpression of H-Ras^{G12V} and SV40-T/t oncogenes was confirmed by Western blot, and PI3KCA^{H1047R} overexpression was confirmed by fluorescent microscopy using coupled YFP. Flow cytometry revealed SV40-T/t antigen overexpression but not H-Ras^{G12V} has a significant effect on differentiation status of cells, causing a shift from differentiated towards luminal progenitor phenotype. Mammosphere assay, which is a surrogate assay for self-renewal of stem cells, revealed much greater growth and irregularity in PI3KCA^{H1047R} and H-Ras^{G12V} overexpressing cells compared to the control. Similar studies with an immortalized BRCA1 mutated cell line are currently underway, as are further studies of in vivo tumorigenicity, tumor histotypes, metastasis patterns and drug sensitivity.