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PARP inhibition potentiates the cytotoxic activity of C-1305, a selective inhibitor of topoisomerase II, in human BRCA1-positive breast cancer cells☆

Józefa Węsierska-Gądek [a,*], Nora Zulehner [a], Franziska Ferk [b], Andrzej Składanowski [c], Oxana Komina [a], Margarita Maurer [a]

[a] Cell Cycle Regulation Group, Dept. of Medicine I, Div.: Institute of Cancer Research, Comprehensive Cancer Center, Medical University of Vienna, Borschkegasse 8a, A-1090 Vienna, Austria
[b] Umweltoxikologie, Dept. of Medicine I, Div.: Institute of Cancer Research, Comprehensive Cancer Center, Medical University of Vienna, Borschkegasse 8a, 1090 Vienna, Austria
[c] Dept. of Pharmaceutical Technology and Biochemistry, Gdańsk University of Technology, Narutowicza 11/12, 80-952 Gdańsk, Poland

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ABSTRACT

Two cellular proteins encoded by the breast and ovarian cancer type 1 susceptibility (*BRCA1* and *BRCA2*) tumor suppressor genes are essential for DNA integrity and the maintenance of genomic stability. Approximately 5–10% of breast and ovarian cancers result from inherited alterations or mutations in these genes.

Remarkably, *BRCA1/BRCA2*-deficient cells are hypersensitive to selective inhibition of poly(ADP-ribose)polymerase 1 (PARP-1), whose primary functions are related to DNA base excision repair; PARP-1 inhibition significantly potentiates the cytotoxicity of various anti-cancer drugs, including inhibitors of topoisomerase I and II.

In the present study, we examined the anti-proliferative and pro-apoptotic effects of C-1305, a selective inhibitor of topoisomerase II, on human breast cancer cell lines with different *BRCA1* and *p53* statuses. BRCA1-competent breast cancer cell lines exhibited different responses to topoisomerase II inhibition. BT-20 cells that express high levels of BRCA1 levels were most resistant to C-1305 than other tested cells. Surprisingly, pharmacological interference with PARP-1 activity strongly inhibited their proliferation and potentiated the efficacy of C-1305 treatment. In contrast, PARP-1 inhibition only weakly affected the proliferation of BRCA1-deficient SKBr-3 cells and was not synergistic with the effects of C-1305. Further experiments revealed that the inhibition of PARP-1 in BT-20 cells caused the accumulation of DNA strand breaks and induced caspase-3 dependent apoptosis. These results seem to indicate that PARP-1 inhibition can potentiate the cytotoxicity of anti-cancer drugs in cancer cells with functional BRCA1 and suggest that mutations in other DNA repair proteins may render cancer cells more sensitive to interference with PARP-1 activity.

1. Introduction

Breast cancer is the most common malignancy in women and the second most prevalent cause of cancer-linked death in women (for reviews, see [1,2]). It is a conglomerate of diseases of the breast and arises from the misregulation of several essential cellular pathways (notably, those controlling cellular metabolism, cell cycle progression, cell proliferation, and apoptosis), with different variants having different signature characteristics and family histories (for reviews, see [3,4]). The identification of molecular signatures for different types of breast cancers over the last two decades has facilitated the development of targeted therapeutic strategies (for a review, see [5]).

Individuals with first-degree relatives having germline mutations in genes such as breast and ovarian cancer type 1 or 2 susceptibility (*BRCA1* or *BRCA2*) genes are subject to an increased risk of developing breast cancer [6]. Approximately 5–10% of breast and ovarian cancers result from inherited alterations or mutations in these genes. The BRCA1 and BRCA2 proteins are both involved in repairing double stranded DNA breaks by homologous recombination (HR), a process that is essential for DNA integrity and the maintenance of genomic stability [7–9].

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Abbreviations: BER, base excision repair; BRCA1, breast cancer type 1 susceptibility protein; HMC, Hoffman modulation contrast; HR, homologous recombination; NER, nucleotide excision repair; NHEJ, non-homologous end joining; MMP, mitochondrial membrane potential; PARP-1, poly(ADP-ribose)polymerase-1; PD, Petri dish; PVDF, polyvinylidene difluoride; TOPO, topoisomerase; WCL, whole cell lysate; WT, wild-type.

* Corresponding author. Tel.: +43 1 40160 57592; fax: +43 1 40 60 957592.
E-mail address: Jozefa.Gadek-Wesierski@meduniwien.ac.at (J. Węsierska-Gądek).

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In this context it is important to note that most conventional anti-cancer drugs such as alkylating agents, cross-linking agents, and topoisomerase inhibitors affect the integrity of DNA either directly or indirectly, resulting in the formation of single or double stranded breaks (for reviews, see: [10]). If these breaks are not repaired, the resulting persistent DNA damage can give rise to mutations and genomic instability that subsequently cause secondary cancers [11–13] or cell death [14], with the precise outcome depending on the overall extent of breakage. Interestingly, it has been found that DNA-repair deficient cancer cells (such as those with *BRCA 1/2* mutations) are more sensitive to inhibitors of poly(ADP-ribose)polymerase 1 (PARP-1), whose primary functions are related to DNA base excision repair (BER) [15–19]. Based on this observation, a new therapeutic approach termed "synthetic lethality" has been developed that relies on the conditional blockage of BER in DNA-repair deficient cancer cells [20]. Treatment with selective inhibitors of PARP-1 (a nuclear enzyme involved in the signaling of DNA damage and BER) in conjunction with radiation or cytotoxic anti-cancer agents such as topoisomerase (TOPO) type I or II inhibitors can induce severe genomic instability that leads to cell death. In recent years, the synergistic benefit of combining PARP-1 inhibition with anti-cancer drug treatment has been demonstrated in several pre-clinical models, and multiple PARP-1 inhibitors for use in treatments of this kind have been developed.

This paper describes an investigation into the sensitivity of breast cancer cells to C-1305, a selective inhibitor of TOPO II. A range of cells that differed in terms of the functional status of *BRCA1* and *p53* were considered. Different BRCA1-competent breast cancer cell lines exhibited different responses to C-1305. BT-20 cells expressing high levels of BRCA1 were most resistant to C-1305. However, pharmacological inhibition of PARP-1 activity strongly inhibited their proliferation and potentiated the efficacy of C-1305 treatment. In contrast, PARP-1 inhibition had only modest effects on the proliferation of BRCA-1-deficient SKBr-3 cells. These unexpected results indicate that interference with BER can potentiate the cytotoxicity of anti-cancer drugs in cancer cells with functional BRCA1 and suggest that mutations in other DNA repair proteins render cancer cells sensitive to inhibition of PARP-1 activity.

2. Material and methods

2.1. Drugs and chemicals

The triazoloacridone compound C-1305 used in this work was synthesized at the Department of Pharmaceutical Technology and Biochemistry (Gdańsk University of Technology) by Dr. Barbara Horowska. A stock solution of triazoloacridone (base-free) was prepared in 0.2% lactic acid. NU1025, an inhibitor of PARP-1 from AXON Medchem BV (Groningen, Netherlands) and camptothecin CPT), a quinoline alkaloid which inhibits topoisomerase I, from Calbiochem-Novabiochem Corporation (La Jolla, CA), were stored as a stock solution in DMSO. All drugs were stored at −20 °C until use.

2.2. Cells and treatment

Human primary breast cancer cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA). The following cell lines were used: human MCF-7, BT-20 [21], and SKBr-3 [22] breast carcinoma cells. MCF-7 cells were grown as a monolayer in phenol red-free Dulbecco's medium supplemented with 10% fetal calf serum (FCS) at 37 °C under an atmosphere containing 8% CO_2 [23]. SKBr-3 cells were cultivated in DMEM medium with 10% FCS, and BT-20 cells in RPMI with 10% FCS. Twenty-four hours after plating (at 60–70% confluence), the cells were treated with the triazoloacridone compound C-1305 at concentrations ranging from 1 to 10 μM, and with NU1025 at a final concentration of 100 or 200 μM. The two drugs were applied separately or simultaneously, for the periods of time indicated in Figs. 2–10.

2.3. Antibodies

The following specific primary antibodies were used to detect the relevant proteins: mouse monoclonal anti-p53 antibody DO-1 and rabbit polyclonal anti-H2AX antibody (from BioLegend, San Diego, CA), anti-BRCA-1 rabbit polyclonal antibody (from Upstate Cell Signaling Solutions, Lake Placid, NY), anti-ER-α rabbit polyclonal antibody (from Sigma–Aldrich, St. Louis, MO), anti-phospho-histone H2AX (Ser139) rabbit polyclonal antibody (from Cell Signaling Technology Inc., Danvers, MA), anti-DBC-1 mouse monoclonal antibody (from Abcam plc, Cambridge, UK), and mouse monoclonal anti-actin antibody (clone C4, ICN Biochemicals, Aurora, OH). Appropriate secondary antibodies linked to horseradish peroxidase (HRP) were obtained from R&D Systems (Minneapolis, MN).

2.4. Detection of chromatin changes in individual cells by fluorescence microscopy

Cells grown in 35 mm Petri dishes were treated with C-1305, NU1025 or a combination of the two for the indicated lengths of time and then washed three times in PBS. The washed cells were immediately fixed in 3.7% paraformaldehyde in PBS, then washed 4 times in PBS and stained with Hoechst 33258 dissolved in PBS at a final concentration of 1.5 μg/ml [24]. The stained cells were inspected under a fluorescence microscope (Eclipse TE300 inverted microscope, Nikon Corporation, Tokyo).

2.5. Determination of numbers of living cells

The numbers of viable human breast cancer cells and their sensitivities to the tested drugs at various concentrations were determined using CellTiter-Glo™ assays (Promega Corporation, Madison, WI). As described previously [25], the CellTiter-Glo™ luminescent cell viability assay measures luminescent signals, which are correlated with cellular ATP levels. Tests were performed at least in quadruplicate, and the cells' luminescence was measured using a Wallac 1420 Victor multilabel, multitask plate counter (Wallac Oy, Turku, Finland). Each data point represents the mean ± SD (bars) of replicates from at least three independent experiments (Figs. 2–4). The effects of the combined C-1305 and NU1025 treatments are shown in Fig. 4.

2.6. Quantitative analysis of the mitochondrial membrane potential by flow cytometry

Mitochondrial depolarization was monitored using the cationic carbocyanine dye JC-1 (Molecular Probes Inc., Eugene, OR) as previously described [26]. Control and drug-treated cells were harvested, washed and incubated with the dye at a final concentration of 10 μM for 5 min followed by extensive washings in PBS and immediate two-color analysis by flow cytometry. JC-1 accumulates in the mitochondria of intact cells if the mitochondrial membrane potential is within the normal range, forming fluorescent red aggregates known as J-aggregates that emit at 570 nm in response to excitation at 488 nm. Mitochondrial depolarization in apoptotic cells causes the aggregates to dissociate into green fluorescent monomers