의학박사 학위논문

Radiation sensitizing effect of combined use of olaparib and PI-103 and its mechanism in triple negative breast cancer

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2014 년 7 월

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이 논문을 의학박사 학위논문으로 제출함

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Abstract

Radiation sensitizing effect of combined use of olaparib and PI-103 and its mechanism in triple negative breast cancer

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Introduction: Triple negative breast cancer (TNBC) shows aggressive clinical behavior but the treatment options are limited due to lack of a specific target. TNBC shares many clinical and pathological similarities with BRCA deficient breast cancer, for which poly(ADP-ribose) polymerase (PARP) inhibitor is effective, but PARP inhibitor alone failed to show clinical effects in patients with sporadic TNBC. Radiation induces DNA double strand break and the phosphoinositide 3-kinase (PI3K) signaling pathway has been known to regulate steady state levels of homologous recombination. Recent preclinical study showed that PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient TNBC to PARP inhibition. Taken together, we assessed the radiation sensitizing effect of combined olaparib and PI-103 in BRCA-proficient triple negative breast cancer cells and investigated its mechanism of action.

Methods: MDA-MB-435s cells were divided into four treatment groups; irradiation (IR) alone, olaparib + IR, PI-103 + IR, olaparib + PI-103 + IR. Cells were exposed to the drugs for 2 hours prior to irradiation, and the cell survival curve was obtained using a clonogenic assay. Western blotting and immunofluorescent detection of γH2AX foci were performed. Xenograft and bioluminescence imaging were performed to assess in vivo radiosensitivity.

Results: Combined use of olaparib and PI-103 enhanced the radiation-induced cell death of MDA-MB-435s ($SER_{0.05}$ 1.7) and MDA-MB-231-BR ($SER_{0.05}$ 2.1) cell lines. In addition, the tumor volume was significantly reduced with the use of radiation plus the two-drug combination in the xenograft models ($p < 0.001$). Treatment with PI-103 showed prolonged γH2AX foci, indicating delayed DNA strand break repair. PI-103 was found to induce upregulation of phosphorylated extracellular signal–regulated kinase (ERK), increase of poly(ADP-ribose), and downregulation of BRCA1.

Conclusions: Combined use of olaparib and PI-103 enhanced the radiation-induced cell killing effect in BRCA-proficient MDA-MB-435s and MDA-MB-231-BR cell lines and xenograft. TNBC patients have high incidences of locoregional relapse and distant metastasis, and radiation therapy is involved not only in locoregional control, but also in the treatment of distant recurrences such as brain metastasis or other oligometastasis. Targeting of the PI3K signaling pathway combined with PARP inhibition could be a reasonable approach to enhance effects of radiation in BRCA-proficient TNBC.

Keywords: Triple negative breast cancer, radiotherapy, olaparib, PI-103, PARP inhibitor, PI3K inhibitor

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Introduction

Triple negative breast cancer (TNBC) is defined as a tumor which does not express the estrogen receptor (ER), progesterone receptor, or human epidermal growth factor 2 (HER-2). Aggressive clinical behaviors such as early distant metastasis and lack of specific treatment targets, i.e. ER or HER-2, have been obstacles to the treatment of TNBC (1). Cytotoxic chemotherapy or a combination with a targeted agent such as bevacizumab or cetuximab has been used for treatment, but the outcomes were not satisfactory (2). Meanwhile, the introduction of poly(ADP-ribose) polymerase (PARP) inhibitor was expected to provide a promising new therapeutic strategy for TNBC.

PARP is a family of enzymes involved in DNA repair, cell proliferation and death, and genomic stability (3-5). PARP-1 is the most abundant type of PARP, and is involved with base excision repair (BER) (6). When a DNA strand break occurs, PARP-1 rapidly binds to the break site and induces autophosphorylation. Auto-poly(ADP-ribosyl)ation creates a negative charge, which recruits the enzymes required for BER (6, 7). Synthetic lethality occurs when two otherwise nonlethal mutations together result in an inviable cell (8). When DNA damage occurs, cells with mutations in either PARP or BRCA, which is involved in homologous recombination (HR), can survive, while cells with mutations in both cannot (9).

This strategy is very effective for treatment of breast cancer patients with BRCA mutations, but the problem is that incidence of BRCA related breast cancer is less than 10% (10). Recently, some investigators have used the term “BRCA-ness” of TNBC, since BRCA deficient breast cancer and sporadic basal-like or TNBC share many clinical and pathological similarities (11, 12).

Applying these similarities, clinical trials have tested the effectiveness of the PARP inhibitor on BRCA-proficient TNBC patients. However, the PARP inhibitor alone failed to show clinical effects in patients with TNBC (13). Subsequent studies are ongoing to test the efficacy of the combined use of the PARP inhibitor and cytotoxic chemotherapy or radiotherapy.

Radiation induces DNA double strand breaks (DSB), and the phosphoinositide 3-kinase (PI3K) signaling pathway has been known to regulate steady state levels of HR (14). Furthermore, Ibrahim et al. published interesting research results that PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient TNBC to PARP inhibition (15). Inhibition of the PI3K signaling pathway induces feedback upregulation of extracellular signal-regulated kinase (ERK) and subsequent increased activation of ETS1 as an ERK-related transcription factor. ETS1 suppresses BRCA1/2 expression and impairs HR, thereby sensitizes the cells to the PARP inhibitor. In addition, preclinical studies showed increased radiosensitivity with use of the PARP inhibitor in replicating cells (16).

Taken together, radiation, PI3K inhibitors, and PARP inhibitors may enhance the tumor cell killing effects of each other, and we postulated that the combined use of a PARP inhibitor and PI3K inhibitor would sensitize cells to the radiation effect. Therefore, we assessed the radiation sensitizing effect of combined treatment with olaparib and PI-103 in BRCA-proficient TNBC cells and investigated its mechanism of action.

Materials and Methods

Cell culture

Triple negative, BRCA-proficient breast cancer cell line MDA-MB-435s and MDA-MB-231-BR (American Type Culture Collection, Rockville, MD, USA) were cultured in RPMI 1640 (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco, Carlsbad, CA, USA) at 37 °C in an atmosphere of 95% air and 5% CO_2.

Pharmacologic inhibitors

Olaparib was obtained from Selleck Chemicals (Houston, TX, USA) and PI-103 (pyridinylfuranopyrimidine inhibitor) was obtained from Calbiochem (Billerica, MA, USA).

siRNA transfection

BRCA1 siRNA was obtained from BioNeer (Alameda, CA, USA). Cells were plated in six-well plate and transfected with 100 nM BRCA1 siRNA using Lipofectamine® RNAiMAX transfection reagent (Life Technologies, Grand Island, NY, USA) according to the manufacturer's protocol.

Clonogenic assay

Clonogenic assay was done according to a previously described protocol (17). Appropriate numbers of cells were plated across the different treatment groups for each radiation dose. Treatment groups were as followed; irradiation alone (IR), olaparib + IR, PI-103 + IR, olaparib + PI-103 + IR. Olaparib (1 μM) and PI-103 (0.4 μM) was treated 2 hours before irradiation (IR) with

reference to the previous studies (18, 19). A specified number of cells were seeded into six-well plates and irradiated with 6 MV X-ray from a linear accelerator (Varian Medical System, Palo alto, CA, USA) at a dose rate of 2.46 Gy/min. After 22 hours incubation, medium was replaced with drug-free, FBS containing medium, and cells were incubated for colony formation for 14 to 21 days. Colonies were fixed with methanol and stained with 0.5% crystal violet; the number of colonies containing at least 50 cells was determined and surviving fraction was calculated. Survival data was fitted to a linear-quadratic model using Kaleidagraph version 3.51 (Synergy Software, Reading, PA, USA). Each point on the survival curves represents the mean surviving fraction (SF) from at least three dishes. Sensitizer enhancement ratio (SER) was defined as the ratio of the isoeffective dose at SF 0.5 in the absence of inhibitors to that in the presence of inhibitors. Average surviving fraction relative to radiation alone group (SF_O) at each radiation dose point was calculated. Expected surviving fraction for two drug combination (SF_E) was calculated as the product of SF_O of each single drug group. Synergistic index (SI) was calculated as the SF_E/SF_O and SI > 1.00 indicates a synergistic effect (20).

Western blotting

Cells were washed, scraped, and resuspended in lysis buffer (iNtRON Biotechnology, Seongnam, Korea). Proteins were solubilized by sonication, and equal amounts of protein were separated by SDS-PAGE and electroblotted onto polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA). Membranes were blocked in PBS containing 0.1% Tween 20 and 5% powdered milk, and probed with primary antibody directed against p-AKT (Ser473), p-ERK (Tyr202/204), Rad51 (Cell Signaling

Technology, Danvers, MA, USA); BRCA1, p-DNA-protein kinases (PKs) (Ser2056), PAR (Abcam, Cambridge, UK), and β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Membranes were washed and incubated with secondary antibody consisting of peroxidase-conjugated goat anti-rabbit or anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) at a dilution of 1:10000 for 1 hour. Membrane washing and Western blot protein detection was performed using the ECL kit (iNtRON Biotechnology, Seongnam, Korea).

Immunofluorescence of γH2AX

Cells were grown and treated on chamber slides. At 17 hours after irradiation, cover slips were rinsed; cells were fixed in 4% paraformaldehyde, and permeablized in methanol for 20 minutes. Cells were subsequently washed and blocked in PBS containing 2% bovine serum albumin for 1 hour. Primary antibody against γH2AX (Cell Signaling Technology) was applied to the cells and incubated overnight. Secondary AlexaFluor 488–conjugated donkey anti-goat antibody (Molecular Probes, Eugene, OR, USA) was applied and incubated for 1 hour. 4′,6-Diamidino-2-phenylindole nuclear counter stain was applied at 1 μg/mL for 5 minutes. Slides were examined on an Axio Scope.A1 Imager fluorescent microscope. Images were captured and acquired using AxioCam MRc5 and the acquisition software AxioVision v.4.4 (Carl Zeiss, Jena, Germany).

In vivo tumor model

Animal experimentation was performed under a protocol approved by institutional animal care and use committee of Seoul National University

Bundang Hospital (No. BA1103-078/015-01).

Cell labeling and implantation

Cell line was transfected with a pGL4 luciferase reporter vector (Promega, Madison, WI, USA) per manufacturer's protocol. After anesthesia, nude mouse was immobilized and prepared transfected MDA-MB-435s cells were subcutaneously implanted. One week after implantation, intraperitoneal administration of olaparib and PI-103 of 10 mg/kg was performed 3 times a week for 2 weeks. After drug treatment, these mice were irradiated 3 times a week with 3 Gy per fraction. Then mice were observed for 2 weeks.

Bioluminescent imaging (BLI)

BLI was obtained using IVIS Lumina II (Caliper, Hopkinton, MA, USA) BLI system following manufacturer's protocol. At 1 week after tumor cell implantation, baseline imaging was obtained and at 2 weeks after IR, follow-up imaging was obtained. After anesthesia, D-luciferin was injected intraperitoneally. Imaging was obtained at 5 minutes after luciferin injection was repeated every few minutes to determine the maximum luminescence intensity in photons/seconds. After image acquisition, a volume of interest was drawn around each tumor, and a background volume of interest was selected in an area free from tumor to generate a background-corrected bioluminescence flux value. The maximum background-corrected value for that tumor during the 30-minute imaging session was used as the maximum bioluminescent value.

Results

Combined use of olaparib and PI-103 enhanced the radiation-induced cell killing effect in TNBC cells

Pretreatment with combined olaparib and PI-103 resulted in a significant increase of the radiation-induced cell death of both MDA-MB-435s ($SER_{0.05}$ 1.7) and MDA-MB-231-BR cells ($SER_{0.05}$ 2.1) (Fig. 1A and B). Colony formation in the combined olaparib and PI-103 group without radiation was also decreased compared to the control group ($p = 0.03$), but was not significantly different from the single drug groups ($p > 0.05$). Radiation-induced cell death at 8 Gy was significantly enhanced in the combination group compared to the single drug groups for both MDA-MB-435s cells ($p = 0.005$ for PI-103 and $p < 0.001$ for olaparib) and MDA-MB-231-BR cells ($p < 0.001$ for PI-103 and $p = 0.01$ for olaparib) by *t*-test. The synergistic index of combination treatment at each radiation dose point was > 1.00 (1.07/1.27/1.20/1.81 at 2/4/6/8 Gy, respectively).

To compare these results with BRCA-deficient cells, MDA-MB-435s cells were transfected with BRCA1 siRNA. As shown in Fig 1C, there seemed to be a radiation sensitizing effect with combined use of olaparib and PI-103 in BRCA1 downregulated cells ($SER_{0.05}$ 1.24) compared with the control. However, adding PI-103 to the olaparib treatment did not result in further enhancement of the radiation-induced cell killing effect compared with olaparib alone.

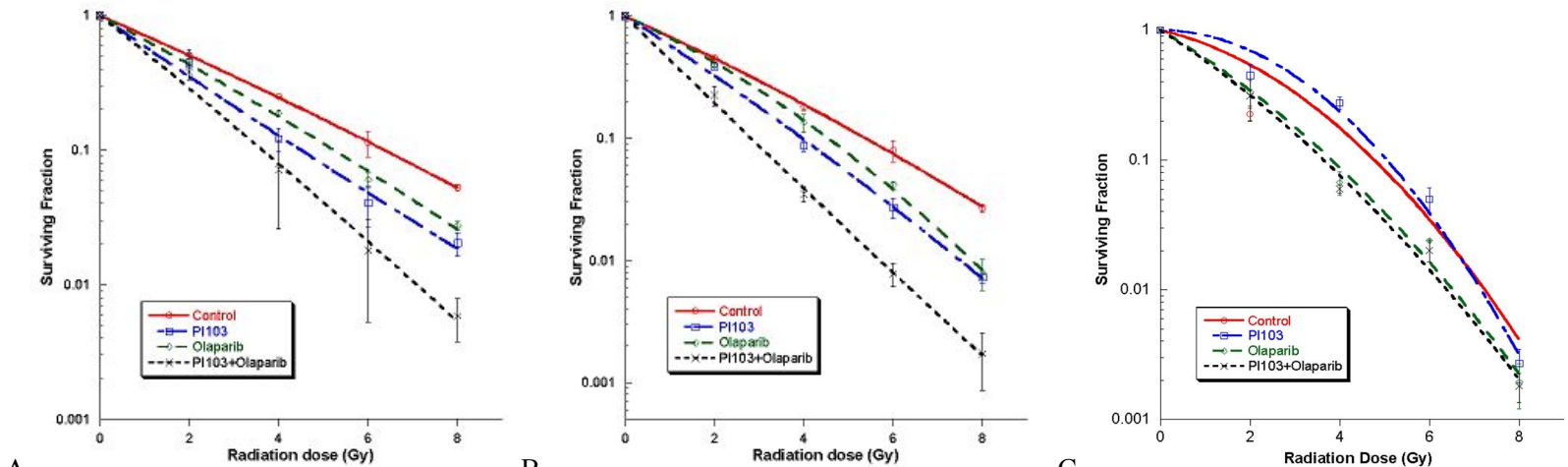


Fig. 1. Effect of olaparib and PI-103 on radiosensitivity of MDA-MB-435s (A), MDA-MB-231 (B), and MDA-MB-435s cells transfected with BRCA1 siRNA (C). Western blotting showed decreased BRCA1 with BRCA1 siRNA transfection (D). Cells were pretreated with 1 μM of olaparib and 0.4 μM of PI-103 for 2 hours according to the treatment groups and irradiated.

Combined use of olaparib and PI-103 enhanced in vivo radiation-induced cell killing effect

After confirming the in vitro radiosensitizing effect of the combined use of olaparib and PI-103, we investigated the in vivo effect. MDA-MB-435s cells transfected with a luciferase reporter vector were implanted into nude mice, which were treated according to the treatment groups. At 2 weeks after commencement of the treatment, the in vivo tumor size was examined using the IVIS Lumina II BLI system. As shown in Fig. 2, a marked decrease of tumor volume was induced by adding olaparib and PI-103, compared to radiation alone ($p < 0.001$ by *t*-test). Means of ROI values were significantly different between the groups ($p < 0.001$ by one way analysis of variance).

PI-103 induced prolongation of γH2AX foci formation

Pretreatment with PI-103 and olaparib plus PI-103 caused marked prolongation of γH2AX foci formation, indicating delayed DNA repair, while irradiation and pretreatment with olaparib alone showed relatively less foci at 17 hours following treatment (Fig. 3A).

Because pretreatment with PI-103 resulted in prolongation of radiation-induced γH2AX foci, we evaluated the molecules involved in DNA damage repair. Pretreatment with PI-103 was associated with decreased p-DNA-PK and Rad51 (Fig 3B).

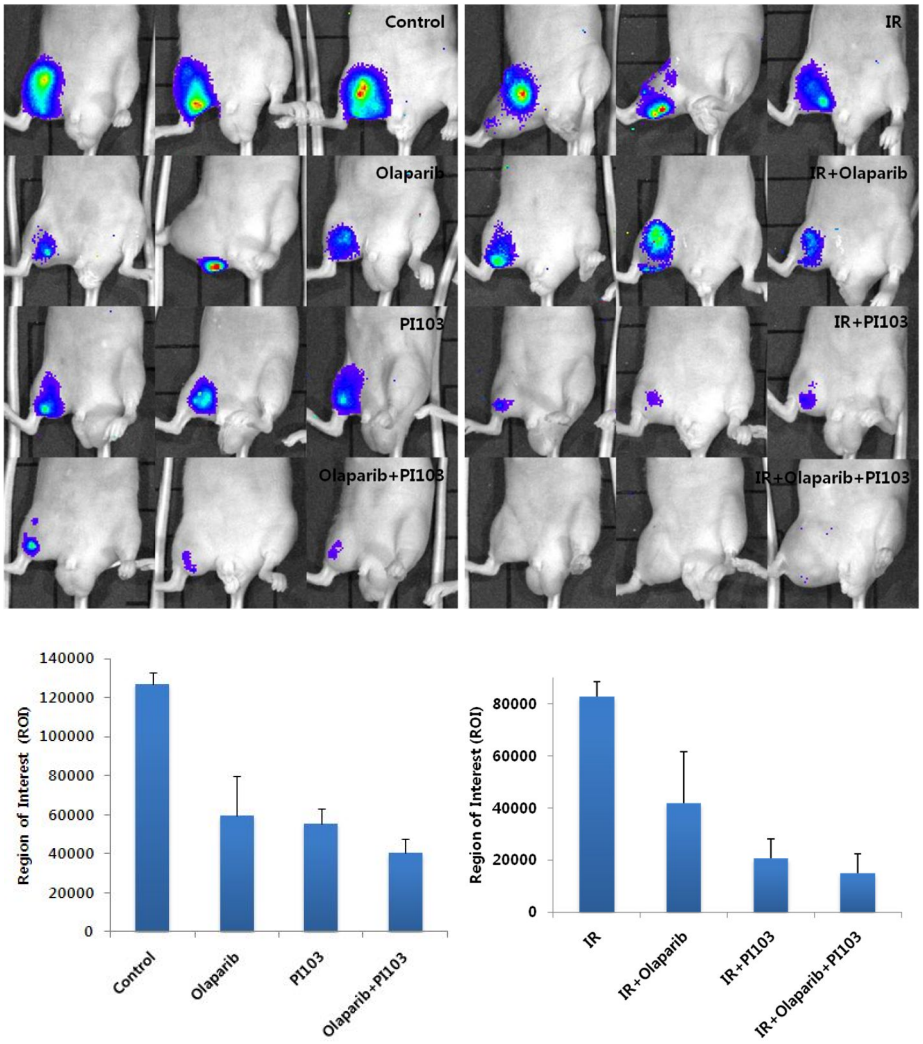
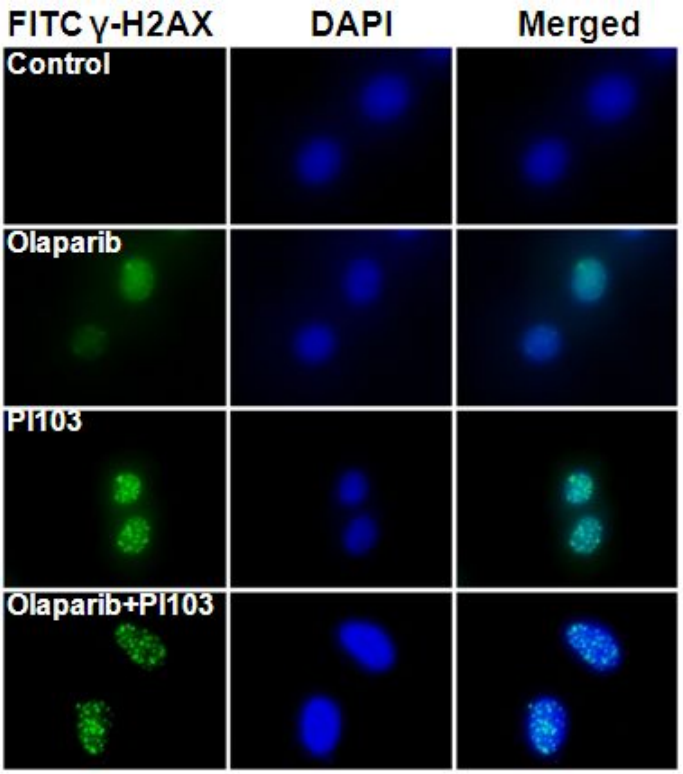


Fig. 2. In vivo radiosensitizing effect of combined use of olaparib and PI-103. MDA-MB-435s cells were transfected with pGL4 luciferase reporter vector and implanted into nude mice. After intraperitoneal administration of olaparib and PI-103, IR was performed. In vivo BLI was obtained using the IVIS Lumina II BLI system 2 weeks after treatment. Means of ROI values were significantly different between groups ($p < 0.001$).

A



B

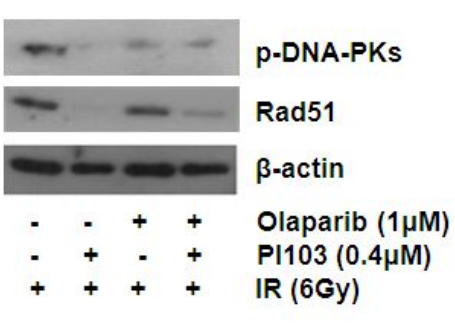


Fig. 3A. Immunofluorescent detection of γH2AX. MDA-MB-435s cells were treated with olaparib (1 μM) and PI-103 (0.4 μM) according to the treatment groups 2 hours before irradiation (IR) of 6 Gy. After 17 hours of IR, γH2AX was detected by immunofluorescence. 3B. Western blotting of cell lysates from MDA-MB-435s cells.

PI-103 induced upregulation of ERK and downregulation of BRCA1

Decreased level of p-AKT and PAR, induced by treatment with PI-103 and olaparib, respectively, showed that the drugs were working well (Fig 4A). To investigate the possible mechanisms of radiosensitization, we analyzed changes of the candidate proteins with reference to the study of Ibrahim et al (15). It is a well-known phenomenon that inhibition of the PI3K signaling pathway induces activation of the ERK pathway (21, 22). As expected, treatment with PI-103 induced p-ERK elevation. Pretreatment with PI-103 was associated with activation of ERK and downregulation of BRCA1, while increased PAR observed with PI-103 treatment disappeared with the addition of olaparib (Fig 4).

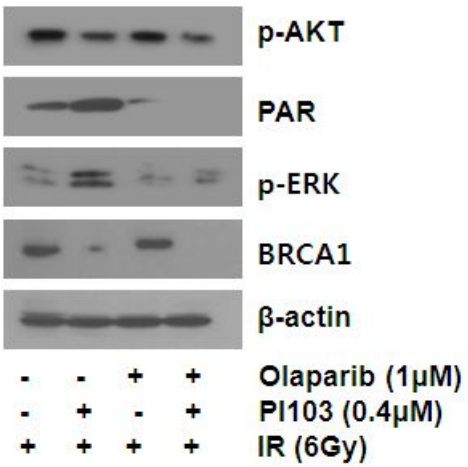


Fig. 4. Western blotting of cell lysates from MDA-MB-435s cells according to the treatment groups. Olaparib (1 μM) and PI-103 (0.4 μM) was treated 2 hours before irradiation (IR) of 6 Gy.

Discussion

TNBC is known to have aggressive clinical behaviors and high mortality rates (1). A high incidence and early development of distant metastasis to areas such as brain or lung in TNBC or basal-like breast cancer cases has been reported in the literature, while the median duration of survival after distant metastasis was significantly shorter than that of other subtypes (23). In addition, the basal-like subtype was associated with an increased risk of local and regional relapse after surgery on multivariate analysis (24). Furthermore, lack of specific treatment targets, such as ER or HER-2, presents a major problem for the treatment of TNBC patients. To reduce locoregional relapse and to treat brain metastasis or other oligometastasis, effect of radiation on TNBC needs to be enhanced.

PARP inhibitor has shown a significant clinical benefit in BRCA related TNBC patients (25). However, in clinical situations, carriers of BRCA mutation account for only a part of TNBC or basal-like breast cancer patients, so we also need to focus on the treatment of BRCA-proficient TNBC. As BRCA mutated breast cancer and sporadic basal-like or TNBC share many clinical and pathological similarities (11, 12), the PARP inhibitor was expected to bring on a new era in the treatment of TNBC. However, clinical outcomes of the PARP inhibitor alone were disappointing for sporadic TNBC. To enhance the efficacy, studies on the combined use of PARP inhibitor and other treatments are ongoing.

Herein, we assessed the radiation sensitizing effect of combined treatment with olaparib and PI-103 in BRCA-proficient TNBC cell lines, which were derived from metastatic sites. Survival curves obtained by the clonogenic assay showed increased radiation-induced cell death with the combined

treatment with olaparib and PI-103 in both MDA-MB-435s (Fig. 1A) and MDA-MB-231-BR (Fig. 1B) cell lines. The MDA-MB-231-BR cell line is a subclone of MDA-MB-231 that selectively metastasizes to the brain, demonstrating that this combined targeting strategy may be applied to enhance the effects of radiation on brain metastasis of TNBC patients.

Therapeutic radiation induces DNA damage with single strand breaks (SSB) and DSBs. PARP-1 detects SSBs, binds to the DNA break sites, is then autophosphorylated and recruits the enzymes required to form the BER multi-protein complex (6, 7). DSBs are repaired by nonhomologous end joining (NHEJ) and HR. NHEJ repairs most radiation induced DSBs, does not require a template, and may occur during any stage of the cell cycle. In contrast, HR is an error prone process and requires a sister chromatid as a template, thus can only occur during the S and G2 phase (16, 26).

Under normal conditions, SSBs are efficiently repaired and do not lead to significant cell death. However, unrepaired SSBs or delay in the repair of SSBs during the DNA replication process can induce DSBs, which must be repaired by HR. Therefore, PARP inhibition is an effective treatment strategy for tumors with HR deficiency, such as those with a BRCA mutation. As expected, knockdown of BRCA1 by siRNA caused a high degree of radiosensitization in itself, and addition of olaparib further enhanced the effects of radiation as shown in results (Fig 1C).

Radiation induces DNA damage, and the PARP inhibitor suppresses DNA repair. Thus, radiation and PARP inhibitor would have synergistic effects for killing tumor cells, and many preclinical studies have shown increased radiosensitization with the use of the PARP inhibitor on replicating cells (16). Generally, tumors have a higher proportion of replicating cells than the surrounding normal tissues, making this strategy very attractive in the field of radiation oncology. Consistent with other studies, we also observed in vitro

and in vivo radiosensitizing effects of PARP inhibition.

In addition, targeting of the PI3K signaling pathway is a well-known strategy to enhance radiation sensitivity based on the findings that PI3K controls DNA DSB repair (14). PI-103 is a potent inhibitor of class I PI3Ks/mTOR/DNA-PK. Pretreatment with PI-103 could impair DNA repair via inhibition of the PI3K signaling pathway and DNA-PK. As expected, PI-103 delayed DNA repair and was related with decreased RAD51 and p-DNA-PK in the MDA-MB-435s cells tested as shown in current study (Fig. 3). Under impaired conditions of DNA DSB repair caused by PI-103, cells may become more dependent on BER. Consequently, pretreatment with PI-103 induced increased PAR levels (= increased PARP activity) in this study (Fig. 4), as seen in other studies (15, 27). This increased activation of PARP was completely blocked by adding olaparib, and the combined use of PI-103 and olaparib showed increased effects of radiation treatment in both in vitro and in vivo models.

It is well known that inhibition of the PI3K signaling pathway induces compensatory activation of the ERK pathway (21, 22), and results also revealed elevated p-ERK after treatment with PI-103 (Fig. 4). Abnormally elevated ERK activity was generally thought to be related with cancer cell survival and progression (28), but the recent study of Ibrahim et al. showed interesting results that PI3K inhibition impaired BRCA1/2 expression via elevated ERK and sensitized BRCA-proficient TNBC to PARP inhibition (15). They measured BRCA1/2 mRNA levels in several BRCA-proficient TNBC cell lines (MDA-MB-468, MDA-MB-231, HCC1143, and HCC70) treated with NVP-BKM120 (pan-PI3K inhibitor) by quantitative real-time PCR, and decreased BRCA1/2 mRNA level occurred in all of the tested cell lines. Inhibition of the PI3K signaling pathway induces feedback upregulation of ERK and subsequent increased activation of ETS1, as an ERK-related

transcription factor. ETS1 is a negative regulator of BRCA1/2 expression, and elevated ETS1 suppresses BRCA1/2 expression. Consequently, the impaired HR sensitizes the cells to PARP inhibitors. As in Ibrahim's study, it was also revealed that PI-103induces downregulation of the PI3K pathway, upregulation of p-ERK, and decreases BRCA1 levels (Fig 4). In addition, adding PI-103 to olaparib did not result in further enhancement of the radiation-induced cell killing effect compared to olaparib alone in MDA-MB-435s transfected with BRCA1 siRNA (Fig 1C), supporting the hypothesis that PI3K inhibition results in HR impairment via BRCA downregulation.

In summary, the combined use of olaparib and PI-103 enhanced the radiation-induced cell killing effect in BRCA-proficient MDA-MB-435s and MDA-MB-231-BR cell lines and xenografts. TNBC patients have high incidences of locoregional relapse and distant metastasis, and radiation therapy is involved not only in locoregional control, but also in the treatment of distant recurrences such as brain metastasis or other oligometastasis. Targeting of the PI3K signaling pathway combined with PARP inhibition could be a reasonable approach to enhance effects of radiation on BRCA-proficient TNBC.

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국 문 초 록

삼중음성 유방암에서 olaparib과 PI-103 동시 사용 시의 방사선감수성 증강작용 확인 및 그 기전에 대한 연구

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서 론: 삼중음성 유방암은 공격적인 임상양상을 보이지만 특별한 치료 목표물이 없어서 치료 방법에 제한이 있어 왔다. 삼중음성 유방암이 BRCA 돌연변이가 있는 유방암과 많은 비슷한 임상적, 병리적 특성을 가진다는 점과 poly(ADP-ribose) polymerase (PARP) 억제제가 BRCA 돌연변이 환자에게서 임상적으로 성과를 거둔 점에 착안하여 PARP 억제제를 삼중음성 유방암에 도입해보았으나, 실제 임상에서 PARP 억제제 단독 사용은 효과가 없었다. 방사선은 DNA 이중나선절단을 유도하고 phosphoinositide 3-kinase (PI3K) 신호전달체계는 상동재조합 (homologous recombination)을 유지 조절하는데 필요한 것으로 알려져 있으며, 최근의 전임상연구에 따르면 PI3K를 억제하면 BRCA1/2의 발현이

억제되어서 BRCA 돌연변이가 없는 삼중음성 유방암에서도 PARP 억제제의 감수성을 증가시킨다고 한다. 이를 바탕으로 BRCA 돌연변이가 없는 삼중음성 유방암에서 olaparib과 PI-103을 동시에 사용하여 방사선감수성을 증강시킬 수 있는지 확인하고 그 기전에 대해 연구해보았다.

방법: MDA-MB-435s 세포를 방사선치료 단독, olaparib + 방사선치료, PI-103 + 방사선치료, olaparib + PI-103 + 방사선치료, 이렇게 4개의 치료군으로 나누어 실험하였다. 세포를 방사선조사 2시간 전에 약물처리 하였으며, 세포생존곡선은 집락형성 분석법을 이용하여 구하였다. 웨스턴 블로팅과 γH2AX foci에 대한 면역형광법을 실시하고, 생체내 방사선감수성 평가를 위해 쥐에 세포주를 이종이식한 뒤 생체발광영상을 이용하였다.

결과: Olaparib과 PI-103의 동시 사용은 MDA-MB-435s ($SER_{0.05}$ 1.7)와 MDA-MB-231-BR ($SER_{0.05}$ 2.1) 세포주를 이용한 생체외 실험에서 방사선감수성을 증가시켰으며 또한 이종이식 모델에서도 종양부피를 현저하게 감소시켰다. ($p < 0.001$). PI-103을 처리했을 때 DNA 나선절단의 복구가 지연되었음을 의미하는 지속된 γH2AX foci가 관찰되었으며, phosphorylated extracellular signal-regulated kinase과 poly(ADP-ribose)의 상승과 BRCA1의 감소가 나타났다.

결론: Olaparib과 PI-103을 동시 사용은 MDA-MB-435s, MDA-MB-231-BR 세포주와 이종이식 모델에서 모두 방사선감수성을 증가시켰다. 삼중음성 유방암은 국소재발과 원격전이 확률이 모두 높으며, 최근의 방사선치료는 국소질환뿐

아니라 뇌전이나 소수 전이암 같은 원격전이 질환에 대해서도 중요한 역할을 담당하고 있다. PARP 억제제와 함께 PI3K 신호전달체계를 억제하는 치료 전략은 이러한 BRCA 야생형 삼중음성 유방암 환자들에게 있어서 방사선치료 효과를 높이는데 응용할 수 있는 기초 전략이 될 수 있을 것으로 생각된다.

주요어: 삼중음성 유방암, 방사선치료, olaparib, PI-103, PARP 억제제, PI3K 억제제

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