Disulfiram Enhances Palbociclib and Abemaciclib Activity Through MGMT and CDK4/6 Inhibition in Breast Cancer Cells

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PROBLEM
Endocrine therapy is the main upfront option for hormone receptor positive breast cancer but some of the patients develop hormone refractory disease. Therefore, it is important to identify ways to restore estrogen receptor function and/or treat hormone refractory disease.

BACKGROUND
MGMT (O6-methylguanine DNA methyltransferase), a DNA repair protein leading to chemotherapy resistance, is increasingly studied for its cell cycle regulatory functions, also known to control ER expression and function, is overexpressed in a majority of cancers, including breast cancer. MGMT inhibition has been reported to restore ER function and sensitivity to hormonal therapy in tamoxifen resistant breast cancer. CDK4/6 is a cell cycle regulator targeted by a new class of drugs in the treatment of breast cancer in patients who had progressed during prior endocrine therapy. We investigated a potential correlative role between MGMT and CDK4/6 expression/activity. In this therapeutic context MGMT inhibition would have the dual role of increasing/restoring effect of endocrine therapy and facilitate activity of CDK4/6 inhibitors (Palbociclib and Abemaciclib).

OBJECTIVE
To investigate better therapeutic option for hormonal sensitive and hormonal refractory breast cancer.

METHODS
We have tested the effect of Antabuse (disulfiram, DSF), as an MGMT inhibitor, at nontoxic doses, on the expression of CDK4/6, or in combination with Palbociclib (PB) or Abemaciclib (LY2835219 - LY) on ER+ breast cancer cells.

RESULTS
DSF at very low doses (achievable in human serum with standard DSF clinical dosing) decreases ER+ breast cancer cell growth (MCF7, T47D and ZR75) in a dose-dependent manner. DSF further sensitizes breast cancer cells to PB or LY and significantly inhibits breast cancer growth without causing unwanted side effects on the normal breast epithelial cells. Dose effect and isobologram studies confirm synergistic activity of DSF + LY and moderate synergism for DSF + PB. DSF, alone or in combination with PB (DSF + PB) or LY (DSF + LY), significantly inhibits expression of MGMT, CDK4/6, ERα and aldehyde dehydrogenase activity – all involved in breast cancer cell cycle proliferation and tumorigenesis. Furthermore, PB and LY dose dependently decreased MGMT and CDK4 expression in breast cancer cells and significantly accumulated breast cancer cells in G1 phase of the cell cycle. DSF, alone or in combination with PB (DSF + PB) and/or LY (DSF + LY) caused significant apoptosis in breast cancer cells. DSF inhibited colony formation which was further enhanced by addition of PB/LY (DSF + PB/LY). Similarly, DSF alone or in combination with PB (DSF + PB) and/or LY (DSF + LY) decreased the metastatic potential of breast cancer cells.

CONCLUSIONS
- DSF at very low doses (achievable in human serum with standard DSF clinical dosing) decreases ER+ breast cancer cell growth (MCF7, T47D and ZR75) in a dose-dependent manner.
- DSF further sensitizes breast cancer cells to PB or LY and significantly inhibits breast cancer growth without causing unwanted side effects on the normal breast epithelial cells.
- Dose effect and isobologram studies confirm synergistic activity of DSF + LY and moderate synergism for DSF + PB.
- DSF, alone or in combination with PB (DSF + PB) and/or LY (DSF + LY), significantly inhibits expression of MGMT, CDK4/6, ERα and aldehyde dehydrogenase activity – all involved in breast cancer cell cycle proliferation and tumorigenesis.
- Furthermore, PB and LY dose dependently decreased MGMT and CDK4 expression in breast cancer cells and significantly accumulated breast cancer cells in G1 phase of the cell cycle.
- DSF, alone or in combination with PB (DSF + PB) and/or LY (DSF + LY) caused significant apoptosis in breast cancer cells.
- DSF inhibited colony formation which was further enhanced by addition of PB/LY (DSF + PB/LY).
- Our findings suggest that DSF as an MGMT inhibitor significantly enhances the antitumor effect of CDK4/6 inhibitors (PB + LY) in ER+ breast cancer.

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