

Regulation of Breast Cancer Progression by Vitamin D Compounds via the TP63 Pathways

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The Tp63 gene is part of the p53 tumor suppressor family. It has two main isoforms, TAp63 and Δ Np63, that have been shown to exert opposite functions on tumorigenic breast cancer cells. The TAp63 isoform acts to inhibit metastasis, promote apoptosis, and decrease the breast cancer stem cell population by promoting terminal differentiation while the Δ Np63 isoform promotes metastasis, cell survival, and stemness. Furthermore, Vitamin D has previously been shown to prevent the transition of breast cancer to invasive ductal carcinoma by down-regulating genes involved in invasion and breast cancer stem cell maintenance although the pathways by which it achieves these functions is generally unknown. We therefore hypothesize that Vitamin D prevents the progression of breast cancer to invasive ductal carcinoma by directly up-regulating the TAp63 and down-regulating the Δ Np63 pathways, respectively. MCF10DCIS, a breast cancer cell line, will be treated with 100 nM of $1\alpha,25(\text{OH})_2\text{D}_3$ or 10 nM of BXL0124 for 2 days. RT-qPCR and Western Blot analyses will be performed for TAp63; LKB1, a downstream target of TAp63 in the hippo pathway; Δ Np63; and FZD7, a downstream target of Δ Np63 in the Wnt pathway. In additional MCF10DCIS cell lines, CRISPR will be used to knockdown each isoform separately and then together. These cells will be treated as in the previous experiment, and RT-qPCR and western blot analyses will be used for Sox9, a cancer stem cell marker; vimentin, a basal marker; E-cadherin, an epithelial marker; and keratin 8, a luminal marker. From the first experiment, an increase in TAp63 and LKB1 is expected as well as a decrease in Δ Np63 and FZD7 expression. In the TAp63 knockdown experiment as well as in the simultaneous knockdown of both isoforms, an increase in Sox9 and vimentin is expected accompanied by a decrease in E-cadherin and keratin 8 while the reverse is anticipated in the Δ Np63 knockdown cells. The results of this experiment will provide a crucial understanding of the pathways by which Vitamin D achieves its potentially life-saving functions allowing for better prevention treatments against invasive ductal carcinoma. Funded by Busch Biomedical Grant, New Jersey Health Foundation, and EOHSI CEED Pilot Grant, R25ES020721.

