

Estrogen receptor mRNA directed therapy for triple negative breast cancer

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BACKGROUND

Triple negative breast cancer (TNBC) occurs in 10-20% of all diagnosed breast cancer cases, and is negative for protein expression of estrogen receptor, progesterone receptor, and HER2/neu receptor. Most TNBCs manifest as high grade invasive ductal carcinomas, of which 70-80% display a basal-like pattern of gene expression. TNBC patients who have a pathological complete response (pCR) to therapy usually demonstrate long-term progression free survival. The majority of patients, however, have residual disease after treatment and are at a high risk of relapse, development of metastatic disease, and demonstrate shorter overall survival. Additionally, the major cause for residual disease after treatment is primary or acquired multidrug resistance to chemotherapy. Due to the absence of hormone receptor expression, TNBC is resistant to hormone therapies including tamoxifen, anastrozole, exemestane, letrozole, and fulvestrant. TNBC is additionally resistant to HER2 targeted therapies (trastuzumab, lapatinib). This greatly limits the available treatment options for these patients, and currently there is no standard chemotherapeutic applications for patients with TNBC. The majority of chemotherapies investigated in the context of TNBC are not targeted therapies, and include anti-mitotic agents (e.g. docetaxel, vinorelbine), DNA-damaging agents (e.g. cisplatin, doxorubicin), and inhibitors of DNA synthesis (e.g. capecitabine, gemcitabine, irinotecan). Some targeted therapies have been evaluated, including anti-angiogenic agents (e.g. bevacizumab, sunitinib, sorafenib) and EGFR inhibitors (e.g. cetuximab), and have shown some clinical benefit when used in combination with chemotherapy. Adverse side effects are common, and management of toxicity is crucial.

STUDY OBJECTIVES

Introduction: Patients with TNBC do not respond to hormone therapy, and therefore are limited to chemotherapeutic treatment regimens. Interestingly, a significant number of histologically triple negative breast cancer (TNBC) cells express substantial estrogen receptor (ER) mRNA, but do not express ER protein as a result of rapid proteolytic degradation. Other TNBC models appear to suppress ER expression via epigenetic silencing of ER transcription. In this study we assessed whether re-expression of ER in TNBC would sensitize cancer cells to antiestrogen targeted therapy.

Methods: Part 1- Data from the Katzenellenbogen laboratory elegantly demonstrate that in high ER mRNA MDA-MB-468 cells, ER protein is rapidly degraded via proteosomal activity (Bhatt et al. MCB, 2012). Utilizing an established human TNBC cell line that expresses high ER mRNA, BT-20, and a newly established human TNBC cell line from our laboratory, MCJB2, we investigated a therapeutic strategy using the proteasome inhibitor carfilzomib in combination with the antiestrogen tamoxifen. Part 2- We have previously shown that combined epigenetic therapy [methyltransferase inhibitor (decitabine) plus a histone deacetylase inhibitor (HDACi; romidepsin)] provided antitumor synergy in MDA-MB-231 cells via upregulation of the soluble Wnt suppressor, secreted frizzled protein 1 (sFRP1; Cooper et al. CCR, 2012). We also showed that this epigenetic therapy restores silenced ER mRNA and protein levels in MDA-MB-231 cells. Here we further evaluate the effects of epigenetic re-expression of ER combined with antiestrogen therapy on tumor cell growth *in vitro* and *in vivo*.

RESULTS

Figure 1: Transcriptional regulation of Estrogen Receptor

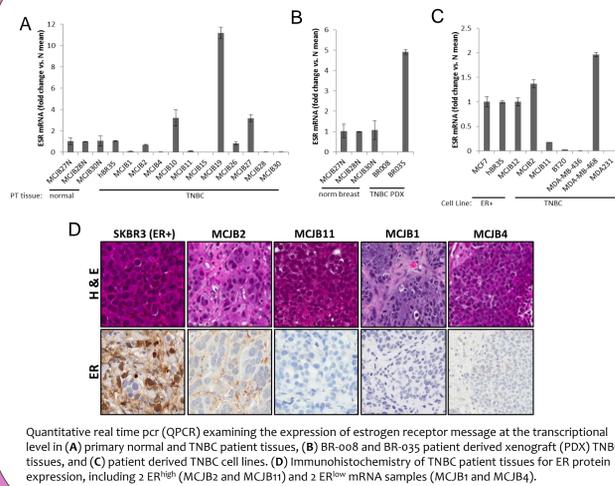


Figure 2: Proteasome inhibitor mediated re-expression of ER in ER^{high} cell models

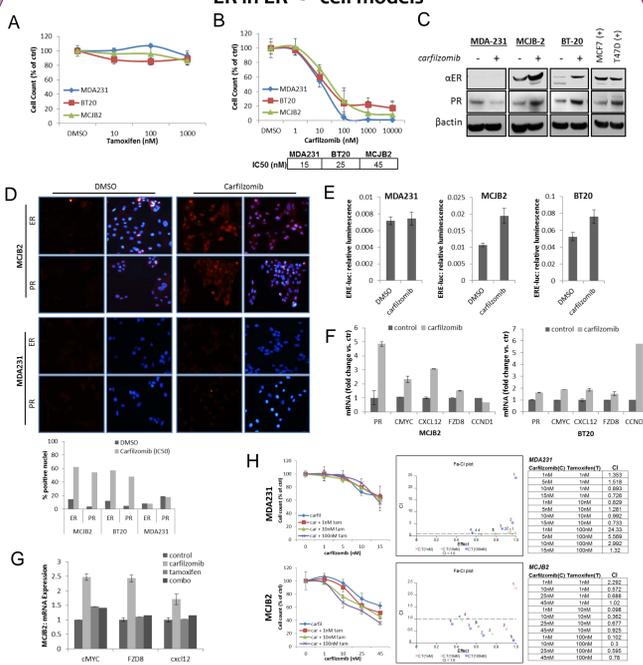


Figure 3: In vitro assessment of epigenetic re-expression of ER in an ER^{low} cell model

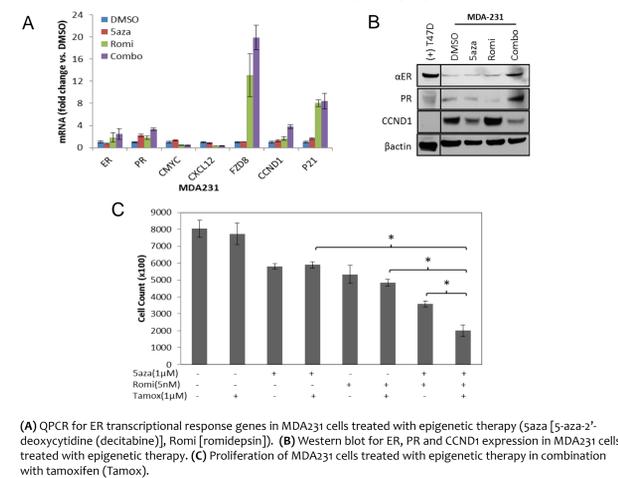
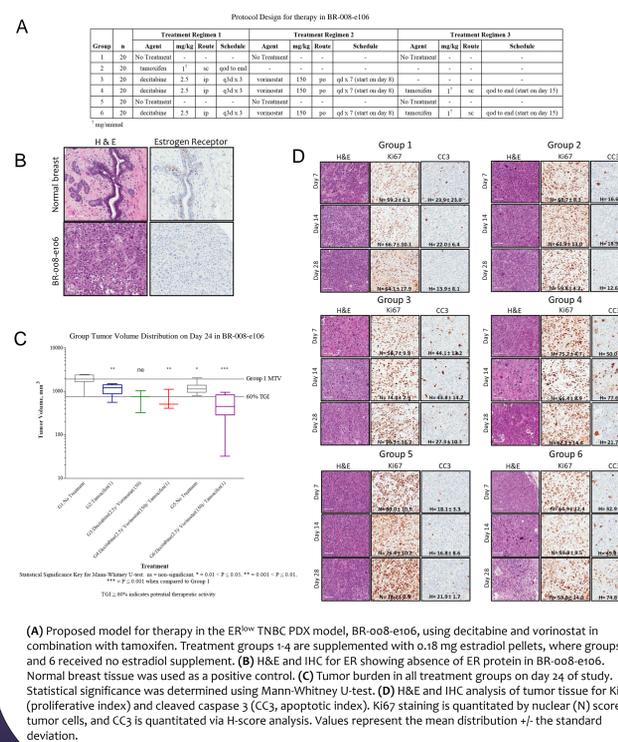


Figure 4: In vivo assessment of epigenetic therapy in an ER^{low} TNBC PDX model



CONCLUSIONS

Results: Administration of carfilzomib in TNBC cell lines with high ER mRNA (ER^{high}) restored ER protein expression, and upregulation of ER transcriptional targets. Carfilzomib treatment combined with antiestrogen (tamoxifen) led to enhanced growth suppression in ER^{high} cells. TNBC MDA-MB-231 cells, which express no ER mRNA (ER^{low}), demonstrate ER re-expression with epigenetic therapy. Tamoxifen in combination with epigenetic therapy provided further growth suppression in these cells. Additionally, this treatment regimen lead to increased tumor cell death, decreased tumor cell proliferative capacity, and decreased tumor burden in an *in vivo* PDX model of ER^{low} TNBC: BR-008-e106.

Conclusions: We propose that ER mRNA expression level in TNBC patients could guide therapeutic choice, whereby, ER^{low} mRNA TNBC tumors could respond to epigenetic therapy followed by antiestrogen while ER^{high} mRNA TNBC tumors could respond to a proteasome inhibitor followed by antiestrogen therapy. These results identify a novel epigenetic biomarker as predictive of therapeutic response.

FUTURE DIRECTIONS

- Evaluate the efficacy of epigenetic therapy mediated estrogen receptor re-expression in combination with anti-estrogen therapy in additional models of ER^{low} TNBC.
- Evaluate proteasome inhibitor mediated re-expression of ER in combination with antiestrogen therapy in an *in vivo* model of ER^{high} TNBC.
- Identify the molecular mechanisms by which combinatorial therapy leads to enhanced tumor cell death in both proteasome inhibitor and epigenetic therapy mediated re-expression of ER combined with antiestrogen.
- Further assess the mechanisms by which estrogen receptor is downregulated in TNBC tumor cells, both at the proteolytic and epigenetic level.

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