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Inhibition of Rad51 sensitizes breast cancer cells with wild-type PTEN to olaparib

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ABSTRACT

PTEN is a tumor suppressor gene well characterized as a phosphatase. However, more evidences demonstrate PTEN functions in DNA repair independent of its phosphatase activity, which affects the efficacy of DNA damage anti-tumoral drugs in treating cancer cells with PTEN variations. Using BT549 breast cancer cells, we studied the roles of PTEN in DNA repair and in sensitization of breast cancer cells to olaparib, a poly(ADP-ribose) polymerase (PARP) inhibitor. Comet assay showed PTEN promoted DNA repair. PTEN-deficient BT549 cells are sensitive to olaparib, which shows the synthetic lethality between PTEN and PARP1. We expressed PTEN in BT549 cells and found PTEN-proficient BT549 cells resist to olaparib. Western blot showed that PTEN up-regulated Rad51 expression, suggesting PTEN promotes DNA repair through Rad51-dependnent homologous recombination. We used 5 μM olaparib or 5 μM RI-1, a Rad51 inhibitor, to treat PTEN-proficient BT549 cells respectively. The immunofluorescent analysis showed the combination of olaparib and RI-1 induced more than 4-fold of γH2AX foci than either of them. MTT assay showed 5 μM RI-1 did not change the survival of PTEN-proficient BT549 cells, however, this dose of RI-1 sensitized PTEN-proficient BT549 cells to olaparib. Consequently, these results demonstrate that inhibition of Rad51 can sensitize BT549 cells with wild type PTEN to olaparib, which would contribute to using PARP inhibitors in individual treatment of breast cancer patients with PTEN variations.

1. Introduction

Phosphatase and tensin homologue (*PTEN*) was identified as a tumor suppressor gene located on 10q23.3 in human genome [1]. It was well characterized to antagonize the activity of PI3 kinase (PI3 K) through its lipid phosphatase activity [2]. Recently, PTEN reportedly plays an essential role in repair of DNA damage, independent of its phosphatase activity [3–6]. The mechanism of PTEN in DNA repair is not well defined. Evidences show that PTEN is physically associated with replication protein A1 (RPA1) to protect DNA replication and induces transcription of Rad51 which leads to double-strand breaks (DSB) repair by homologous recombination (HR) [7–9]. Thus, PTEN is possibly involved in DNA repair through multiple targets, which was confirmed in sensitization of PTEN-deficient cancer cells to various DNA damage anti-tumoral drugs including iron radiation, cisplatin and poly (ADP-ribose) polymerase (PARP) inhibitors [7]. Dysregulation of PTEN is frequently observed in various types of cancers, thus it would be efficient to use DNA damage anti-tumoral drugs to treat PTEN-deficient cancer cells [10–14]. In this study, we analyzed the effect of PTEN in DNA repair, especially in Rad51-dependnent HR.

PARPs are enzymes that transfer ADP-ribose groups to target proteins and are involved in many nuclear and cytoplasmic processes, including DNA transcription, cell-cycle regulation, and DNA repair [15,16]. The N-terminal zinc finger domains enable PARPs to bind to DNA single strand breaks (SSB) that are repaired through the interaction of PARPs and other nuclear proteins [15]. When cells lose the activity of PARPs, accumulation of unrepaired SSBs will form DNA double strand breaks (DSB) which is highly toxic to cells. Therefore, inhibition of PARP becomes a promising therapy for cancer treatment, especially to cancer cells with BRCA mutants [17–19]. HR pathway is a precise DSB repair pathway, in which Rad51 is essential to promote strand invasion of

Abbreviations: PTEN, Phosphatase and tensin homologue; PARP, poly(ADP-ribose) polymerase; PI3K, PI3 kinase; RPA1, replication protein A1; DSB, double-strand breaks; HR, homologous recombination; SSB, single strand breaks; γH2AX, Phospho-histone H2AX; PMSF, phenylmethylsulfonylfluoride; IR, iron radiation; EB, ethidium bromide; DAPI, 4′,6-diamidino-2-phenylindole.

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homologous sequences. In this study, we analyzed the sensitization of breast cancer cells with PTEN variations to olaparib, a PARP inhibitor. We also investigated the sensitization of PTEN-proficient cancer cells to olalparib in combination with RI-1, a Rad51 inhibitor.

This study illustrates a synthetic lethality between PTEN and PARP1, and the effect of Rad51-dependent HR in sensitization of PTEN-proficient breast cancer cells to olaprib, which would contribute to individual therapy of breast cancer patients with PTEN variations.

2. Materials and methods

2.1. Antibodies and reagents

Phospho-histone H2AX (γH2AX) antibody was from Cell Signaling (U.S.A). Antibodies against PTEN, RAD51, ACTIN were purchased from Proteintech (U.S.A). Alexa fluor 488-conjugated secondary antibody was also from Proteintech (U.S.A). Olaparib and RI-1 were from Selleck (U.S.A).

2.2. Cell lines

Human breast cancer cell lines BT549 were from ATCC (U.S.A). PTEN cDNA was inserted in *pcDNA3.1-Flag* (Invitrogen, U.S.A) and sequenced (Sangon, China). pcDNA3.1-Flag-PTEN was stably transfected into BT549 to generate PTEN-WT cells.

2.3. Western blot

Protein was extracted utilizing ice-cold RIPA buffer (0.05 M Tris-HCl, pH7.4, 0.15 M Nacl, 0.25% deoxycholic acid, 1% NP-40, 1 mM EDTA) supplemented with 1 mM phenylmethylsulfonylfluoride (PMSF), 1× protease inhibitor (Selleck) and 1× phosphatase inhibitor (Selleck). Following determined by Fluorometer Qubit 2.0 (Invitrogen, U.S.A), lysates were resolved by 10% SDS-PAGE and transferred to PVDF membrane. Proteins were detected using various antibodies.

2.4. Comet assay

Cells were treated with 10 Gy ionizing radiation (IR) and embed in low melting argarose and immersed in ice-cold lysis buffer (2.5 M Nacl, 100 mM EDTA, 10 mM Tris Base, 1% sodium sarcosinate, 1% Triton X-100, 10% DMSO) for 90 mins. Then, electrophoresis was performed in alkaline buffer (1 mM EDTA, 300 mM NaOH, Tris-Hcl Ph7.5) and samples were stained using 2.5 ug/ml ethidium bromide (EB) for 3 mins. Slides were detected by microscopy (Olympus IX71, Japan)

2.5. Immunofluorescence

Cells were exposed in 10 Gy IR and fixed by 4% formaldehyde for 15 mins. Following blocked by 5% normal goat serum for 1 h, samples were incubated with Phospho-histone H2AX antibody at 4 °C overnight. After rinsed using PBS, samples were incubated with Alexa fluor 488-conjugated secondary antibody and were detected by microscopy. 4′,6-diamidino-2-phenylindole (DAPI) was used to stain nuclei.

2.6. MTT assay

Cell viability was detected by MTT assay. It was performed as previously reported [20]. Briefly, cells were seeded in 96-well microtiter plates and treated with drugs and DMSO as controls. Results were quantified at 492 nm.

2.7. Statistical analysis

Immunofluorescence and comet assay were analyzed by Quantity One software. Statistic analysis was performed by SPSS software.

3. Results

3.1. PTEN promotes DNA damage repair

We stably expressed PTEN in PTEN-deficient BT549 cells, which was measured by western blot (Fig. 1A). Comet assay was used to measure DNA damage repair in PTEN-/- and PTEN-WT cells. At 8 h after iron radiation (IR) stimulation, comet assay showed PTEN promoted about 4-fold DNA repair (Fig. 1B).

3.2. PTEN-WT cells resist to olaparib

MTT assay was used to measure the growth of PTEN-/- and PTEN-WT cells, in which PTEN-/- and PTEN-WT cells showed similar growth curve (Fig. 2A). After treatment of olaparib for 48 h, MTT assay showed PTEN-/- cells are sensitive to olaparib, however, the expressed PTEN helps BT549 cells resist to olaparib. This result shows that PTEN promotes DNA repair and helps cancer cells to resist to PARP inhibitors.

3.3. PTEN up-regulates rad51 expression

HR is a precise DSB repair pathway, which can restore the DNA damage induced by anti-tumoral drugs. Rad51 is an essential factor in HR, thus we next analyzed the effect of PTEN to Rad51 expression levels. Western blot showed PTEN obviously up-regulated Rad51 expression in BT549 cells stimulated by IR

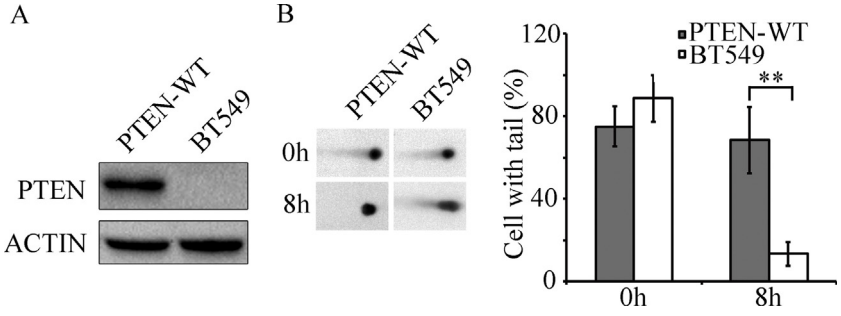

Fig. 1. PTEN promotes DNA repair. A. The expression of PTEN in BT549 (PTEN-WT) harboring wild type PTEN and in BT549 transfected with empty plasmid (BT549) was detected using western blot. B. Comet assay displayed DNA repair in BT549 and BT549 (PTEN-WT) at 0 and 8 h after treating with 10 Gy irradiation (IR).