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Panobinostat sensitizes cyclin E high, homologous recombination-proficient ovarian cancer to olaparib

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HIGHLIGHTS

- Panobinostat downregulates DNA damage repair genes and homologous recombination (HR) efficiency in cyclin E-overexpressing, HR-proficient ovarian cancer cells.
- Panobinostat synergizes with the poly (ADP-ribose) polymerase inhibitor olaparib to inhibit growth and viability in HR-proficient ovarian cancer cells.
- Panobinostat combined with olaparib promotes DNA damage and apoptosis in HR-proficient ovarian cancer cells in vitro and in vivo.

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ABSTRACT

Objective. Homologous recombination (HR) proficient ovarian cancers, including CCNE1 (cyclin E)-amplified tumors, are resistant to poly (ADP-ribose) polymerase inhibitors (PARPi). Histone deacetylase inhibitors (HDACi) are effective in overcoming tumor resistance to DNA damaging drugs. Our goal was to determine whether panobinostat, a newly FDA-approved HDACi, can sensitize cyclin E, HR-proficient ovarian cancer cells to the PARPi olaparib.

Methods. Expression levels of CCNE1 (cyclin E), BRCA1, RAD51 and E2F1 in ovarian tumors and cell lines were extracted from The Cancer Genome Atlas (TCGA) and Broad-Novartis Cancer Cell Line Encyclopedia (CCLE). In HR-proficient ovarian cancer cell line models (OVCAR-3, OVCAR-4, SKOV-3, and UWB1.289 + BRCA1 wild-type), cell growth and viability were assessed by sulforhodamine B and xenograft assays. DNA damage and repair (pH2AX and RAD51 co-localization and DRGFP reporter activity) and apoptosis (cleaved PARP and cleaved caspase-3) were assessed by immunofluorescence and Western blot assays.

Results. TCGA and CCLE data revealed positive correlations (Spearman) between cyclin E E2F1, and E2F1 gene targets related to DNA repair (BRCA1 and RAD51). Panobinostat downregulated cyclin E and HR repair pathway genes, and reduced HR efficiency in cyclin E-amplified OVCAR-3 cells. Further, panobinostat synergized with olaparib in reducing cell growth and viability in HR-proficient cells. Similar co-operative effects were observed in xenografts, and on pharmacodynamic markers of HR repair, DNA damage and apoptosis.

Conclusions. These results provide preclinical rationale for using HDACi to reduce HR in cyclin E-overexpressing and other types of HR-proficient ovarian cancer as a means of enhancing PARPi activity.

1. Introduction

Ovarian cancer is the deadliest gynecologic malignancy and the fifth leading cause of cancer death among women in the US [1]. High-grade serous ovarian cancer is the most common and fatal subtype. Treatment options are limited for women with recurrent ovarian cancer, particularly those with chemoresistant disease. Poly ADP ribose polymerase inhibitors (PARPi) are promising new drugs that have shown clear advantage in BRCA-mutated ovarian cancer [2,3] and in tumors with deficiencies in other homologous recombination (HR) DNA repair genes [4,5]. By inhibiting single-strand break repair machinery, PARPi cause synthetic lethality in HR-deficient cells. Despite some activity, PARPi are far less effective in the 50% of high-grade serous tumors that retain HR proficiency [2,6–8]. Developing strategies to expand the use of

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PARPi and other DNA damaging drugs to HR-proficient tumors is a critical clinical need.

Mutually exclusive to HR-deficient phenotypes are ~20% of high-grade serous HR-proficient ovarian tumors with *CCNE1* (cyclin E) overexpression by amplification or upregulation. Ovarian tumors with cyclin E amplifications have high levels of HR proficiency, are relatively resistant to DNA damaging drugs, and have poor clinical outcomes in most studies [6,9–14]. Amplified cyclin E is a known oncogenic driver of unchecked replication, which causes replicative stress and enormous genomic instability [6,10–12,15]. To escape sensors that detect and destroy cells with DNA damage, cyclin E amplified ovarian tumors depend on robust mechanisms to promote HR DNA repair. The major partner kinase of cyclin E, CDK2, phosphorylates Rb and displaces it from a complex with E2F1, which promotes E2F1-dependent transcription of BRCA1 and other DNA damage repair genes [6,15]. To date, no drugs directly target cyclin E. Further, indirect targeting of cyclin E with currently available CDK2 inhibitors is limited by the development of chemoresistance that occurs in part through E2F1 upregulation [16,17]. An alternate and emerging paradigm is to convert HR-proficient tumors to HR-deficient phenotypes by using epigenetic drugs [4,9].

Our group has generated multiple lines of evidence demonstrating that histone deacetylase inhibitors (HDACi) improve responses to DNA damaging drugs in ovarian cancer cells [4,18,19]. We have shown that vorinostat downregulates HR gene expression in HR-proficient ovarian cancer cells and sensitizes chemoresistant cells to the PARPi olaparib both in vitro and in vivo [4]. The newest FDA-approved HDACi, panobinostat, is structurally similar to vorinostat but is more potent, with superior pharmacokinetics [20]. Here, we show that panobinostat treatment downregulated cyclin E, E2F1, and HR pathway genes. Consistent with this finding, established markers of HR repair efficiency were reduced in cyclin E amplified HR-proficient ovarian cancer cells with panobinostat treatment alone and in combination with olaparib. Panobinostat synergized with the cytotoxic effects of olaparib in HR-proficient ovarian cancer cells in vitro and in vivo. Further, panobinostat combined with olaparib induced robust and prolonged activation of pH2AX, indicative of deficient DNA damage repair. Our results indicate that targeting HR pathways with HDACi is a promising strategy for improving PARPi efficacy in cyclin E high and other types of HR-proficient ovarian cancer.

2. Materials and methods

2.1. Cell culture and compounds

The epithelial ovarian cancer cell lines SKOV-3, OVCAR-3, UWB1.289 + BRCA1 wild-type (BRCA1 WT) and UWB1.289 BRCA1 null (BRCA1 Null) cell lines (American Type Culture Collection, Manassas, VA), and OVCAR-4 (National Cancer Institute, Bethesda, MD) were maintained in culture as previously described [4,19,21–23]. Cell lines were authenticated by the Vanderbilt VANTAGE Genomics Core using the GenePrint 10 kit (Promega, Madison, WI). All cell lines used tested negative for mycoplasma. Panobinostat was synthesized at the Broad Institute (Cambridge, MA) and AZD-2281 (olaparib) provided by Astra Zeneca Pharmaceuticals (Wilmington, DE). For in vitro experiments, combination panobinostat/olaparib treatment was as follows unless specifically noted: cells were pre-treated for 24 h with vehicle (0.01% DMSO), followed by 24–72 h treatment with vehicle (*Con*) or 10 μM olaparib (*Ola*); cells were also pre-treated for 24 h with panobinostat (25 nM), followed by 24-72 h treatment with 25 nM panobinostat (*Pano*) or 25 nM panobinostat plus 10 μM olaparib (*Pano + Ola*). Clinically achievable doses of olaparib (10 μM) were used in these experiments [24]. Separately, cells were treated with cisplatin (Sigma Chemical Co, St Louis, MO) and/or panobinostat.

2.2. Cell proliferation, cytotoxicity and clonogenic assays

Sulforhodamine B (SRB) assays were used to measure cell growth and viability as described [4]. The interaction between fixed ratios of panobinostat and olaparib was measured with the Combination Index (CI) method [25]. Clonogenic assays were performed and quantified as described [26].

2.3. Immunofluorescence

Following drug treatment and/or transient transfection with the HR reporter plasmid pDRGFP and endonuclease encoding pCBASce1 (I-Sce1) (both gifts from Maria Jasin; Addgene plasmids #26475 and #26477, respectively) [27,28] plasmids using Lipofectamine 2000 according to manufacturer's instructions (Invitrogen Corp., Carlsbad, CA), ovarian cancer cells were fixed, permeabilized and visualized for GFP expression, or stained with mouse monoclonal anti-phospho(p)-H2AX (Ser139) (pH2AX) (Millipore, Billerica, MA), rabbit polyclonal anti-RAD51 (Millipore), and rabbit polyclonal anti-cleaved caspase-3 (Cell Signaling Technology, Beverly, MA) as described [19]. Secondary antibodies, and image acquisition and analysis was as described [19].

2.4. Western blotting

Whole cell protein isolation from cultured cells and harvested tumors, hydrochloric acid extraction of histones, western blotting and signal detection were as described [19]. Antibodies used were rabbit polyclonal anti-cyclin E (Abcam, Cambridge, MA), rabbit polyclonal anti-E2F1 (DBA Acris Antibodies, Inc., Rockville, MD), rabbit polyclonal anti-RAD51 (Millipore), mouse monoclonal anti-BRCA1 (Millipore), rabbit polyclonal anti-PARP (Cell Signaling Technology), mouse monoclonal anti-PCNA (Santa Cruz Biotechnology, Inc., Dallas, TX), rabbit polyclonal anti-cleaved caspase-3 (Cell Signaling Technology), and mouse monoclonal anti-pH2AX (Ser139) (Millipore). Loading controls were mouse monoclonal anti-histone H3 (Millipore) and mouse monoclonal β-actin (Sigma) for histones and total proteins, respectively.

2.5. Animals

Experiments performed were approved by the Vanderbilt University Institutional Animal Use and Care Committee, and female athymic Nude-*Foxn1*^{nu} mice (Harlan Laboratories, Indianapolis, IN) were maintained in accordance to guidelines of the American Association of Laboratory Animal Care. 5×10^6 SKOV-3 tumor cells in a 200 μL of mixture of PBS and Matrigel (1:1 v/v) (BD Biosciences, San Jose, CA) were injected subcutaneously into the right flank. After the tumors reached approximately 200mm^3, mice were randomized into one of 4 treatment groups ($n = 10$). Two treatment groups received panobinostat pre-treatment over one week (2.5 mg/kg five times weekly IP) and two received vehicle only (0.01% DMSO in PBS five times weekly IP). Following pre-treatment, mice were treated for 3 weeks with: *Vehicle* (vehicle pre-treatment & 0.01% DMSO five times weekly IP and PO); *Panobinostat* (panobinostat pre-treatment & panobinostat 2.5 mg/kg five times weekly IP, 0.01% DMSO five times weekly PO); *Olaparib* (vehicle pre-treatment & olaparib 100 mg/kg five times weekly PO & 0.01% DMSO five times weekly IP); and the *Panobinostat*/*Olaparib* combination (panobinostat pre-treatment & panobinostat 2.5 mg/kg five times weekly IP, olaparib 100 mg/kg five times weekly PO). Animals were examined biweekly for the effects of tumor burden and tumor growth, and tumor measurements were performed weekly. Weekly tumor volume measurements were calculated from caliper measurements of the smallest (SD) and largest diameter (LD) volume $= [LD \times SD^2] \times \pi/6$ [4]. 24 h after the final dose of drug, mice were euthanized according to protocol and tumors excised and weighed.