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 half maximal 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 showed significant cytotoxicity after 48 hours for all treatments. MDA BCCs exhibited significant cell death in chemotherapy treatment with 0.9 ug/ml DOX or 7.5 uM TAM, while MCF-7 BCCs had significant cell death at 0.92 ug/ml DOX for 7.5 uM for TAM. However, these results were less consistent when multiple assays were performed on MCF-7 BCCs treated with DOX. Many attempts 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 shRNA treated samples. Overall, these results indicate that TAM is a strong cytotoxic agent against MDA and MCF-7 BCCs, while 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 chemotherapy resistance in breast cancer cells

Hypothsis

References

Table

Chemotherapy cytotoxicity in MCF-7 and MDA-MB-231 breast cancer cells

WST-1 assay indicated significant cell death of MCF-7 BCCs treated with greater than or equal to 0.92 ug/ml doxorubicin (p<0.0001). Significant cell death was seen in MDA-MB-231 BCCs treated with greater than or equal to 0.9 ug/ml doxorubicin (p<0.0001).

Cell proliferation analysis of HMF and MDA co-cultures treated with tamoxifen

Tamoxifen treated MCF-7 BCCs showed significant cell death at concentrations greater than or equal to 0.1 μM (p<0.0001). Increased significant cell death was seen at concentrations of 7 μM tamoxifen or greater in treated MDA-MB-231 BCCs (p<0.0009).

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Conclusions

These findings indicate that tamoxifen is an effective cytotoxic agent for both MDA and MCF-7 BCCs, while doxorubicin is a strong agent against MDA BCCs. Although murine mammary gland fibroblasts were not isolated despite repeated attempts, transfection of HMFs with cav-1 shRNA was a useful tool in the downregulation of cav-1 protein. Lastly, the HMF and MDA co-cultures suggest that the downregulation of cav-1 in stromal mammary fibroblasts may play a role in endocrine chemotherapy resistance of MDA-MB-231 breast cancer cells.

ΔΔCT analysis of HMF and MDA co-cultures treated with tamoxifen

HMFs transfected with GFP protein showed positive fluorescence indicating successful transfection. Tryptosinized followed by the addition of puromycin selective media ensured only transfected cells remained adherent to cell culture plates. GFP transfection was maintained even after cell subculture following puromycin selection.

Western blot analysis of cav-1 shRNA-mediated transfection of HMFs indicated significant overall downregulation of cav-1 protein expression when compared to HMFs plated in transfection medium only or transduced with control shRNA (p=0.0510).

Cell proliferation analysis of HMF and MDA co-cultures treated with tamoxifen

WST-1 analysis of HMF and MDA co-cultures (indicated very significant increase in cell proliferation of the shRNA treated HMFs co-cultured with MDA cells compared to TM only HMFs co-cultured with MDA cells (p<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).

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