MGMT Inhibition by Disulfiram Sensitizes ER+ Breast Cancer Cells to Temozolomide and Cyclophosphamide

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PROBLEM

MGMT (O6-methylguanine-DNA methyltransferase), a DNA repair protein leading to chemoresistance, is overexpressed in a majority of cancers, including breast cancer. MGMT expression directly correlates with ER expression and tamoxifen resistance making ER-positive breast cancer resistant to alkylating agents like Temozolomide and Cyclophosphamide. Alkylating Depolymerizing Agents (ADAs) activity, as a chemotherapy-resistant cell trait, has also been reported to inversely correlate with MGMT expression in other cancers, has also been linked to chemotherapy and radiation resistance.

BACKGROUND

In breast tumours, MGMT expression is elevated at levels that are 3-4 fold higher than in the normal counterparts [1, 2, 3]. MGMT has been recognized as a critical determinant of tumor resistance to alkylating agents and represents an important target for inhibitor development [4]. Reduction of the role of Disulfiram (DSF), as an MGMT inhibitor, either alone or in combination with alkylators (Temozolomide/Cyclophosphamide) in the treatment of breast cancers, has been suggested for many years [5, 6]. Disulfiram (DSF), a yellow/orange crystalline, also known as Antabuse, is a carbamate derivative clinically used for treating alcoholism. DSF is a modestly nonselective (ERL, TAM, and Tamoxifen) modulator of apoptosis in drug resistant breast cancer [5, 6]. Alkylating agents, with widely different carbon chain lengths [5, 6], have demonstrated antitumor activity against a broad range of tumor types (glioma, melanoma, non-small cell lung cancer, and carcinomas of the esophagus and colon) [5, 6]. Cyclophosphamide is an alkylating agent with dose dependent biological effects which has been for decades successfully used to treat a variety of cancers. At higher doses, it is associated with increased cytotoxicity and immunosuppression, while at lower doses, it shows immunostimulation and antitumorigenic properties [5, 6]. These observations suggest the way to investigate the effect of alkylators, such as Temozolomide/Cyclophosphamide in presence or absence of MGMT inhibition in breast cancer cells.

OBJECTIVE

In this study, we investigated whether Disulfiram, either alone or in combination with Temozolomide or Cyclophosphamide can inhibit breast cancer growth and establish whether use of DSF as MGMT inhibitor would allow for using lower doses of Tamoxifen or Clinical Refills.

METHODS

To test the effectiveness of Antabuse (Disulfiram, DSF), as a dual MGMT and ALDH inhibitor, at breast cancers, in combination with Temozolomide (TMZ) or Cyclophosphamide (CP) on ERα breast cancer cells.

RESULTS

Effect of Disulfiram on Normal Breast Epithelial Cells and Breast Cancer Cells: Normal breast epithelial cells (MCF10A), normal breast cells (MCF7), and model breast cancer cell lines (MCF7, MDA-MB-231, and MDA-MB-468) were treated with different concentrations of DSF. Forty eight hours post treatment cell viability assays were performed. Results reveal that DSF has no effect on normal breast epithelial cells (MCF10A) whereas ERα positive breast cancer cell line was dose dependently inhibited (Figure 1).

In vitro Studies: We used Chou & Talalay isobologram method to investigate synergy between DSF in combination with Cyclophosphamide and tamoxifen. Results reveal that synergistic activity was resulted between DSF and Cyclophosphamide and moderate synergistic activity was resulted between DSF and Tamoxifen in all ER alpha positive breast cancer cells (Figure 2).

Combination Therapeutic Effect on MGMT, ERα, CDK12, and AKT1 in Breast Cancer Cells: Breast cancer cells were treated with single agents (DSF, Cyclophosphamide and Tamoxifen) and combination of the drugs. Forty eight hours after the treatment, cells were harvested and protein were isolated and western blots analysis was performed. Results reveal that either alone at or in combination with Cyclophosphamide and Tamoxifen decreased MGMT expression, ERα, AKT1, CDK12, and CDK12 and Src1450 (需Viewer) expression in MCF7 breast cancer cells (Figure 3).

Combination Therapeutic Effect on Colony Formation: We used colony formation assay to determine the effectiveness of these drug treatments on breast cancer cells. We plotted breast cancer cells (MCF7 & MDA) in 6 well plates and treated with Temozolomide, Cyclophosphamide and DSF alone or in combination. Results revealed that DSF inhibited the colony formation of these cells and DSF in combination with Temozolomide significantly decreased the colony formation of these cells (Figure 4).

CONCLUSIONS

- DSF at very low doses (achieved in human serum with standard DSF clinical dosing) decreases ER+ breast cancer cell growth (MCF7 and 4T10) in a dose-dependent manner.
- DSF further sensitizes breast cancer cells to TMZ (cyclophosphamide) and significantly inhibits breast cancer growth without causing unwanted side effects on the normal breast epithelial cells.
- Dose effect and in vivo studies suggest consistent activity of DSF & CP and may be utilized in combination therapy.
- DSF, alone or in combination with TMZ (cyclophosphamide) and/or CP (cyclophosphamide), significantly inhibits expression of GSH, alkylation-dependent, Tax1 and Bax1 gene (conclusively) and is highly effective in breast cancer treatment.
- DSF, alone or in combination with TMZ (cyclophosphamide) and/or CP (cyclophosphamide) significantly inhibits apoptotic genes in breast cancer cells.
- In a dose-dependent manner, DSF inhibited colony formation, effect which was further enhanced by addition of TMZ (cyclophosphamide) and/or CP (cyclophosphamide).

- Similarly, DSF alone or in combination with TMZ (cyclophosphamide) and/or CP (cyclophosphamide) decreased the metastatic potential of breast cancer.

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