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Mutations in the Estrogen Receptor Alpha Hormone Binding Domain Promote Stem Cell Phenotype through Notch Activation in Breast Cancer Cell Lines

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Abstract

The detection of recurrent mutations affecting the hormone binding domain (HBD) of estrogen receptor alpha (ERα/*ESR1*) in endocrine therapy-resistant and metastatic breast cancers has prompted interest in functional characterization of these genetic alterations. Here, we explored the role of HBD-*ESR1* mutations in influencing the behaviour of breast cancer stem cells (BCSCs), using various BC cell lines stably expressing wild-type or mutant (Y537N, Y537S, D538G) ERα. Compared to WT-ERα clones, mutant cells showed increased $CD44^{+}/CD24^{-}$ ratio, mRNA levels of stemness genes, Mammosphere Forming Efficiency (MFE), Self-Renewal and migratory capabilities. Mutant clones exhibited high expression of NOTCH receptors/ligands/target genes and blockade of NOTCH signaling reduced MFE and migratory potential. Mutant BCSC activity was dependent on ERα phosphorylation at serine 118, since its inhibition decreased MFE and NOTCH4 activation only in mutant cells. Collectively, we demonstrate that the expression of HBD-*ESR1* mutations may drive BC cells to acquire stem cell traits through ER/NOTCH4 interplay. We propose the early detection of HBD-*ESR1* mutations as a challenge in precision medicine strategy, suggesting the development of tailored-approaches (i.e. NOTCH inhibitors) to prevent disease development and metastatic spread in BC mutant-positive patients.