The Role of Caveolin-1 Deficient Human Mammary Fibroblasts on Chemotherapy Resistance in Breast Cancer Cells

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Abstract

Chemotherapy is one of the most widely used treatments of various breast cancers; however, it has been shown that carcinoma-associated fibroblasts (CAFs) induce chemotherapy resistance of breast cancer cells (BCCs). Previous research has shown that the downregulation of caveolin-1 (cav-1), a structural component of membrane caveolae, promotes a CAF-like phenotype in stromal cells. This study was performed to evaluate the effect that downregulation of caveolin-1 in mammary fibroblasts imparts on chemotherapy resistance in BCCs. To determine the halflife inhibitory concentration (IC50) of chemotherapy agents on BCCs, MDA-MB-231 (MDA) and MCF-7 BCCs were treated for 24 and 48 hours with doxorubicin (DOX) or tamoxifen (TAM); then cell viability was measured. The WST-1 cell viability assay indicated significant cytotoxicity after 48 hours for all treatments. MDA BCCs exhibited significant cell death in tamoxifen treatment with 0.92 uM, whereas TAM, MCF-7 BCCs had significant cell death at 0.92 uM for DOX and 7.5 uM for TAM; however, those results were less consistent when multiple assays were performed on MCF-7 BCCs treated with DOX. Many alterations were made to isolate mammary gland fibroblasts from cav-1/− and cav-1+/+ mice with no success. Instead, human mammary fibroblasts (HMFs) were transfected with cav-1 shRNA to induce downregulation of cav-1 protein expression. HMFs transfected with cav-1 shRNA were positively selected with puromycin, then subcultured to ensure only transfected cells remained adherent. After a 72-hour growth period, protein lysate was collected and a western blot was done to assess cav-1 expression. Results showed a significant downregulation of cav-1 in HMFs treated samples. Overall, these results indicate that TAM is a strong cytotoxic agent against MDA and MCF-7 BCCs, whereas DOX is more effective against MDA BCCs. Also, the use of shRNA transfection proved to be a beneficial technique for downregulation of cav-1 in HMFs.

Introduction

Loss of fibroblast cav-1 will induce chemotherapeutic resistance in breast cancer cells.

References

1. Martinez-Oturshok, U., et al. Loss of caveolin-1 in vivo and in activated cAFs contributes to the secretion of growth factors, cytokines, and metabolites that stimulate breast cancer mitochondrial metabolism, decrease the pH within the breast cancer tumor microenvironment, and stimulate tumorogenesis (Luo, H., et al.). Previous research has also shown that downregulation of cav-1 promotes a CAF-like phenotype in stromal fibroblasts (Casazza, A., et al.). The downregulation of cav-1 at the protein level in stromal fibroblasts has been linked to breast cancer tumor progression and poor patient outcome (Sotgia, F., et al.).

Hypothesis

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1. For 500 cells/mL, purified with 50 uM of TAM, MDA BCCs were transfected with cav-1 shRNA and treated with 7.5 uM TAM. The dose of 7.5 uM for MDA BCCs was used, as this was the half-life inhibitory concentration (IC50) of TAM for MDA BCCs.

2. While the use of TAM is not a common treatment for breast cancer, the use of TAM was included as a control to determine differences in cell proliferation and viability of the shRNA treated HMFs co-cultured with MDA cells compared to MDA only with 0.0062. Additionally, there was a significant increase in cell proliferation of the shRNA treated HMFs co-cultured with MDA cells versus MDA cells treated with tamoxifen alone (p=0.0207).