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 upregulated 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 alkylation agents like Temozolomide and Cyclophosphamide. Alkaloid Diphosphorine (DA) activity, as a chemosensitizer, cell growth, and metastasis has also been reported to increase sensitivity to MGMT expression in other cancers, but has also been linked to chemotherapy and radiation resistance.

BACKGROUND

In breast tumors, MGMT expression is elevated at levels that are 3.4 fold higher than in the normal tissue (2, 3, 4). MGMT has been recognized as an important determinant of tumor resistance to alkylating agents and therefore important for the investigation of the role of Disulfiram (DSF), an MGMT inhibitor, either alone or in combination with alkylators (Temozolomide/Cyclophosphamide) in the treatment of breast cancer. Disulfiram (DSF) is a thioetherthiourea compound, also known as Antabuse, is an ancient derivative clinically used for treating alcoholism. DSF is a poorly water-soluble, Temozolomide (TMZ), an alkylating agent, with therapeutic, anti-inflammatory properties, has demonstrated antineoplastic activity against a wide range of tumor types (glioma, melanoma, non-small cell lung cancer, and cancers of the breast and colon) (5, 6). Cyclophosphamide is an alkylating agent with dose-dependent biological actions 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 low, continuous doses, it shows immunostimulatory and antitumorogenic properties (7). These observations paved the way to investigate the effect of alkylators such as Temozolomide/Cyclophosphamide in presence or absence of MGMT blockers (DSF) in breast cancer cells.

OBJECTIVE

To investigate 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 lower doses of Tamoxifen or Taxane.

METHODS

We have tested the effect of Antabuse (Disulfiram, DSF), as a dual MGMT and ALDH inhibitor, at various doses, in combination with Temozolomide (TMZ) or Cyclophosphamide (CP) on ER+ breast cancer cells.

RESULTS

Effect of Disulfiram on Normal Breast Epithelial Cells and Breast Cancer Cells: Normal breast epithelial cells (HMEC) were treated with various concentrations of DSF. Forty eight hour post treatment cell viability assays were performed. Results reveal that DSF has minimal effect on normal breast epithelial cells (HMEC101A) whereas ER alpha positive breast cancer cell was dose dependently inhibited (Figure 1).

Alkylphosphorine Antibody: MGMT breast cancer cells were treated with different concentrations of DSF and 48 hr post treatment cell viability assays were carried out and ALDH activity was measured. Results reveal that DSF dose dependently decreased ALDH activity in breast cancer cells. In another experiment, MGMT cells were treated with single agents (DSF, Cyclophosphamide, and Temozolomide) in combination of these drugs and 48 post treatment ALDH activity was measured. Results reveal that single agents (DSF, Cyclophosphamide and Temozolomide) decreased ALDH activity compared to control and combination therapy further decreased it (Figure 5).

Combination Therapeutic Effect on Apoptotic Cell Death of Breast Cancer Cells: Breast cancer cells were treated with single agents (DSF, Cyclophosphamide and Temozolomide) and combination of the drugs. Forty eight hour post treatment, cell were harvested and protein were isolated and western blot analysis performed. Results reveal DSF either alone or in combination with Cyclophosphamide and Temozolomide decreased MGMT expression, ER alpha,123F, 123F, 123F, and Survivin (BIRC5) expression in MGMT breast cancer cells (Figure 4).

Combination Therapeutic Effect on Colony Formation: We used colony formation assays to determine the effectiveness of these drug treatments on breast cancer cells. We plated breast cancer cells (MCF-7 and ZR-75) in 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 7).

CONCLUSIONS

DSF at low dose (achivable in human serum with standard DSF clinical dosage) decreases ER+ breast cancer cell growth (MCF-7 and ZR-75) in a dose-dependent manner.

DSF further sensitizes breast cancer cells to TEM (CAF) and significantly inhibits breast cancer growth without causing severe side effects on the normal breast epithelial cells.

Combination of DSF and AKT inhibitors may provide synergistic effects on breast cancer cell proliferation.

In a dose dependent manner, DSF inhibited colony formation, effect which was further enhanced by addition of Temozolomide (TMZ) or Cyclophosphamide (CP).

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

REFERENCES


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