이학석사 학위논문

# *BRCA2* 돌연변이 췌장암에서 PARP 억제로 인한 Oxaliplatin의 항종양효과 규명 연구

Oxaliplatin Exerts a Synergistic Antitumor Effect with a PARP Inhibitor in *BRCA2*-Mutant Pancreatic Cancer

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2022 년 2 월

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## Abstract

**Purpose:** Cisplatin and gemcitabine with or without veliparib, a poly-(ADP-ribose) polymerase (PARP) inhibitor, significantly inhibit *BRCA2*-mutant pancreatic cancer. However, the relative efficacy of these treatment regimens compared to that of FOLFIRINOX, a new chemotherapeutic regimen for treating pancreatic cancer, is unknown. BRCAness-associated mutations may be promising prognostic markers for oxaliplatin-based regimens such as FOLFIRINOX. In this study, we evaluated the combined effects of a PARP inhibitor, olaparib, and oxaliplatin for the treatment of *BRCA2*-mutant pancreatic cancer, using a *BRCA2*-deficient pancreatic cancer cell line (Capan-1).

**Materials and Methods:** Olaparib, a PARP inhibitor, has been evaluated in the *BRCA2*-deficient pancreatic cancer cell line (Capan-1) and the *BRCA2* wild-type pancreatic cancer cell line (Miapaca-1). The antitumor effects of Olaparib and Oxaliplatin in Capan-1 were further analyzed by cell viability, combination index, western blotting, Immunofluorescence stating, flow cytometry, apoptosis assays and *in vivo* study.

**Results:** Capan-1 cells were more sensitive to olaparib than the *BRCA2* wild-type pancreatic cancer cell line (MIA PaCa-2). Compared to cisplatin, oxaliplatin showed better synergistic effects in Capan-1 cells when combined with olaparib. Olaparib showed synergistic effects with oxaliplatin in Capan-1 cells alone. The combination of oxaliplatin and olaparib inhibited molecules involved in the DNA repair pathway and

increased the level of apoptosis and led to the accumulation of DNA damage markers. Additionally, this combination confirmed significant *in vivo* antitumor activity in a Capan-1 cell xenograft model compared to monotherapy.

**Conclusion:** The olaparib, a PARP1 inhibitor, combined with oxaliplatin exhibited significant anti-tumor effects in pancreatic cells with *BRCA2* mutations. Unlike cisplatin, oxaliplatin induced BRCAness by inhibiting CDK1 function, allowing the PARP inhibitor to function effectively in Capan-1 cells. Further studies are required to assess the combination of PARP inhibitor with DDR inhibitors for anti-cancer efficacy in Homologous recombination DNA repair proficient BRCA wild-type pancreatic cancer cells.

**Keywords**: pancreatic cancer; *BRCA2*; PARP inhibitor; olaparib; oxaliplatin; DNA damage; synthetic lethality

## Introduction

DNA double-strand breaks (DSBs), one of the most fatal error of DNA lesions, disrupt the sugar-phosphate DNA backbone, thereby damaging the structural stability of chromosomes [11]. DSBs are highly genotoxic and have a high tendency to cause mutations in the genome, leading to cancer [12]. Cells with genomic instability caused by DNA damage are detected and repaired by the DNA damage repair (DDR) pathway [12]. A dysfunctional DDR pathway may lead to cell cycle arrest and apoptosis. Hence, efficient repair processes are important for cell survival and inhibition of tumorigenesis through the maintenance of cell genomic stability [12-14].

DSBs are repaired by non-homologous end-joining (NHEJ) and homologous recombination (HR) pathways [15]. The NHEJ pathway is a relatively simple DSB repair pathway. Ku70 and Ku80 bind to free DNA ends, recruit DNA-dependent protein kinases (DNA-PKcs), and phosphorylate downstream targets. This activates the DNA damage response and initiates the NHEJ pathway [16]. The HR pathway is an error-free repair mechanism that uses genetic information contained in homologous sequences [18]. The MRE11-RAD50-NBS1 (MRN) complex detects DSBs and initiates a DNA damage response by activating the Ataxia–telangiectasia mutated (ATM) kinase. The activation of CtIP-mediated nuclease leads to the formation of single-stranded DNA (ssDNA) from 5′ to 3′ DNA ends. The exposed ssDNA is coated with DNA replication protein A (RPA) and activates Ataxia-telangiectasia and RAD3-related protein (ATR)

response to promote HR repair. Then, RPA is replaced with RAD51 by BRCA2 to form filaments. DSBs are then repaired by performing homology sequence search, strand invasion, and final recombination [17,18]. Therefore, deficiency of HR repair molecules is essential in cancer, and hence they may be targets for therapy. In particular, the most common target gene is *BRCA1/2*, and there have been many studies on this gene in breast and ovarian cancer [7].

BRCA protein is a tumor suppressor involved in homologous DNA repair mechanism in the double-stranded DDR (dsDDR) pathway. BRCA plays an important role in maintaining genome integrity, and loss of BRCA function causes genome instability, thereby promoting cancer [8]. BRCA1 plays a role in DNA damage signaling and cell cycle checkpoint regulation. In contrast, BRCA2 regulates activation of the recombinase RAD51, and is directly involved in the HR pathway that functions to repair DSBs [9]. In particular, cancers with BRCA mutations undergo inefficient DNA repair and thus are sensitive to platinum-based anti-cancer drugs [35]. *BRCA1/2* mutations are observed in breast and ovarian cancers, as well as in melanoma, prostate cancer, and pancreatic cancer. Synthetic lethality strategies that target such *BRCA1/2* mutations are promising therapeutic methods [38]. Synthetic lethality occurs when two genetic lesions that are not individually lethal are combined and thus made lethal [31]. Treatment using a synthetic lethality approach can selectively target genetically modified cancer cells. Defects in *BRCA1/2* genes required for HR repair become synthetically lethal when poly (ADP-ribose) polymerase (PARP) is inhibited.

Among the synthetic lethality partners of *BRCA1/2* mutations, PARP is an

enzyme that catalyzes ADP ribosylation of various cellular factors [31] and is a DNA nick sensor that is involved in DNA repair, maintenance of genomic integrity, cell apoptosis, and cell survival. In particular, PARP uses NAD, recruits various DNA repair effectors such as the scaffold protein XRCC1 to lesion sites [7], and induces base excision repair (BER) for single-stranded DNA break repair (SSBR). PARP1 is activated through PARylation in the cell nucleus [24]. PARP inhibitors bind to the catalytic site of PARP1 at the lesion site and trap PARP1. This prevents the inactivation of PARP1, causing its dysfunction. Thus, the PARP1/DNA nucleoprotein complex causes the accumulation of unrepaired SSBs and delays the progression of the replication fork, leading to highly cytotoxic DSBs [39].

When DNA is damaged in normal cells, lack of PARP activation due to the PARP inhibitor leads to the accumulation of DNA damage and suppresses the DNA replication fork at the damaged site. As a result, SSBs are converted to DSBs and get accumulated. However, the HR repair pathway is initiated to repair DSBs [30]. On the other hand, cells with *BRCA1/2* mutations (deficiency) cannot prevent the accumulation of DNA damage due to the lack of HR repair. Thus, PARP inhibition induces PARP trapping, which causes replication stress and genomic instability. This leads to loss of viability in HR-deficient cells [38]. Therefore, cells with *BRCA1/2* mutations affected by PARP inhibitors are killed by synthetic lethality, and cells lacking BRCA1/2 proteins show hypersensitivity to PARP inhibitors [25,26]. Olaparib, a PARP inhibitor, is an FDA approved drug for the treatment of breast and ovarian cancer with BRCA mutations [27, 28, 29]. The effects of PARP inhibitors in BRCA mutant ovarian and

breast cancers, as well as prostate, pancreatic, and non-small cell lung cancer (NSCLC) have been shown in many clinical trials conducted after 2009 [32].

In Korea, pancreatic cancer is the leading cause of cancer deaths among those in among those in the top 10 cancers, with the lowest 5-year overall survival rate of 12.6% [1]. Surgical treatment is the only available treatment in the early stages of the disease; however, only 15% of pancreatic cancer patients can undergo resection, and the disease recurs within 5 years in 75% of patients [2]. Other treatment options include standard treatment using FOLFIRINOX (fluorouracil, oxaliplatin, leucovorin, and irinotecan) or gemcitabine with nab-paclitaxel for advanced-stage pancreatic cancer. Currently, pancreatic cancer patients with *BRCA1/2* mutations are likely to benefit from targeted therapy [4].

In this study, we evaluated the effects and mechanism of combined therapy using a PARP inhibitor (olaparib) and oxaliplatin in *BRCA2*-deficient pancreatic cancer cells.

## Materials and Methods

### Cell lines and culture

The human pancreatic cancer cell line Capan-1 was obtained from Korea Research Institute of Bioscience and Biotechnology, KRIBB (Korea). The human pancreatic cancer cell line MIA PaCa-2 was obtained from Asan Preclinical Evaluation center for cancer therapeutiX, APEX (Korea). The cells were cultured in RPMI-1640 medium (Gibco) supplemented with 10% fetal bovine serum (WELGENE) and 1% penicillin/streptomycin (Gibco). All cells were maintained in an incubator at 37 °C with 5% $CO_2$.

### Cell viability assay and $IC_{50}$ assay

The cells were seeded in triplicate at a density of $1 \times 10^3$ cells in 50 μL per well in 96-well cell culture plates and incubated for 24 h in a 37 °C incubator with 5% $CO_2$. Olaparib (Selectchem), cisplatin and oxaliplatin (SIGMA-ALDRICH) were diluted to 2x of the final concentration, and 50 μL of each drug solution was added to the cells. Cisplatin, oxaliplatin, and olaparib were used at concentrations of 1.56, 3.12, 6.25, 12.5, 25, 50, and 100 μM for 24, 48, 72, 96, and 120 h. After treatment of cells with the drugs, 20 μL of CellTiter-Glo® Luminescent Cell Viability Assay solution (Promega) was added into each well of the 96-well plate. The solution in 96-well plates was mixed on a

shaker for 2 min and then incubated for 10 min at room temperature. Then, 100 μL of the solution was transferred to white 96-well plates. The luminescence was read using PerkinElmer VICTOR X2 multilabel plate reader. The results are represented as the mean values from three independent experiments performed in triplicate. The half-maximal inhibitory concentration ($IC_{50}$) values were calculated using dose-response curves and GraphPad Prism software v5.01 (GraphPad software).

**Analysis of Synergistic Effects**

The Capan-1 and MIA PaCa-2 cells ($1 \times 10^3$ cells in 50 μL per well) were seeded in 96-well plates and incubated for 24 h at 37 °C. After 24 h, the cells were exposed to olaparib for 6 h before treatment with oxaliplatin or cisplatin. Combination effects were analyzed using a constant drug combination ratio (2x) at the $IC_{50}$ concentration of a single drug. After 48 h, cell viability was assessed using the CellTiter-Glo® Luminescent Cell Viability Assay kit (Promega). For cell viability data analysis, the number of cells was normalized to the negative control values (vehicle control). The potential synergism between olaparib and oxaliplatin or cisplatin was assessed by calculating the combination index (CI) values based on the Chou–Talalay method (CalcuSyn software, Biosoft) [51]. CI values below 0.9 indicate synergy.

**Western blotting**

The proteins, quantified by BCA assay (Thermo Scientific), were separated on NuPAGE™ 4-12% Bis-Tris Protein Gels (Invitrogen) by SDS-PAGE in MES SDS Running Buffer (Invitrogen). The proteins, separated on the gel, were subsequently transferred onto a polyvinylidene difluoride membrane (Invitrogen) in transfer buffer (Novex) and blocked with 5% skim milk or BSA in Tris-buffered saline containing Tween-20 (TBST) (iNtRON Biotechnology) for 1 h at room temperature. The membranes were incubated with primary antibodies overnight at 4 °C followed by incubation with horseradish peroxidase-conjugated secondary antibodies (Cell signaling) for 2 h at room temperature. The membranes were washed with TBST for 15 min each, three times. The enhanced signals by SuperSignal ELISA Femto Stable Peroxide Buffer (Thermo Scientific) were detected using a Chemiluminescence Imaging System (ATTO). The antibodies used are as follows; PARP (Cell Signaling, #9532), phospho-histone H2A.X ser139 (Cell Signaling, #80312), phospho-BRCA1 ser1524 (Cell Signaling, #9009), cdc2 (POH1) (Cell Signaling, #9116), XRCC1 (Cell Signaling, #2735), 53BP1 (Cell Signaling, #4937), phoshpo-cdc2 Tyr15 (Cell Signaling, #9111), β-actin (SIGMA-ALDRICH, A5441), BRCA1 (Santa Cruz Biotechnology, sc-6954), XRCC4 (Santa Cruz Biotechnology, sc-271087), RPA70 (Abcam, ab79398), PAR (Trevigen, 4335-MC-100).

**Immunofluorescence staining**

Capan-1 cells ($2 \times 10^4$ cells in 200 μL per well) were seeded on glass coverslips (Nunc) in eight-well plates and incubated for 24 h at 37 °C. After 24 h, the cells were exposed to olaparib for 6 h before treatment with oxaliplatin. Subsequently, the cells were fixed with 4% paraformaldehyde at room temperature for 10 min. After fixation, the cells were permeabilized with 0.1% Triton X 100/PBS for 10 min. Then, the cells were washed three times with PBS and blocked with 1% skim milk in 0.1% Tween20/PBS containing 22.52 mg/mL glycine for 30 min at room temperature. The cells were incubated with primary antibodies overnight at 4 °C. The cells were then washed three times with 0.1% Tween20/PBS and incubated with the secondary antibody (1:1000 dilution; Alexa 488-conjugated antibody, Invitrogen) at 37 °C for 1 h. Nuclei were stained with 4',6-diamidino-2-phenylindole (Invitrogen). The slides were rinsed with distilled water (D.W.) and mounted using Vecta mount solution (Vector Laboratories). The specimens were viewed using a laser scanning confocal microscope (Carl Zeiss; LSM880). The antibodies used are as follows; phospho-histone H2A.X ser139 antibody (1:200 dilution; Cell Signaling), 53BP1 (1:100 dilution; Cell Signaling).

**Flow cytometry analysis**

For evaluating apoptosis, Capan-1 cells were treated with olaparib or oxaliplatin for 48 h. The cells were harvested at a density of $1 \times 10^6$ cells/mL and rinsed with PBS, followed by centrifugation at 300 ×*g* for 10 min at 4 °C. The cells were

washed twice with binding buffer (Miltenyi Biotec) and resuspended in 100 μL of binding buffer, followed by staining with 10 μL of Annexin V-FITC (Miltenyi Biotec) for 15 min in the dark at room temperature. The cells were centrifuged with binding buffer at 300 ×*g* at 4 °C for 10 min. Subsequently, the cells were resuspended in 500 μL of binding buffer and incubated with 5 μL of Propidium Iodide (Miltenyi Biotec) for further 15 min at room temperature in the dark. Finally, the apoptotic cells were analyzed using FACS Canto II flow cytometer (BD Biosciences) and FlowJo V10 software (FlowJo).

**Xenografts**

Capan-1 cells ($5 \times 10^6$) were subcutaneously injected into the right flank of 5 week-old BALB/c female nude mice (Central Lab. Animal Inc., Korea). When the tumors reached approximately 90 to 100 $mm^3$, the animals were randomly divided into vehicle control, oxaliplatin alone, olaparib alone and olaparib-oxaliplatin combination group of 5 mice each. Oxaliplatin (5 mg/kg) was diluted in D.W. and administered intraperitoneally twice a week for 4 weeks. Olaparib (75 mg/kg) was diluted with 3% DMSO/30% PEG-300/dd$H_2O$ buffer and administered orally 5 days a week for 4 weeks. The body weight and tumor volume were measured twice a week. Tumor volume was calculated according to the formula (length × width ×width) / 2. After completing the last administration, all mice were euthanized and the tumors were harvested for further analysis. All experiments were approved by the Institutional Animal Care and Use

Committee of the Asan Institute for Life Science.

**Statistical Analysis**

Data were analyzed by two-tailed Student's *t*-test using GraphPad Prism software v5.01 (GraphPad software). Results with $P < 0.05$ were considered as statistically significant.

## Results

### Capan-1 cells carry loss-of-function mutation in *BRCA2*

*BRCA1/2* mutations in pancreatic cancer cell lines were assessed using the Cancer Cell Line Encyclopedia (CCLE) database. Among various pancreatic cancer cell lines, only Capan-1 cells showed a frameshift mutation (6174delT) causing truncation of the *BRCA2* protein c-terminal (Table 1).

**Table 1. Status of *BRCA2* mutations in pancreatic cancer cell lines.**

| | Capan-1 | MIA PaCa-2 | BxPC-3 | AsPC-1 | Capan-2 |
|---|---|---|---|---|---|
| BRCA1 | wt | wt | wt | wt | wt |
| *BRCA2* | mt (6174delT) | wt | wt | wt | wt |

*BRCA1/2* loss of function mutations were not confirmed in other pancreatic cancer cell lines except Capan-1 in the CCLE (Cancer Cell Line Encyclopedia) database. Abbreviations: wt, wild type; mt, mutant type.

**Capan-1 cells are resistant to oxaliplatin but susceptible to olaparib**

The $IC_{50}$ of DNA cross-linking agents such as oxaliplatin or cisplatin, and PARP1 inhibitors were evaluated to assess whether the response in Capan-1 (*BRCA2*mt) and MIA PaCa-2 (BRCAwt) cells was affected by BRCA mutation profile. Cell viability assays showed that each agent inhibited cell survival in a dose- and time-dependent manner (Table 2 and Fig. 1). The $IC_{50}$ values of cisplatin in Capan-1 and MIA PaCa-2 cells at different time points were determined (Table 2 and Fig. 1A). The $IC_{50}$ values of oxaliplatin at 72 h for Capan-1 and MIA PaCa-2 cells were 5.128 and 2.65 μM, respectively; thus, the $IC_{50}$ value was twice higher in Capan-1 cells than in MIA PaCa-2 cells (Table 2 and Fig. 1B). The $IC_{50}$ of olaparib was 88.75 μM in MIA PaCa-2 cells, which was four times higher than that in Capan-1 cells (20.73 μM). This suggested that Capan-1 pancreatic cells with *BRCA2* mutations were four times more susceptible to olaparib than wild-type MIA PaCa-2 cells (Fig. 1C). Hence, it was expected that the resistance of Capan-1 cells with *BRCA2* mutations to oxaliplatin was caused by susceptibility to olaparib.

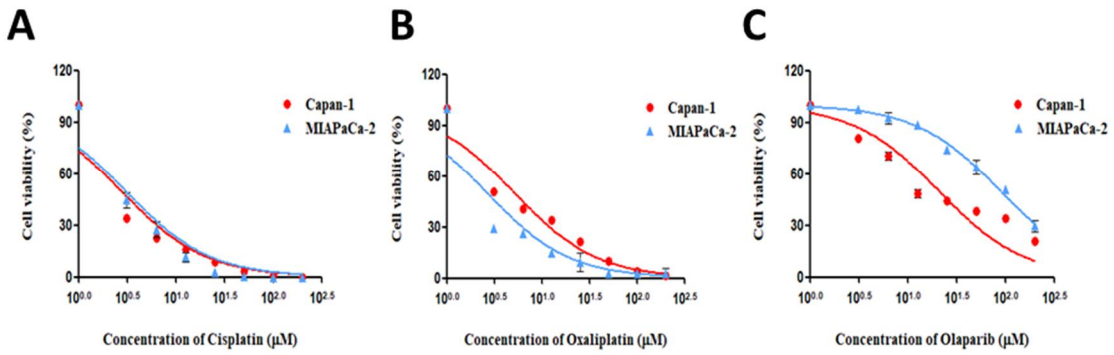



**Figure 1. *BRCA2*-deficient cell lines have high sensitivity to olaparib.**

(A) $IC_{50}$ curve of cisplatin, (B) $IC_{50}$ curve of oxaliplatin, (C) $IC_{50}$ curve of olaparib in *BRCA2*-deficient pancreatic cancer cell line (Capan-1) and *BRCA2* wild-type pancreatic cancer cell line (MIA PaCa-2). Cells were treated with cisplatin, oxaliplatin, or olaparib at the indicated concentrations (1.56-100 μM) for 72 h, and cell viability was measured by CellTiter-Glo assay. Each value indicates the mean ± SD (n=3).

**Table 2. Summary of $IC_{50}$ values in Capan-1 and MIA PaCa-2 cells.**

| | Capan-1 (*BRCA2*mt) | | | MIA PaCa-2 (*BRCA2*wt) | | |
|---|---|---|---|---|---|---|
| Time/μM | Cisplatin | Oxaliplatin | Olaparib | Cisplatin | Oxaliplatin | Olaparib |
| 24 | 71.35±0.04 | 170.1±0.06 | 251.2±0.58 | 20±0.036 | 40.84±0.06 | 180.9±0.05 |
| 48 | 8.73±0.035 | 15.51±0.03 | 107.6±0.05 | 6.11±0.05 | 7.48±0.04 | 137.4±0.05 |
| 72 | 2.72±0.08 | 5.128±0.05 | 20.73±0.06 | 3.02±0.07 | 2.65±0.08 | 88.75±0.02 |
| 96 | 1.93±0.11 | 3.453±0.07 | 10.93±0.05 | 2.17±0.10 | 1.94±0.11 | 30.71±0.03 |
| 120 | 1.89±0.11 | 2.437±0.09 | 4.02±0.08 | 1.78±0.12 | 1.66±0.13 | 10.95±0.04 |

$IC_{50}$ was identified using a wide range of concentrations (1.56-100 μM) of cisplatin, oxaliplatin and olaparib. $IC_{50}$ values varied significantly with time and concentration. Each value indicates the mean ± SD (n=3). Abbreviations: $IC_{50}$, 50% inhibitory concentration; wt, wild-type; mt, mutant.

**Olaparib sensitizes Capan-1 cells to oxaliplatin**

The combination of platinum-based anti-cancer agents and olaparib showed synergistic effects according to the combination index in Capan-1 cells. The combination of olaparib and oxaliplatin (CI 0.26) (Fig. 2A and 2B) showed greater synergistic effects than the combination of olaparib and cisplatin (Table 3, Fig. 2C and 2D). Additionally, the viability of Capan-1 cells gradually decreased as the concentration of olaparib increased with respect to oxaliplatin concentration (Fig. 2E). The viability of Capan-1 cells also decreased as oxaliplatin concentration increased with respect to olaparib concentration (Fig. 2F). On the other hand, MIA PaCa-2 cells showed antagonism towards oxaliplatin and olaparib with CI > 1 (Fig. 2A and 2B). Although the viability of MIA PaCa-2 cells decreased as olaparib concentration increased with respect to oxaliplatin concentration, these cells showed no significant differences in cell viability when treated with oxaliplatin alone or a combination of oxaliplatin and olaparib (Fig. 2G and 2H). Olaparib increased the effects of cell growth inhibition by oxaliplatin in Capan-1 cells (Fig. 2I). PARP1 inhibition by olaparib increased the susceptibility of Capan-1 cells with *BRCA2* mutations to oxaliplatin.

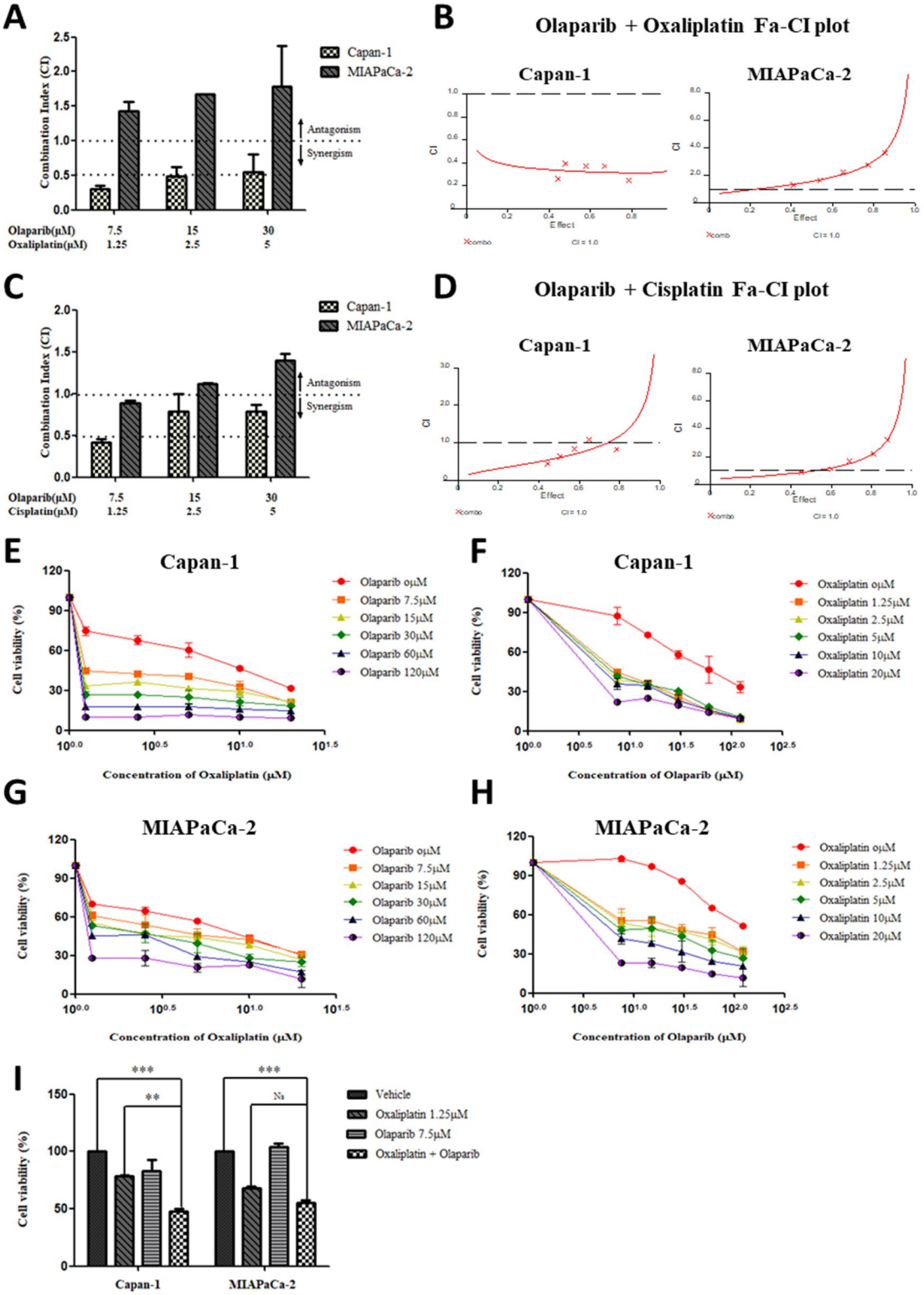

A
Combination Index (CI)
Capan-1
MIAPaCa-2
Antagonism
Synergism
Olaparib(μM) 7.5 15 30
Oxaliplatin(μM) 1.25 2.5 5
B
Olaparib + Oxaliplatin Fa-CI plot
Capan-1
MIAPaCa-2
C
Combination Index (CI)
Capan-1
MIAPaCa-2
Antagonism
Synergism
Olaparib(μM) 7.5 15 30
Cisplatin(μM) 1.25 2.5 5
D
Olaparib + Cisplatin Fa-CI plot
Capan-1
MIAPaCa-2
E
Capan-1
Cell viability (%)
Olaparib 0μM
Olaparib 7.5μM
Olaparib 15μM
Olaparib 30μM
Olaparib 60μM
Olaparib 120μM
Concentration of Oxaliplatin (μM)
F
Capan-1
Cell viability (%)
Oxaliplatin 0μM
Oxaliplatin 1.25μM
Oxaliplatin 2.5μM
Oxaliplatin 5μM
Oxaliplatin 10μM
Oxaliplatin 20μM
Concentration of Olaparib (μM)
G
MIAPaCa-2
Cell viability (%)
Olaparib 0μM
Olaparib 7.5μM
Olaparib 15μM
Olaparib 30μM
Olaparib 60μM
Olaparib 120μM
Concentration of Oxaliplatin (μM)
H
MIAPaCa-2
Cell viability (%)
Oxaliplatin 0μM
Oxaliplatin 1.25μM
Oxaliplatin 2.5μM
Oxaliplatin 5μM
Oxaliplatin 10μM
Oxaliplatin 20μM
Concentration of Olaparib (μM)
I
Cell viability (%)
***
**
***
Ns
Vehicle
Oxaliplatin 1.25μM
Olaparib 7.5μM
Oxaliplatin + Olaparib
Capan-1
MIAPaCa-2

**Figure 2. PARP inhibitor exhibits a synergistic effect with oxaliplatin in *BRCA2*-deficient Capan-1 cells.**

(A, C) Combination index of platinum-based anti-cancer drugs (cisplatin, oxaliplatin) and olaparib in Capan-1 and MIA PaCa-2 cells. The cells were treated with the drugs for 48 h and analyzed by CellTiter-Glo assay. (B) Fa-CI plot of oxaliplatin and olaparib. (D) Fa-CI plots of cisplatin and olaparib. Only Capan-1 cells showed synergistic effects at various concentration combinations (CI < 1). CI below 0.9, 0.9-1.0, and above 1.0 indicate synergistic, additive, and antagonistic effects. (E, G) Cell viability analysis in Capan-1 and MIA PaCa-2 cells after exposure to oxaliplatin and olaparib. The X-axis represents oxaliplatin dose. Cell viability is normalized to non-olaparib (0 μL olaparib) values at different oxaliplatin doses. (F, H) Cell viability analysis in Capan-1 and MIA PaCa-2 cells after exposure to oxaliplatin and olaparib. The X-axis represents olaparib dose. Cell viability is normalized to non-oxaliplatin (0 μL oxaliplatin) values at different olaparib doses. (I) Quantification of cell viability in Capan-1 and MIA PaCa-2 cells treated with the indicated dose for 48 h. n=3, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

**Table 3. Combination index (CI) table showing synergistic effects of cisplatin or oxaliplatin with olaparib in Capan-1 and MIA PaCa-2 cell lines.**

| | Oxaliplatin (μM) | Olaparib (μM) | Combination index |
|---|---|---|---|
| Capan-1 | 1.25 | 7.5 | 0.26 |
| | 2.5 | 15 | 0.39 |
| | 5 | 30 | 0.37 |
| | 10 | 60 | 0.37 |
| | 20 | 120 | 0.25 |
| MIA PaCa-2 | 1.25 | 7.5 | 1.25 |
| | 2.5 | 15 | 1.19 |
| | 5 | 30 | 1.33 |
| | 10 | 60 | 1.75 |
| | 20 | 120 | 2.12 |

| | Cisplatin (μM) | Olaparib (μM) | Combination index |
|---|---|---|---|
| Capan-1 | 1.25 | 7.5 | 0.45 |
| | 2.5 | 15 | 0.63 |
| | 5 | 30 | 0.84 |
| | 10 | 60 | 1.09 |
| | 20 | 120 | 0.83 |
| MIA PaCa-2 | 1.25 | 7.5 | 0.87 |
| | 2.5 | 15 | 1.10 |
| | 5 | 30 | 1.68 |
| | 10 | 60 | 2.20 |
| | 20 | 120 | 3.16 |

The table shows the combination index (CI) values after treatment of Capan-1 and MIA PaCa-2 cell lines with the drugs. Synergistic growth inhibition was observed at most concentrations, but the lowest CI values in Capan-1 cells were seen when treated with olaparib and oxaliplatin. CI below 0.9, 0.9-1.0, and above 1.0 indicate synergistic, additive, and antagonistic effects.

**Oxaliplatin induces hyperactivation of PARP1 in Capan-1 cells with *BRCA2* deficiency**

Our findings suggested that the response of BRCA-2-deficient cells to oxaliplatin was related to the activity of PARP1. Capan-1 and MIA PaCa-2 cells were treated with olaparib and oxaliplatin to evaluate the activation of PARP1. First, PARP expression level in Capan-1 cells under physiological conditions was 50% compared to that observed in MIA PaCa-2 cells (**$P < 0.01$) (Fig. 3A). However, oxaliplatin significantly increased the expression level of PARP in Capan-1 cells than in MIA PaCa-2 cells (**$P < 0.01$) (Fig. 3B). Olaparib inhibited PARP activity in pancreatic cancer cells (Fig. 3B). Capan-1 cells treated with olaparib and oxaliplatin showed a 2.4-fold increase in γH2AX, a DSB marker, compared to cells treated with oxaliplatin alone (**$P < 0.01$) (Fig. 3B). These findings suggested that inhibition of PARP1 by olaparib significantly aggravated oxaliplatin-induced DNA damage and enhanced sensitivity to oxaliplatin in pancreatic cancer cells with *BRCA2* mutations. This showed that malfunction of the DNA repair mechanism through *BRCA1/2* was compensated by another mechanism through PARP1, which increased the drug resistance of the cells.

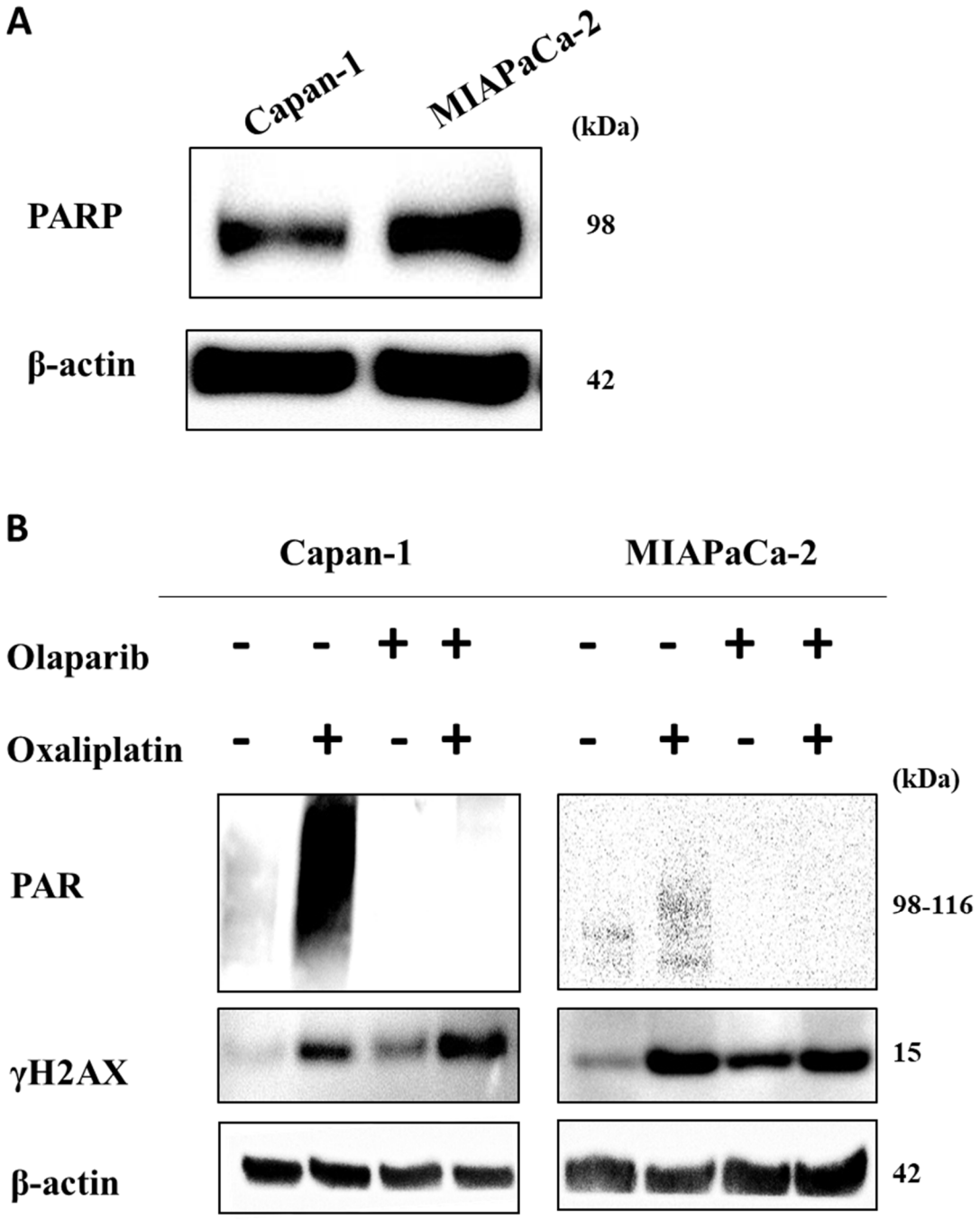

A
Capan-1
MIAPaCa-2
(kDa)
PARP
98
β-actin
42
B
Capan-1
MIAPaCa-2
Olaparib
- - + + - - + +
Oxaliplatin
- + - + - + - +
(kDa)
PAR
98-116
γH2AX
15
β-actin
42

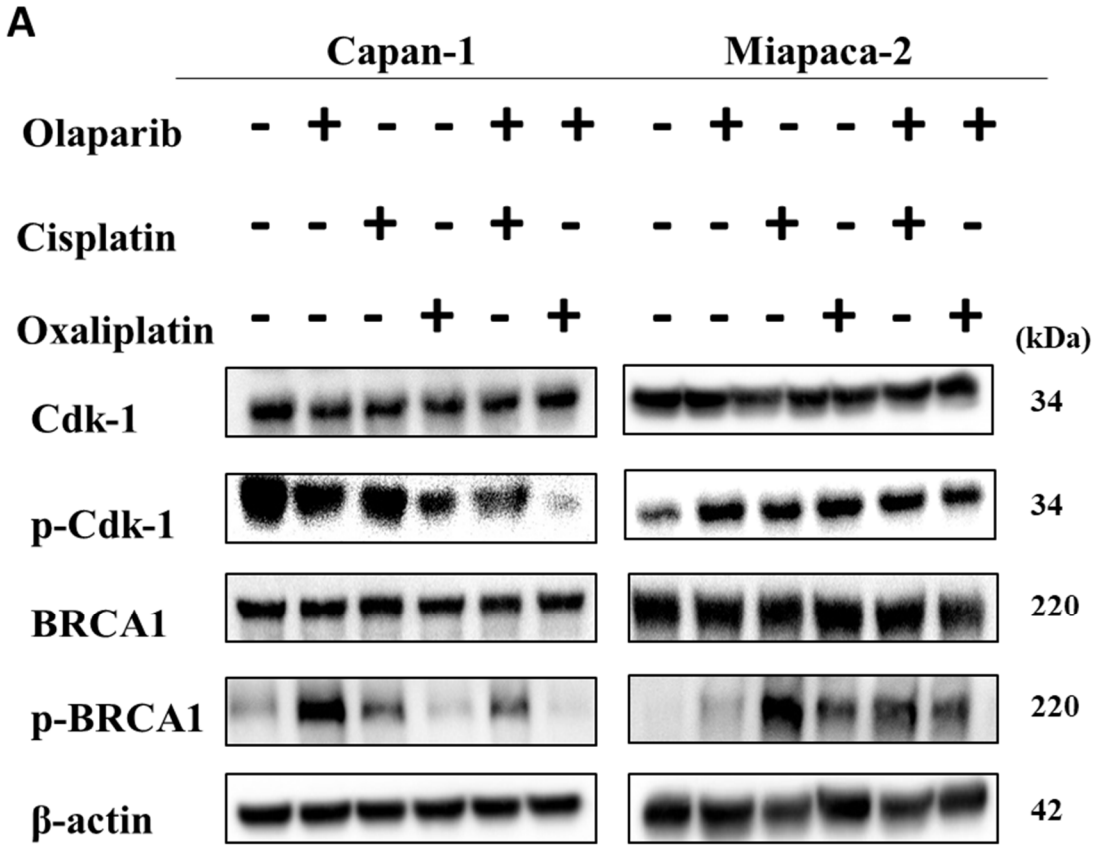
**Figure 3. PARP1 expression and activity under olaparib and oxaliplatin.**

(A) Western blots showing the expression levels of PARP1 in the two cell lines. (B) Western blots showing the activity of PARP1 by detecting PAR in different drug treatment groups. Expression of γH2AX was confirmed.

**Oxaliplatin, unlike cisplatin, induces BRCAness via inhibition of CDK1-BRCA1 axis, which is enhanced by combination with olaparib**

We assessed the reason for greater increase in the synergistic effect of olaparib and oxaliplatin than that of olaparib and cisplatin. Oxaliplatin and cisplatin are platinum-based anti-cancer drugs that induce cytotoxicity by forming platinum adducts on DNAs, and these two drugs exhibit different mechanisms in the cell cycle. Oxaliplatin completely inhibits the G1 and G2 phases, whereas cisplatin slows the S phase and disrupts G2-M phase transition. These two drugs are known to reversibly regulate the protein expression of CDK1 and cyclin B [47]. In a recent study, CDK1 was found to be involved upstream of the DNA damage response pathway [33]. Treatment of Capan-1 (*BRCA2*mt) cells with oxaliplatin decreased the expression level of p-CDK1, the active form of CDK1, by 1.8-fold compared to that in the control group (*$P < 0.05$) (Fig. 4A, lane 4). Additionally, the activity of BRCA1, a protein downstream of CDK1 and involved in HR repair, was also assessed. Oxaliplatin decreased the expression level of p-BRCA1, the active form of BRCA1, by 1.8-fold and induced BRCAness (**$P < 0.01$) (Fig. 4A, lane 4). Combined treatment with oxaliplatin and olaparib reduced the expression levels of p-CDK1 and p-BRCA1 by more than 2-fold compared to oxaliplatin treatment alone (**$P < 0.01$, ***$P < 0.001$) (Fig. 4A, lane 6). To evaluate whether cisplatin could directly inhibit BRCA1 through CDK1, the functional effects of cisplatin were compared. Cisplatin alone and combined treatment with cisplatin and olaparib did not reduce p-CDK1 and p-BRCA1 expression levels (Fig. 4A, lanes 3 and 5). BRCAness through strong inhibition of the CDK1-

BRCA1 axis in *BRCA2*-deficient cell lines was only observed after oxaliplatin and olaparib combination treatment. Western blotting was performed to confirm the effects of olaparib and oxaliplatin combination treatment on molecules acting on various DDR mechanisms. RPA binds to ssDNA and actively promotes replication. Then, RPA recruits *BRCA2* to induce the formation of RAD51 filaments at the damaged site, for HR repair. RPA70 expression level increased 1.7-fold after oxaliplatin treatment, suggesting replication stress induced by DSBs (*$P < 0.05$). Compared to oxaliplatin treatment alone, combined treatment with oxaliplatin and olaparib decreased the expression level of RPA by 5-fold (**$P < 0.01$) (Fig. 4B). In *BRCA2*-deficient cells, RAD51 expression level was not affected by anti-cancer drugs. Trapping of PARP through olaparib at DSB sites reduced the expression levels of XRCC1, a BER effector, and XRCC4, an NHEJ mediator, by more than 2-fold (*$P < 0.05$) (Fig. 4B).


A
Capan-1
Miapaca-2
Olaparib
Cisplatin
Oxaliplatin
(kDa)
Cdk-1
p-Cdk-1
BRCA1
p-BRCA1
β-actin
34
34
220
220
42

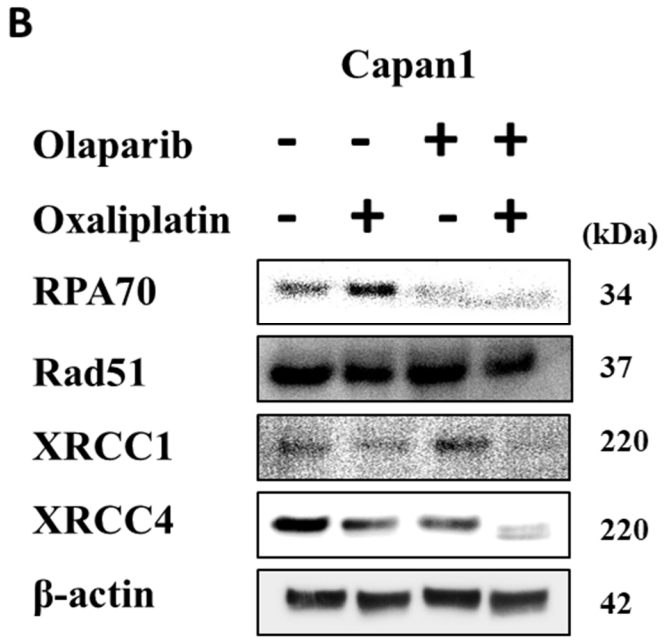

B
Capan1
Olaparib
Oxaliplatin
(kDa)
RPA70
Rad51
XRCC1
XRCC4
β-actin
34
37
220
220
42

**Figure 4. Treatment with oxaliplatin inhibits DSB repair pathways via blocking CDK1-**BRCA1 **activities in *BRCA2*-deficient pancreatic cancer cells.**

(A) Validation by western blotting on the effects of various drug treatments on levels of CDK1 expression and phosphorylation as well as BRCA1 expression and phosphorylation. (B) In Capan-1 cell line, inhibition of the DSB repair pathway upon treatment with oxaliplatin and olaparib was confirmed by western blotting. (n=3, $*P < 0.05$, $**P < 0.01$).

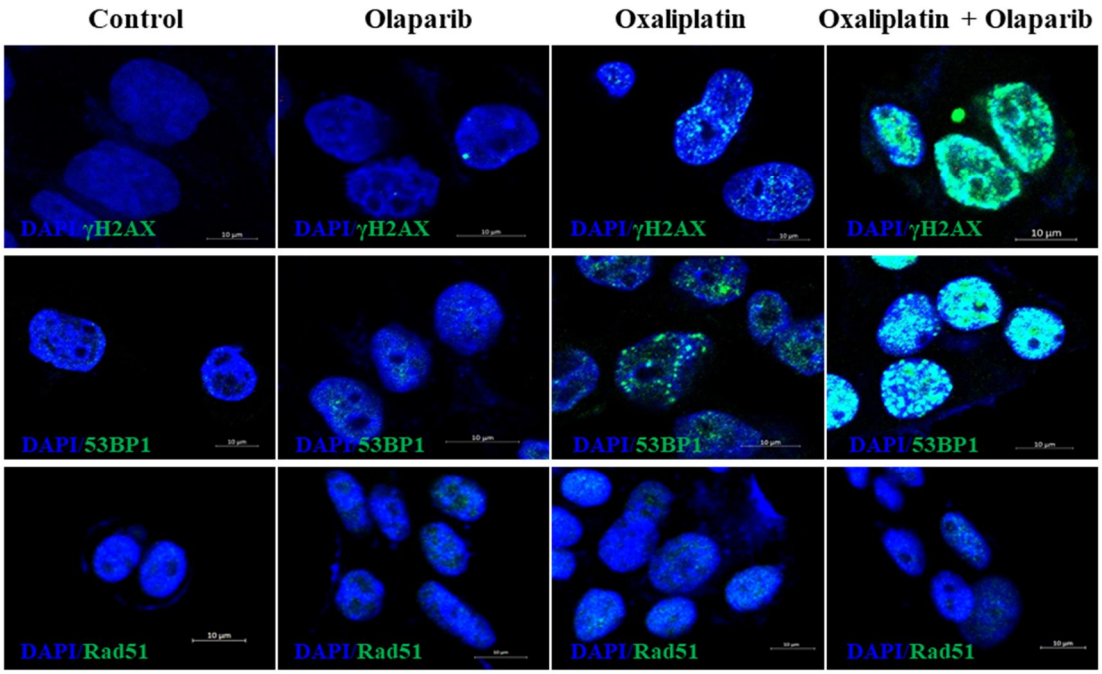
**Combination of olaparib and oxaliplatin induces foci formation by DNA damage marker leading to apoptosis**

Cells were treated with olaparib or oxaliplatin alone or a combination of the two drugs, and the accumulation of γH2AX in the cell nuclei was assessed by immunofluorescence staining. In cells treated with olaparib and vehicle (negative control), γH2AX accumulation was barely observed (Fig. 5A). In contrast, cells treated with oxaliplatin alone showed 40% more accumulation of γH2AX than vehicle control cells. This accumulation of γH2AX increased by 25% after oxaliplatin and olaparib combination treatment (*$P < 0.05$) (Fig. 5B). The DSB repair effector, 53BP1, did not accumulate in the nuclei of cells in the vehicle control group and cells treated with olaparib alone (Fig. 5A). Treatment with oxaliplatin alone increased the accumulation of 53BP1 by 20% in the vehicle control group and olaparib only group. Oxaliplatin and olaparib combination treatment increased the accumulation of 53BP1 in the nucleus by 40% (**$P < 0.01$) (Fig. 5B). Formation of RAD51 foci, which indicates BRCA-dependent DSB repair and loss of PARP1 function [37], was also observed; however, in BRCA-deficient pancreatic cancer cells, RAD51 foci were not formed, regardless of drug treatment ($P > 0.05$) (Fig. 5A and 5B). Increased foci formation due to γH2AX or 53BP1 in the nucleus indicates accumulation of DNA damage, resulting in induction of cell apoptosis. In agreement with our findings regarding γH2AX and 53BP1 foci, the apoptotic effect was greatest in cells treated with a combination of oxaliplatin and olaparib (**$P < 0.01$) (Fig. 6).

Therefore, combined treatment of *BRCA2*-deficient pancreatic cancer cells with

olaparib and oxaliplatin induces DDR blockade and BRCAness through PARP1 inhibition. This prevents DNA repair and induces cell apoptosis due to accumulation of DNA damage.

**A**


Control
Olaparib
Oxaliplatin
Oxaliplatin + Olaparib
DAPI/γH2AX
DAPI/53BP1
DAPI/Rad51
10 µm

**B**

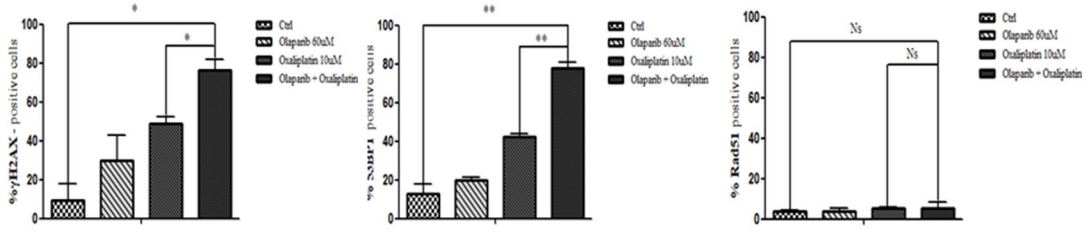
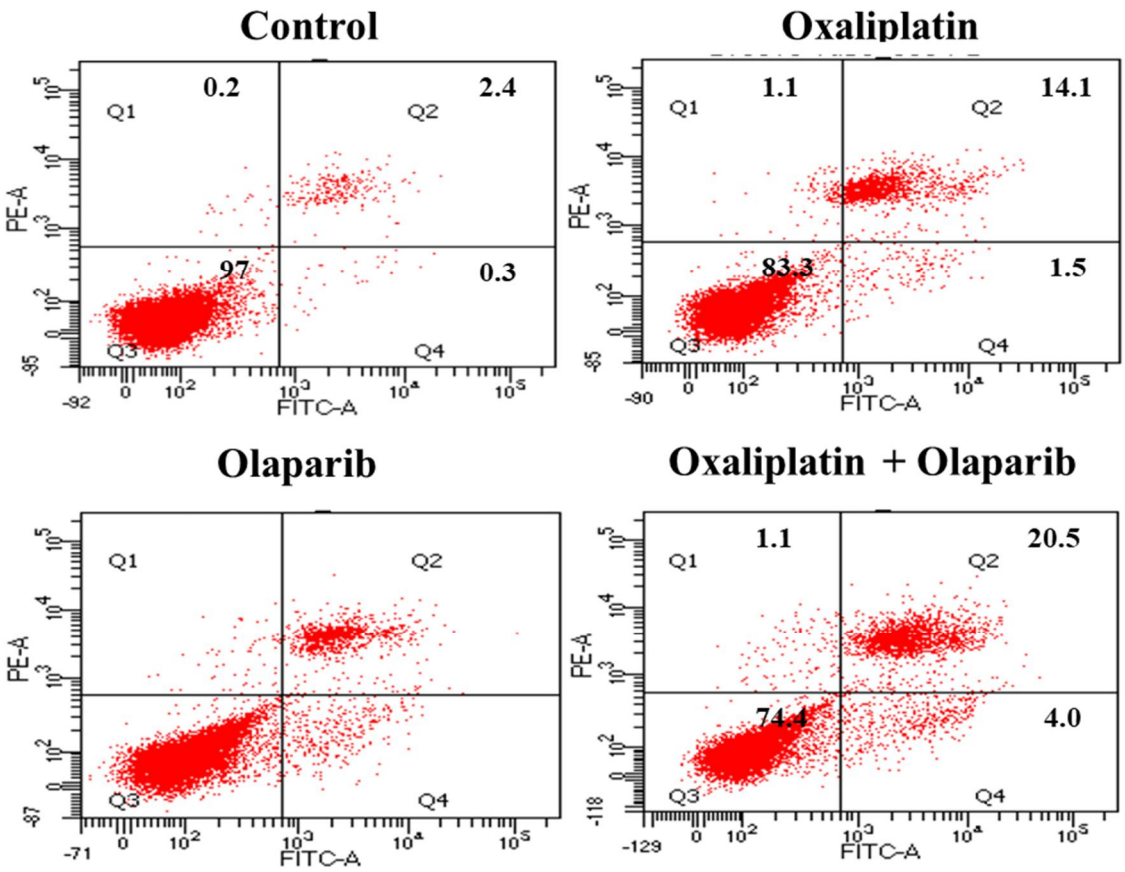

%γH2AX - positive cells
% 53BP1 positive cells
% Rad51 positive cells
Ctrl
Olaparib 60uM
Oxaliplatin 10uM
Olaparib + Oxaliplatin
*
**
Ns

**Figure 5. Combination of olaparib and oxaliplatin induces foci formation by DNA damage markers.**

(A) Effects of different drug combinations, as observed by immunofluorescence staining, showing γH2AX, 53BP1, and Rad51 foci in Capan-1 cells. (B) Quantification of γH2AX, 53BP1, and Rad51 foci formation. The number of cells positive for foci formation was compared with the total cell number (n=3, $*P < 0.05$, $**P < 0.01$).

**A**


Control
0.2
2.4
97
0.3
Oxaliplatin
1.1
14.1
83.3
1.5
Olaparib
Oxaliplatin + Olaparib
1.1
20.5
74.4
4.0
Q1
Q2
Q3
Q4
PE-A
FITC-A

**B**

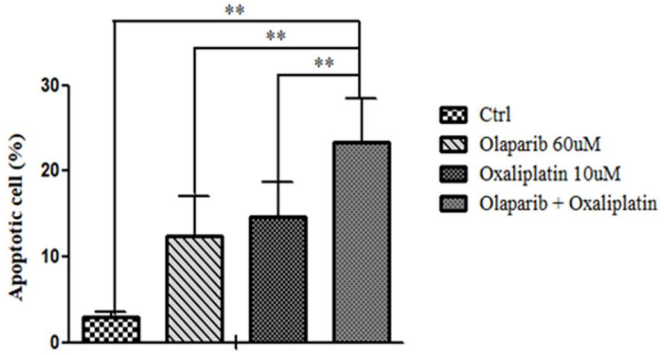

Apoptotic cell (%)
0
10
20
30
**
**
**
Ctrl
Olaparib 60uM
Oxaliplatin 10uM
Olaparib + Oxaliplatin

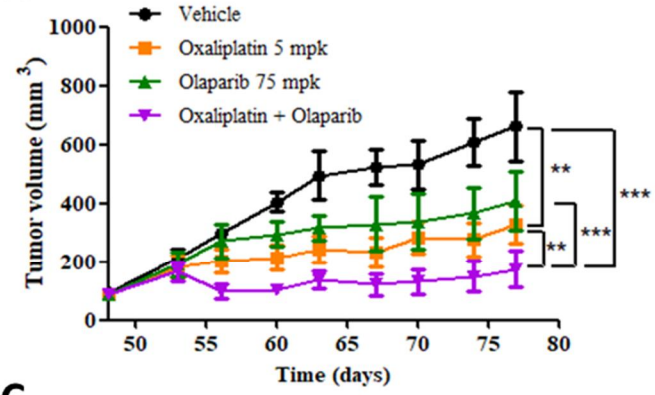
**Figure 6. Combination of oxaliplatin and olaparib increases apoptosis of *BRCA2*-deficient pancreatic cancer cells.**

(A) Effects of different drug combinations on apoptosis of Capan-1 cells. Cells were treated with oxaliplatin or olaparib alone or co-treated with oxaliplatin and olaparib for 48 h. (B) Quantification of apoptosis (n=3, $*P < 0.05$, $**P < 0.01$).

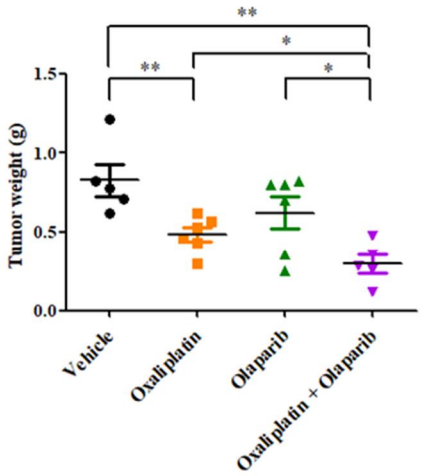
**Combination of olaparib and oxaliplatin inhibits tumor growth in a *BRCA2*-deficient pancreatic cancer xenograft model**

According to the results obtained, *BRCA2*-deficient pancreatic cancer cells can be a therapeutic target for PARP inhibitors. The efficacy of the combination of oxaliplatin and olaparib was validated using a pancreatic cancer xenograft model prepared using capan-1 cells. The oxaliplatin alone (59.2%) or olaparib alone (49.2%) treatment group showed tumor growth inhibition compared to the vehicle control group. And then, the combination treatment group of oxaliplatin and olaparib showed significant tumor growth inhibition compared to the vehicle control group, oxaliplatin, or olaparib alone group (6 mice/group, $*P < 0.05$, $**P < 0.01$, $**P < 0.001$) (Fig. 7). There was no weight loss in either the drug-treated or control groups, indicating that there was no toxicity (Fig. 7C). Together, these findings indicated therapeutic efficacy of combination of oxaliplatin and olaparib *in vivo*.

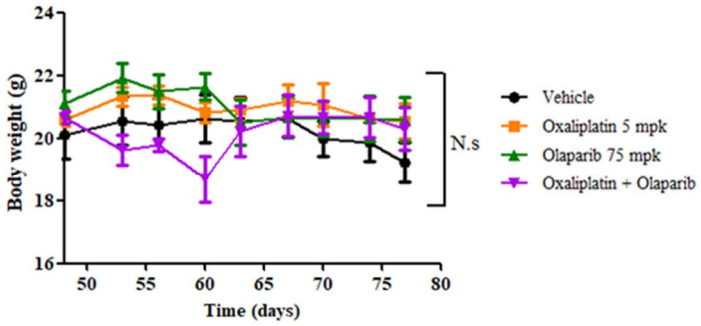
A




B




C




D

| | Tumor volume (Ave±SD, mm³) | TGI (%) |
|---|---|---|
| **Vehicle** | 806.5 ± 264.9 | - |
| **Oxaliplatin 5 mpk** | 329.4 ± 156.9 | **59.2** |
| **Olaparib 75 mpk** | 409.6 ± 243.3 | **49.2** |
| **Oxaliplatin+Olaparib** | 175.8 ± 139.4 | **78.2** |

E

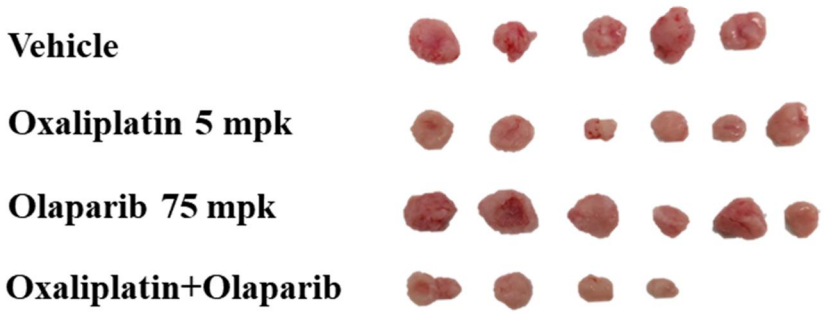


**Figure 7. PARP inhibitors make Capan-1 cells xenografts more susceptible to oxaliplatin chemotherapy.**

The Capan-1 cells xenograft mouse model was treated with vehicle control (3% DMSO/30% PEG-300/dd$H_2O$), oxaliplatin (5 mg/kg), olaparib (75 mg/kg), or a combination of these two agents orally gavage or intraperitoneal injection for 30 days. (A) The resulting tumor volumes were measured on the indicated days of treatment. (B) The tumor weight of end-point mice after 30 days of treatment. (C) The body weight time curve for mice after drug treatment up to day 74. (D) Table of average tumor volume and rate of tumor growth inhibition. (E) Representative pictures of implanted tumors in each group at the time of study termination. (6 mice/group, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

## Discussion

Here, we determined the effects of olaparib, a PARP inhibitor, on pancreatic cancer cells carrying *BRCA2* mutation with oxaliplatin. In the two examined pancreatic cancer cell lines (Capan-1 and MIA PaCa-2), we performed cell viability assays to assess the sensitivity of platinum-based anti-cancer drugs (cisplatin, oxaliplatin) and the PARP inhibitor (olaparib). Based on the combination index values, olaparib was shown to improve the sensitivity of *BRCA2*-deficient pancreatic cancer cells towards oxaliplatin. The mechanism of olaparib sensitization by oxaliplatin was related to CDK1 based on western blotting, immunofluorescence labeling and flow cytometry. These findings suggest that combined therapy with a PARP inhibitor and oxaliplatin should be selectively applied for the treatment of *BRCA2*-deficient pancreatic cancer.

The standard treatment for advanced pancreatic cancer is FOLFIRINOX (fluorouracil, oxaliplatin, leucovorin, and irinotecan). Studies have been conducted to improve the efficacy of FOLFIRINOX therapy by combining it with new targeted therapy drugs [5,6]. A phase 3 POLO trial established a method for using PARP inhibitors as a maintenance therapy for germline *BRCA* mutant PDAC [34]. Monotherapy with veliparib, a PARP inhibitor, and combined therapy with veliparib and cisplatin/gemcitabine revealed 74.1 % and 65.2 % high response rates in patients with germline *BRCA* mutant and PALB2 mutant pancreatic cancer, respectively [35]. These results reflect the relationship between PARP and *BRCA1/2*-deficient cells, which is the

most successful synthetic lethality relationship for HR repair in cancer treatment [18]. In previous studies, PARP inhibitors and cisplatin showed synergistic effects in not only breast cancer but also pancreatic cancer cell lines [41].

In this study, we evaluated the anti-cancer effects of the chemotherapeutic agent, oxaliplatin, and a PARP inhibitor in pancreatic cancer cells lines and animal models with defective HR repair. The anti-cancer effects of the chemotherapeutic drugs, cisplatin and oxaliplatin, and the PARP inhibitor, olaparib, showed different reactivity in various pancreatic cancer cell lines. Olaparib alone had minimal effect on the growth of MIA PaCa-2 (*BRCA2*wt) cells, whereas it inhibited the growth of Capan-1(*BRCA2*mt) cells (Fig. 1).

Platinum-based anti-cancer drugs and PARP interact as platinum-based agents form DSBs and PARP play key roles in DSB repair. Therefore, PARP inhibitors such as olaparib may enhance platinum-based anti-cancer drug-induced tumor cell death. The results of this study showed that olaparib enhanced the susceptibility of Capan-1 (*BRCA2*mt) cells to oxaliplatin by inhibiting PARP1. The combination of olaparib and oxaliplatin had synergistic effects in Capan-1 (*BRCA2*mt) cells (Fig. 2). The CI value for olaparib and oxaliplatin was higher than that of cisplatin and olaparib. This provides the basis for future studies on the clinical use of a PARP inhibitor and oxaliplatin combination therapy. Combination using oxaliplatin with olaparib also inhibited cell growth (Fig. 2); however, the combination of oxaliplatin and olaparib showed maximum inhibition of tumor growth by increasing the expression level of γH2AX (Fig. 3) and inducing enhanced levels of apoptosis (Fig. 2).

DNA damage increases the catalytic activity of PARP1, which results in PARylation, i.e., the synthesis of a long, branched PAR chain. PAR binds to DDR proteins and other proteins involved in DNA metabolism [44,46]. Thus, PAR recruits DDR proteins to the DNA damage site and is involved in DNA repair and cellular apoptosis. In this study, olaparib inhibited PARylation in pancreatic cancer cells, suggesting that this drug significantly inhibits the DNA damage repair process. Moreover, expression level of the PARP protein was higher in MIA PaCa-2 cells than in Capan-1 cells, but the expression level of PAR, an active marker of PARP, was higher in the latter. This indicates an increase in susceptibility to oxaliplatin due to PARP1 inhibition and supports the high $IC_{50}$ concentration (Fig. 1) of oxaliplatin in Capan-1 cells (Fig. 3). HR-deficient cells focus on DNA repair via PARP, and this increases drug resistance.

CDK1 phosphorylated the serine residues of BRCA1, and through this phosphorylation, BRCA1 efficiently forms a focus at the site of DNA damage and promotes checkpoint activation. BRCA1 plays an important role in DNA damage repair by HR. Thus, depletion or inhibition of CDK1 can inhibit homologous recombination, a DNA damage response pathway, by inhibiting BRCA1 phosphorylation [43]. PARP is a DNA repair enzyme that is part of the BER pathway (base cleavage repair). The HR defects in BRCA mutant cancers make them particularly susceptible to inhibition of other DNA repair pathways that compensate for the loss of HR activity [25]. When PARP is inhibited, SSB converts to DSB, causing more lethal cellular damage and the need for requires HR repair. Therefore, cells deficient in BRCA or HR-related factors

are very sensitive to PARP inhibition [24, 25, 54, 55, 56, 58]. According to a recent study, cisplatin did not inhibit CDK1 phosphorylation, [47, 52, 53, 58] but only oxaliplatin inhibited phosphorylated CDK1, thereby inhibiting phosphorylated BRCA1 and cause HR failure [47, 52]. Studies have shown that *BRCA2*-deficient pancreatic cancer cells respond sensitively to cisplatin and PARP inhibitors [56]. However, there were few anticancer evaluations using oxaliplatin.

In our study, oxaliplatin directly inhibited BRCA1 through CDK1 in Capan-1 (*BRCA2*mt) cells, and combined treatment with oxaliplatin and olaparib led to greater inhibition of CDK1-BRCA1 than oxaliplatin alone (Fig. 4). In contrast, cisplatin did not inhibit the function of CDK1 and BRCA1 (Fig. 4). Additionally, the synergistic effects of cisplatin and the PARP1 inhibitor were not significantly different from that of oxaliplatin and the PARP1 inhibitor (Fig. 2). However, since both oxaliplatin and cisplatin could not inhibit CDK1-BRCA1 in MIA PaCa-2 (*BRCA2*wt) cells, this may be a specific response of *BRCA2*-deficient pancreatic cancer cells to oxaliplatin. Therefore, CDK1 plays an important role in BRCAness upon oxaliplatin administration. Furthermore, an increase in the inhibition of HR repair was observed following additional combination of PARP inhibitors. Oxaliplatin and olaparib inhibited DDR markers of BER and HR, such as PARP, XRCC1, RPA, and XRCC4, and aggravated DNA damage (Fig. 4B).

We observed that oxaliplatin decreased XRCC1 expression level. DNA base excision repair (BER) is driven by PARP and the scaffold protein XRCC1. PARP is required to detect single-stranded break intermediates and promote BER. However,

PARP1 tends to bind excessively to the BER intermediate, blocking access and repair by other BER enzymes. A protein complex comprising DNA polymerase β and DNA ligase III assembled by XRCC1 prevents excessive participation and activity of PARP1 during BER. Even in the absence of PARP1, XRCC1 rapidly proceeds with BER through complex recruitment. However, in the absence of XRCC1, PARP1 may be excessively involved in the BER intermediate; the effect is similar to that of a PARP1 inhibitor. XRCC1 prevents PARP1 trapping during BER and XRCC1 is dispensable for DNA base excision repair [45, 59].

This suggests that oxaliplatin impairs BER (Fig. 4B). We observed that oxaliplatin inhibited XRCC1-induced DDR and prevented PARylation in combination with olaparib, increasing the susceptibility to DNA-damaging drugs (Fig. 4B). Previous studies have reported that XRCC1 is regulated by STAT3 activity [48], and oxaliplatin, not cisplatin, inhibits this activity [49,50]. Based on these findings, oxaliplatin may inhibit XRCC1 by inhibiting STAT3. However, in this study, we could not assess whether the decreased expression level of XRCC1 by oxaliplatin was a drug-specific effect in *BRCA2*-deficient pancreatic cancer cells.

PARP inhibitors act through HR-deficient cells and synthetic lethality. Therefore, in this study, the formation of γH2AX, 53BP1, and RAD51 foci in the cell nuclei was assessed to evaluate functional HR recovery and DSB induction by oxaliplatin and olaparib. Upon PARP1 inhibition, oxaliplatin-induced DNA damage generated a greater number of γH2AX (DSB marker) and 53BP1 (DSB repair effector) foci. Combined treatment with olaparib and oxaliplatin induced synergistic DNA

damage in Capan-1 (*BRCA2*mt) cells (Fig. 5). On the other hand, *BRCA2*-deficient cells cleaved RAD51 binding sites. Thus, RAD51 foci formation was not observed regardless of the drug treatment used, and HR repair was not restored. Additionally, we observed that combined treatment with oxaliplatin and olaparib increased the accumulation of unrepaired DSBs and induced apoptosis of Capan-1 (*BRCA2*mt) cells (Fig. 6). Therefore, combined treatment with oxaliplatin and olaparib has significant therapeutic effects in HR-deficient pancreatic cancer cells.

In conclusion, we have reported on the *in vitro* and *in vivo* synergistic effects of a PARP inhibitor and oxaliplatin in a *BRCA2*-deficient pancreatic cancer cell line. Such synergistic effects are mediated by changes in HR due to oxaliplatin treatment, and require PARP activity for a compensatory mechanism of BRCAness through CDK1-BRCA1 inhibition. Furthermore, combination with olaparib (PARP1 inhibitor) induces failure of HR and inhibits a wide range of DNA repair molecules in different repair pathways, subsequently leading to tumor cell death. However, germline mutations such as *BRCA1/2* in the HR pathway are observed in a small proportion of pancreatic cancer patients. Future studies must establish additional biomarkers for treatment and expand the treatment approach by testing various combinations of therapies such as radiation and conventional chemotherapy. Our findings suggest possible biomarkers that might help in selecting patients for combined therapy using oxaliplatin and PARP inhibitors. In addition, we have suggested a new treatment strategy using PARP inhibitors for treating pancreatic cancer.

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## 국문요약 (Korean Abstract)

최근 Cisplatin/gemcitabine 단독과 poly ADP-ribose polymerase (PARP) 억제제인 veliparib의 조합을 평가했고 *BRCA2* 돌연변이 췌장암 환자의 표준치료로써 상당한 활성을 나타냈다[34]. 이 환자 집단에서 cisplatin과 gemcitabine이 췌장암에 대한 최첨단 화학요법인 FOLFRINOX 보다 우수한지 여부는 불분명하다. *BRCA*ness 관련 돌연변이는 oxaliplatin 기반 화학 요법에 대한 유망한 예측 마커가 될 수 있음을 시사 함으로 oxaliplatin과의 PARP 억제제의 조합 요법의 효능을 평가하였다. *BRCA2* 결손 돌연변이 췌장암 세포주(Capan-1)는 *BRCA2* wild type 췌장암 세포주(MIA PaCa-2)에 비해 olaparib에 더 민감하다. Capan-1은 oxaliplatin 및 olaparib과 함께 치료했을 때 cisplatin보다 더 좋은 시너지 효과를 보였다. olaparib은 *BRCA2* 결손 돌연변이 췌장암세포에서만 oxaliplatin과 상승 작용을 하였다. Cisplatin과 달리 oxaliplatin은 Capan-1에서 CDK1 활성과 BRCA1 활성을 억제하였다. 또한 oxaliplatin과 olaparib의 조합은 DNA repair 기전에 관여하는 분자들을 억제하였고 DNA 손상 마커의 축적 증가와 세포자멸사 증가를 확인했다. 또한 동물 실험에서도 이와 같은 효과를 확인 하였다. 이를 통해 Capan-1에서 olaparib과의 병용투여에 oxaliplatin 이 더 효과적일 것으로 판단하였다.

우리의 연구는 oxaliplatin과 조합된 PARP1 억제제가 *BRCA2* 결손

돌연변이가 있는 췌장암세포에서 항종양 효과를 갖는다는 것을 발견했다. 또한 우리 연구는 oxaliplatin 자체가 CDK1의 기능을 억제하여 BRCA1 기능 장애를 일으키고 PARP1 억제제가 효과적으로 기능하도록 함으로써 *BRCA*ness를 일으킬 수 있음을 발견하였다. 본 연구는 oxaliplatin이 *BRCA2* 결손 돌연변이 및 HRD 췌장암세포에서 cisplatin보다 더 효과적일 수 있음을 제안한다.

중심단어: 췌장암; *BRCA2*; PARP 억제제; 올라파립; 옥살리플라틴; DNA 손상; 합성 치사