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 following treatment with 0.9 ug/mL of DOX and 7 uM of TAM. MCF-7 BCC's had significant cell death at 0.92 ug/mL for DOX and 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 cav-1 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

Carcinoma-associated fibroblasts (CAFs) have the largest concentration of all cell types within the breast cancer tumor stroma and have been shown to induce chemotherapy resistance within multiple lines of breast cancer cells (Buchsbaum, R. et al.). CAFs experience a loss of cav-1 through autophagy in response to oxidative stress induced by breast cancer cells (Martinez-Outschoorn, U., et al.). Loss of cav-1 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 tumorigenesis (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.). This 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

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

References

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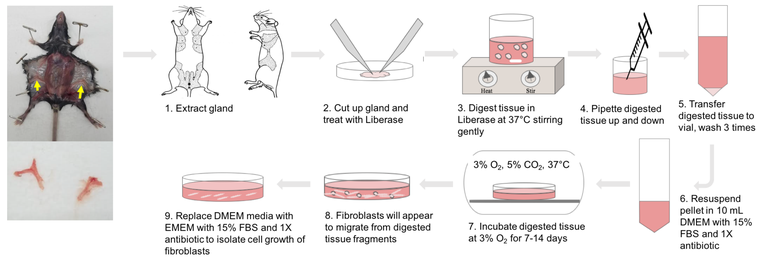
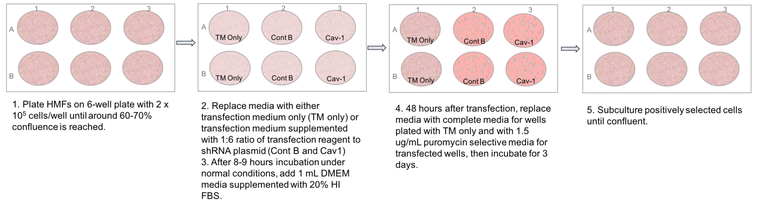
Experimental Methods



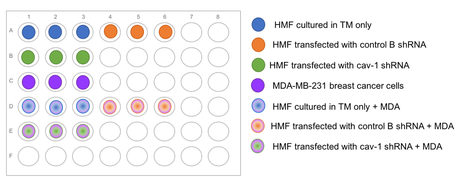
Unable to isolate murine mammary gland fibroblasts. Alternatively downregulated cav-1 in HMFs via shRNA.



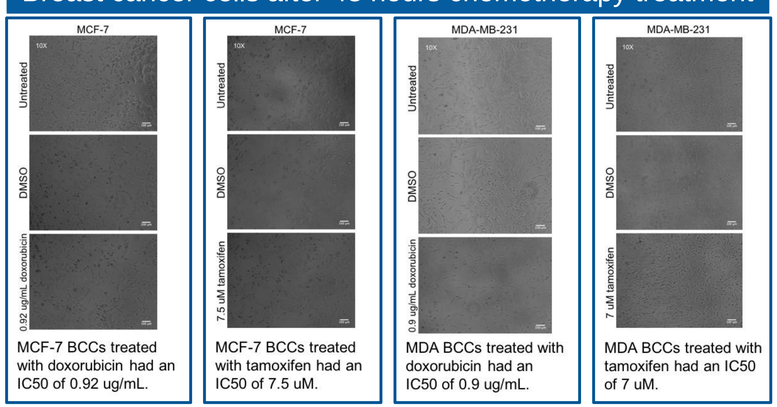
1. Plate HMFs on 6-well plate with 2 x 10^5 cells/well until around 60-70% confluence is reached.
2. Replace media with either transfection medium only (TM only) or transfection medium supplemented with 1:6 ratio of transfection reagent to shRNA plasmid (Cont B and Cav1)
3. After 8-9 hours incubation under normal conditions, add 1 mL DMEM media supplemented with 20% HI FBS.
4. 48 hours after transfection, replace media with complete media for wells plated with TM only and with 1.5 ug/mL puromycin selective media for transfected wells, then incubate for 3 days.
5. Subculture positively selected cells until confluent.

Co-cultures of HMFs and MDA-MB-231 (MDA) breast cancer cells were set up for tamoxifen treatment.

1. Four 48-well culture plates were plated with 2.8 x 10^4 cells/well containing either transfected HMFs only, MDA BCCs only, or a co-culture of transfected HMFs with MDA BCCs.
2. After 24 hours, two of the four plates were treated with 7 uM tamoxifen, while the other two were plated in MDA complete media.
3. One set of treated and untreated plates was analyzed for cell proliferation via a WST-1 assay after 24 hours, the other set of plates was analyzed after 48 hours.



Breast cancer cells after 48 hours chemotherapy treatment



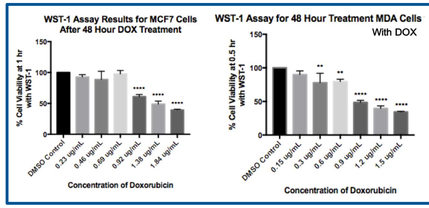
MCF-7 BCCs treated with doxorubicin had an IC50 of 0.92 ug/mL.

MCF-7 BCCs treated with tamoxifen had an IC50 of 7.5 uM.

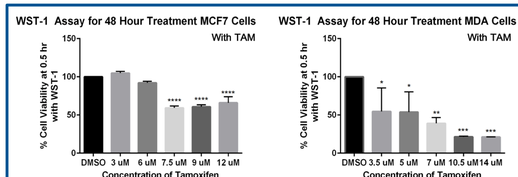
MDA BCCs treated with doxorubicin had an IC50 of 0.9 ug/mL.

MDA BCCs treated with tamoxifen had an IC50 of 7 uM.

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

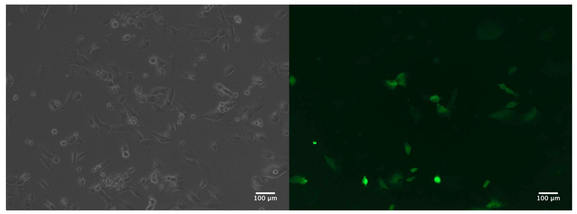


WST-1 assay indicated significant cell death of MCF-7 BCCs treated with greater than or equal to 0.92 ug/mL doxorubicin ($p<0.0001$). Significant cell death was seen in MDA-MB-231 BCCs treated with greater than or equal to 0.9 ug/mL doxorubicin ($p<0.0001$).

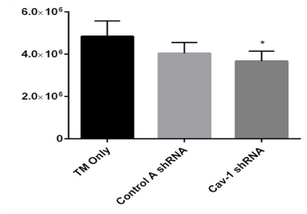


Tamoxifen treated MCF-7 BCCs showed significant cell death at concentrations greater than or equal to 7.5 uM ($p<0.0001$). Increased significant cell death was seen at concentrations of 7 uM tamoxifen or greater in treated MDA-MB-231 BCCs ($p=0.0009$).

shRNA plasmid DNA transfection of HMFs

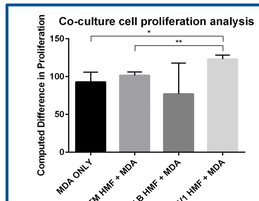


HMFs transfected with GFP protein showed positive fluorescence indicating successful transfection. Trypsinization followed by the addition of puromycin selective media ensured only transfected cells remained adherent to cell culture plates. GFP transfection was maintained even after cell subculture following 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 transfected 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 in cav-1 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 ($p=0.0207$).

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.

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