Identification of GREB1 as a Potential Mutant Estrogen Receptor Coactivator in Breast Cancer

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Over 70% of breast cancer patients' cells express the estrogen receptor (ER), which promotes the proliferation of cancer cells, by activating the expression of tumor-promoting genes. In the normal ER pathway, estrogen binds to ER, stimulating its activity. Drugs have been developed to interfere with ER signaling, thereby inhibiting ER-driven tumor growth. However, due to prolonged use of hormonal therapy in the clinical setting, resistance occurs where cells develop mutations and activate themselves in the absence of estrogen. To identify the mechanism by which one of the mutant ERs, S463P, is able to activate, a RIME analysis revealed that high levels of the GREB1 protein was bound to the mutant S463P ER. Based on these findings, GREB1 was hypothesized to be a potential coactivator of the mutant estrogen receptor. A series of experiments was conducted to better understand the role of GREB1. The data revealed that partial loss of GREB1 using shRNA showed a differential effect on ER activity, as the data showed increase in RNA levels of some target genes but decrease in others. Subsequently, the CRISPR/Cas9 system was used to knockout the expression of GREB1. The GREB1 knockout resulted in a notable decrease of downstream targets, as detected by lighter bands in the western blot and decrease in mRNA levels in qRT-PCR analysis. These findings supported the hypothesis and indicated that GREB1 is a potential coactivator of the mutant S463P ER. Its role as a potential coactivator may be exploited for therapeutic targeting in the clinical setting.