HDAC Inhibitors Sensitized Triple-negative Breast Cancer Cells to PARP Inhibition and Cisplatin Treatment

- HDAC inhibitors indirectly caused DNA damage and impaired DNA repair.
- Cellular conditions created by HDAC inhibition mimicked those in BRCA1-mutated breast cancer cells.
- Findings could have implications for hard-to-treat triple-negative breast cancer.

SAN ANTONIO — In-vitro exposure to an HDAC inhibitor indirectly impaired the ability of triple-negative breast cancer cells to repair damaged DNA and sensitized the cells to treatment with two therapies that have clinical activity in some patients with breast cancer — a PARP inhibitor and cisplatin, according to data presented at the 2012 CTRC-AACR San Antonio Breast Cancer Symposium, held here Dec. 4-8.

“Triple-negative breast cancer is a particularly aggressive breast cancer that is not susceptible to traditional hormone therapies,” said Kapil N. Bhalla, M.D., chief of personalized cancer medicine at the University of Kansas Cancer Center in Kansas City. “That is why it is important to try to find new ways of killing triple-negative breast cancer cells.”

Cells of certain human tumors rely on intact DNA repair pathways for survival. Prior research has shown that proteins such as ATR, CHK1 and BRCA1 are essential parts of a cell’s response to DNA damage and its subsequent repair of the damage. These three proteins are controlled or chaperoned by heat shock protein 90 (hsp90).

Bhalla and colleagues previously found that treatment with an HDAC inhibitor renders hsp90 inactive, thus impeding the DNA damage response that involves the ATR, CHK1 and BRCA1 proteins. Therefore, HDAC inhibition creates an environment within cells that is similar to that seen in breast cancer cells with BRCA1 mutations.
“In simple terms, we are trying to cause a ‘BRCAness’ so that you confer on triple-negative breast cancer cells the sensitivity to PARP inhibitors or platinum therapy seen when BRCA1 mutations are present,” Bhalla said.

The researchers examined the mechanism of action of HDAC inhibitors and determined that inhibition of HDAC3 specifically rendered hsp90 inactive and consequently inhibited repair of damaged DNA.

“The icing on the cake, so to speak, was that in addition to inhibiting the DNA damage response through depletion of DNA repair proteins, HDAC inhibitors induced DNA damage,” Bhalla said. “By using HDAC inhibitors, we were targeting the cancer two ways at once.”

The researchers also tested whether treatment with the HDAC inhibitors vorinostat or panobinostat would sensitize triple-negative breast cancer cells to PARP inhibition. Combined treatment with either of the HDAC inhibitors plus the PARP inhibitor ABT888 resulted in the death of triple-negative breast cancer cells with or without BRCA1 mutation. In addition, vorinostat treatment made the triple-negative breast cancer cells more susceptible to treatment with cisplatin.

If further validated, the findings of this study could have implications for women with triple-negative breast cancer and possibly even women with ovarian cancer, which has a genetic makeup similar to that of triple-negative breast cancer, according to Bhalla.

“If you have a patient with triple-negative breast cancer who does not have a BRCA1 mutation, you could consider a clinical trial using an HDAC inhibitor in combination with a PARP inhibitor and cisplatin,” Bhalla said.

Bhalla has received clinical research support from Novartis Oncology, which makes panobinostat, the HDAC inhibitor used in the studies.

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The mission of the 2012 CTRC-AACR San Antonio Breast Cancer Symposium is to produce a unique and comprehensive scientific meeting that encompasses the full spectrum of breast cancer research, facilitating the rapid translation of new knowledge into better care for patients with breast cancer. The Cancer Therapy & Research Center (CTRC) at The University of Texas Health Science Center at San Antonio, the American Association for Cancer Research (AACR) and Baylor College of Medicine are joint sponsors of the San Antonio Breast Cancer Symposium. This collaboration utilizes the clinical strengths of the CTRC and Baylor and the AACR’s scientific prestige in basic, translational and clinical cancer research to expedite the delivery of the latest scientific advances to the clinic. For more information about the symposium, please visit www.sabcs.org.
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Title: Treatment with Histone Deacetylase Inhibitors Creates .BRCAness. and Sensitizes Human Triple Negative Breast Cancer Cells to PARP Inhibitors and Cisplatin

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Body: DNA damage induces DNA damage response (DDR), which regulates cell cycle transit, DNA repair and apoptosis. During DDR, stability and activity of ATR and CHK1 is essential for the cell cycle arrest and subsequent DNA repair through homologous recombination (HR) in which BRCA1 protein is involved. We have previously reported that ATR, CHK1 and BRCA1 are hsp90 client proteins and treatment with hsp90 inhibitor sensitizes cancer cells to DNA damage. We have also previously shown that treatment with pan-HDAC inhibitors, including vorinostat (VS) and panobinostat (PS), induces hyperacetylation of hsp90, thereby inhibiting its chaperone function. In the present studies we determined that treatment with VS or PS induced hyper-acetylation and inhibition of chaperone function of nuclear hsp90, leading to proteasomal degradation and depletion of ATR, CHK1 and BRCA1. This led to inhibition of DDR and DNA repair following ionizing radiation (IR) through destabilization of ATR-CHK1 and BRCA1 proteins. Based on this, we hypothesized that treatment with VS or PS would create 'BRCAness' in breast cancer cells. Specific siRNA-mediated knockdown of HDAC3 but not of HDAC1 or HDAC2 also induced hyper-acetylation of the nuclear hsp90 and depletion of ATR and CHK1, indicating that among the class I HDACs, HDAC3 is the deacetylase for the nuclear hsp90. We next determined whether, by depleting DDR proteins and BRCA1 and inducing 'BRCAness', treatment with VS would sensitize the triple negative breast cancer (TNBC) SUM59PT, MB-231 and HCC1937 cells to the PARP inhibitor ABT888 (veliparib). Indeed, combined treatment with VS or PS with ABT888 (10 to 20 μM) for 48 hours synergistically induced apoptosis (Combination indices by isobologram analysis being < 1.0) of TNBC cells with (HCC1937) or without BRCA1 mutation (MB-231 and SUM159PT cells). As compared to treatment with each agent alone, co-treatment with VS and ABT888 also induced significantly more DNA strand breaks, as demonstrated by higher γ-H2AX levels. Combined treatment also induced markedly greater tail moment, as determined by the Comet assay that evaluates the SYBR green-stained DNA tails by fluorescent microscopy. Co-treatment with VS and ABT888 also induced more BH3 domain-only pro-death protein BIM, while knock-down of BIM by shRNA significantly reduced the apoptosis induced by co-treatment with VS and ABT888. It is noteworthy that treatment with VS also sensitized the TNBC cells to cisplatin (2.0 to 10 μM)-induced apoptosis. Moreover, co-treatment with VS and ciplatin synergistically induced apoptosis of TNBC cells (CIs < 1.0). These findings indicate that treatment with pan-HDAC inhibitors VS or PS creates BRCAness, and in combination with a PARP inhibitor or cisplatin synergistically induces apoptosis in human TNBC cells. These findings support a
compelling rationale to confirm whether the combination of VS or PS with ABT888 and cisplatin would be a highly active treatment in the in vivo models of human TNBC cells, irrespective of their expression of mutant BRCA1.