BMC Cancer

RESEARCH

Open Access

The combination of poly(ADP-ribose) polymerase inhibitor and statin inhibits the proliferation of human castration-resistant and taxane-resistant prostate cancer cells in vitro and in vivo

Yoshitaka Sekine[1*], Daisuke Oka[1], Akira Ohtsu[1], Hiroshi Nakayama[1], Takeshi Miyao[1], Yoshiyuki Miyazawa[1], Seiji Arai[1], Hidekazu Koike[1], Hiroshi Matsui[1], Yasuhiro Shibata[1] and Kazuhiro Suzuki[1]

Abstract

Background Olaparib exhibits antitumor effects in castration-resistant prostate cancer patients with germline mutations in DNA repair genes. We previously reported that simvastatin reduced the expression of DNA repair genes in PC-3 cells. The efficacy of combination therapy using olaparib and simvastatin as "BRCAness" in castration-resistant and taxane-resistant prostate cancers was evaluated in this study.

Methods PC-3, LNCaP, and 22Rv1 human prostate cancer cell lines were used to develop androgen-independent LNCaP cells (LNCaP-LA). mRNA and protein expression levels were evaluated by quantitative real-time polymerase chain reaction and western blot analysis, respectively. Cell viability was determined using the MTS assay and cell counts. All evaluations were performed on cells treated with simvastatin with or without olaparib.

Results The mRNA levels of BRCA1, BRCA2, RAD51, FANCD2, FANCG, FANCA, BARD1, RFC3, RFC4, and RFC5, which are known DNA repair genes, were downregulated by simvastatin in androgen-independent prostate cancer cells, such as PC-3, LNCaP-LA, and 22Rv1 cells. In contrast, the expression of all these genes remained unchanged in androgen-dependent LNCaP cells following treatment with simvastatin. Furthermore, simvastatin increased the expression of above stated genes in normal prostate stromal cells (PrSC). The reduction in BRCA1 and BRCA2 expression following siRNA transfection increased the cytocidal effects of Olaparib in PC-3 and LNCaP-LA cells. The combination of olaparib and simvastatin further inhibited cell proliferation compared to monotherapy with either drug in PC-3, 22Rv1, and LNCaP-LA cells but not in PrSC cells. In a 22Rv1-derived mouse xenograft model, the combination of olaparib and simvastatin enhanced the inhibition of cell proliferation. Moreover, we established a 22Rv1 cell line with acquired resistance to Cabazitaxel (22Rv1-CR). In 22Rv1-CR cells, simvastatin also decreased the expression of BRCA1, BRCA2,

*Correspondence:
Yoshitaka Sekine
ysekine@gunma-u.ac.jp

Full list of author information is available at the end of the article

and FANCA, and the combination of olaparib and simvastatin further enhanced the inhibition of cell proliferation compared with treatment with either of the drugs alone.

Conclusions Simvastatin altered the expression of several genes associated with DNA repair in castration-resistant and taxane-resistant prostate cancer cells. The combination of poly (ADP-ribose) polymerase inhibitors and drugs that decrease DNA repair gene expression can potentially affect castration-resistant and taxane-resistant prostate cancer growth.

Keywords Prostate cancer, Statin, Simvastatin, Olaparib, DNA repair gene, BRCA1, BRCA2

Background

Androgen deprivation therapy plays an important role in the treatment of metastatic prostate cancer. In addition, new anti-androgen therapies, such as enzalutamide, abiraterone, apalutamide, and darolutamide, have been introduced for castration-resistant prostate cancer (CRPC) [1–4]. However, most CRPC cases are difficult to control after a few years. Although docetaxel and cabazitaxel have been cited as other treatments for CRPC, these therapies are also ineffective. Therefore, new therapeutic methods are required for CRPC treatment.

Statins comprise a class of drugs used to treat hyperlipidemia and prevent cardiovascular disease. Previous studies have demonstrated that statins reduce the risk of metastatic prostate cancer and mortality in prostate cancer patients [5, 6]. Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A, which decreases intracellular cholesterol levels in prostate cancer cells [7]. Cholesterol is necessary for cancer cells, and its deficiency inhibits the progression of prostate cancer cells [8, 9]. Additionally, statins have been reported to exert antitumor effects through a variety of mechanisms [10–12]. Statins appear to possess pleiotropic effects in prostate cancer.

Olaparib is an inhibitor of poly(ADP-ribose) polymerase (PARP)1 and PARP2, which play fundamental roles in the DNA damage response. PARP inhibitors exhibit therapeutic effects in some cancers with Breast Cancer 1 (BRCA1) and Breast Cancer 2 (BRCA2) deficiencies through the mechanism of synthetic lethality [13, 14]. In prostate cancer, olaparib has been reported to be effective in CRPC patients with germline mutations in DNA repair genes [15, 16]. In addition, combination therapy with PARP and androgen receptor inhibitors reduces the expression of DNA repair genes and inhibits the growth of androgen-independent prostate cancer cells [17]. These results demonstrated that pharmaceutically induced "BRCAness" opens the possibility of the application of PARP inhibitors as agents for CRPC treatment [17].

In a previous study [12, 18], we investigated the effect of statins on gene expression in PC-3 cells. Using microarray data, pathway analysis revealed that simvastatin downregulated DNA repair genes. In this study, we investigated the efficacy of combination therapy with olaparib and statins for castration-resistant and taxane-resistant prostate cancers.

Methods

Cells and chemicals

Human prostate cancer cell lines PC-3, LNCaP, and 22Rv1 were purchased from DS Pharma Biomedical (Osaka, Japan) and cultured in RPMI 1640 (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS) (Moregate, Bulimba, Australia). Normal human prostate stromal cells (PrSC) were purchased from Lonza (Walkersville, MD) and cultured in SCBM (Lonza) supplemented with 10% FBS. LNCaP-LA cells generated from LNCaP cells were cultured in a medium containing 10% charcoal-stripped FBS (CS-FBS) for >3 months. To induce cabazitaxel resistance in the prostate cancer cell line 22Rv1, we referred to our previously reported method [19]. Cells that grew in 5-nmol/L cabazitaxel were designated as 22Rv1-CR. We used the above culture medium for each cell line in all experiments.

Quantification of mRNA levels

mRNA levels were quantified using the Applied Biosystems 7300 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Waltham, MA, USA). Complementary DNA synthesis was performed [20], and PCR amplification was conducted using 2 µL of complementary DNA, and the BRCA1, BRCA2, RAD51, Fanconi Anemia Complementation Group D2 (FANCD2), Fanconi Anemia Complementation Group G (FANCG), Fanconi Anemia Complementation Group A (FANCA), BRCA1 Associated RING Domain 1 (BARD1), Replication Factor C Subunit (RFC) 3, RFC4, and RFC5 primers (Hs01556193_m1, Hs00609073_m1, Hs00947967_m1, Hs00276992_m1, Hs00184947_m1, Hs01116668_m1, Hs00184427_m1, Hs00161357_m1, Hs00427469_m1 and Hs00738859_m1, respectively; Applied Biosystems). Next, PCR was performed for one cycle of 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 60 s at 60 °C. β-actin (Applied Biosystems) transcript levels were used as internal controls. Fold changes in mRNA expression were determined using the comparative CT ($2^{-\Delta\,\Delta Ct}$) cycle (ΔCt) method [21].

Western blotting assays

Cell lysates were prepared in radio-immunoprecipitation assay buffer (Pierce) containing protease inhibitors (Complete without ethylenediamine tetraacetic acid, Roche Diagnostics, Basel, Switzerland). Equal amounts of proteins (20–40 μg/lane) were electrophoresed on a 4–12% sodium dodecyl sulfate-polyacrylamide gel and transferred onto nitrocellulose membranes. Rabbit anti-BRCA1 polyclonal antibody, Rabbit anti-RAD51 Recombinase (RAD51) monoclonal antibody, mouse anti-BARD1 monoclonal antibody, rabbit anti-FANCA monoclonal antibody, rabbit anti-γH2AX monoclonal antibody and rabbit anti-human β-actin monoclonal antibody were purchased from Santa Cruz (Dallas, USA), Cell Signaling (Beverly, USA), Cell Signaling (Beverly, USA), Cell Signaling (Beverly, USA), Cell Signaling (Beverly, USA) and A&G Pharmaceutical (Columbia, USA), respectively. Each membrane was incubated with one of the following antibodies at 4 °C overnight: BRCA1 (1:100), RAD51 (1:1000), BARD1 (1:100), FANCA (1:200), γH2AX (1:1000), and β-actin (1:1000). Blots were developed using a 1:2000 dilution of horseradish peroxidase-conjugated secondary antibody (Cell Signaling). Proteins were visualized using Immobilon Western horseradish peroxidase Reagent (Millipore, Burlington, MA, USA). For BRCA1, protein lysates (60–100 μg/lane) were electrophoresed on a 3–8% sodium dodecyl sulfate-polyacrylamide gel and transferred onto PVDF membranes. A representative of three independent experiments is shown in each figure.

SiRNA transfection experiments

Approximately 4×10^3 PC-3 cells per well or 7×10^3 LNCaP-LA cells per well were seeded into a 96-well microtiter plate in a 100-μL medium for 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2 H-tetrazolium (MTS) assay (Celltiter 96 Aqueous one solution cell proliferation assay, Promega, Madison, WI), and approximately 3×10^5 PC-3 cells per well or 6×10^5 LNCaP-LA cells per well into a 6-well microtiter plate in a 2300-μL medium for Western blotting for 24 h at 37 °C in a 5% CO_2 atmosphere. Subsequently, the cells were transfected with the ON-TARGETplus Non-targeting Pool (No. D-001810-10-05, Dharmacon, Lafayette, CO, USA), ON-TARGETplus BRCA1 siRNA (No. L-003461-00-0005, Dharmacon), or ON-TARGETplus BRCA2 siRNA (No. L-003462-00-0005, Dharmacon) using DharmaFECT (Dharmacon). Following transfection, the cells were incubated for 48 h before RNA levels were measured via qPCR or protein levels analyzed by western blotting.

Cell proliferation assay

Approximately 3×10^3 PC-3 cells per well, 6×10^3 LNCaP-LA cells per well, 7×10^3 LNCaP-LA cells per well, 7×10^3 22Rv1 cells per well, 7×10^3 PrSC cells per well or 8×10^3 22Rv1-CR cells were plated onto a 96-well plate in 100-μL of culture medium. After 24 h, the cells were incubated with a medium containing simvastatin (PC-3, 2 μM; other cells, 5 μM) and/or olaparib (10 μM). After incubation for each iteration, the number of live cells was measured using an MTS assay. The optical density of the cell lysate was expressed as fold change.

Animal studies

Five- to eight-week-old male BALB/c/nu mice (Charles River Laboratories Japan, Yokohama, Japan) were injected subcutaneously into the right flank with approximately 3×10^6 22Rv1 cells in BD Matrigel basement membrane matrix (BD Biosciences, USA), and castration surgery was performed. After 2 weeks, mice with well-established tumors (0.6–1.0 cm long and 0.6–1.0 cm wide) were orally dosed with 100 mg/kg simvastatin and/or 50 mg/kg olaparib once daily. Control mice received methylcellulose gavage and phosphate-buffered saline. The tumor sizes and weights were measured weekly for 8 weeks, and tumor volumes were calculated using the formula $A \times B \times C \times \pi/6$, where A, B, and C represent the length, width, and height of the tumor, respectively. Finally, the mice were euthanized 56 days after the xenograft, and the tumors were collected. Cervical dislocation was used to euthanize the mice without anesthesia, and the procedure was performed by researchers who had received appropriate training.

Statistical analysis

All data, unless otherwise indicated, are expressed as mean ± standard deviation. Differences between values were evaluated by one-way ANOVA using Tukey's post hoc analysis. In all analyses, $p < 0.05$ was considered statistically significant.

Results

Expression of DNA repair genes in androgen-independent prostate cancer cells after treatment with simvastatin

Microarray and pathway analyses were performed on PC-3 cells after simvastatin treatment in our previous studies [12, 18]. BRCA1, BRCA2, RAD51, FANCD2, FANCG, FANCA, BARD1, RFC3, RFC4, and RFC5, which are known DNA repair genes [15], were downregulated by simvastatin [18]. For validation, a quantitative reverse transcription-polymerase chain reaction (RT-PCR) was performed for these genes. Initially, we compared the basal mRNA and protein levels of these genes in LNCaP, which is androgen-dependent; PC-3, 22Rv1, and LNCaP-LA, which are androgen-independent; and

PrSC, which represents normal prostate cells. BRCA1, BRCA2, RAD51, RFC3, RFC4, and RFC5 mRNA levels were lower in PrSC cells than in other cells (Fig. 1A). In PC-3 cells, we were unable to detect protein expression of BRCA1 by western blotting (Fig. 1B). The relationship between mRNA and protein expression levels depends on the cell type. BRCA1 and RAD51 protein expression was not detected in PrSC (data not shown). The mRNA levels of BRCA1, BRCA2, RAD51, FANCD2, FANCG, FANCA, BARD1, RFC3, RFC4, and RFC5 were significantly reduced by simvastatin treatment in PC-3 cells. Simvastatin decreased the mRNA expression of BRCA1, RAD51, FANCD2, FANCG, FANCA, and RFC3 in 22Rv1 cells and in BRCA2, RAD51, BARD1, RFC3, and RFC5 in LNCaP-LA cells. In contrast, the expression of all these genes remained unchanged in androgen-dependent LNCaP cells after treatment with simvastatin. Moreover, simvastatin increased the expression of all these genes in PrSC cells (Fig. 1C). The protein levels of BRCA1 were evaluated by western blotting. Simvastatin decreased the protein levels of BRCA1 in both LNCaP-LA and 22Rv1 cells (Fig. 1D). In contrast, simvastatin did not alter the protein levels of BRCA1 in LNCaP cells (Fig. 1D).

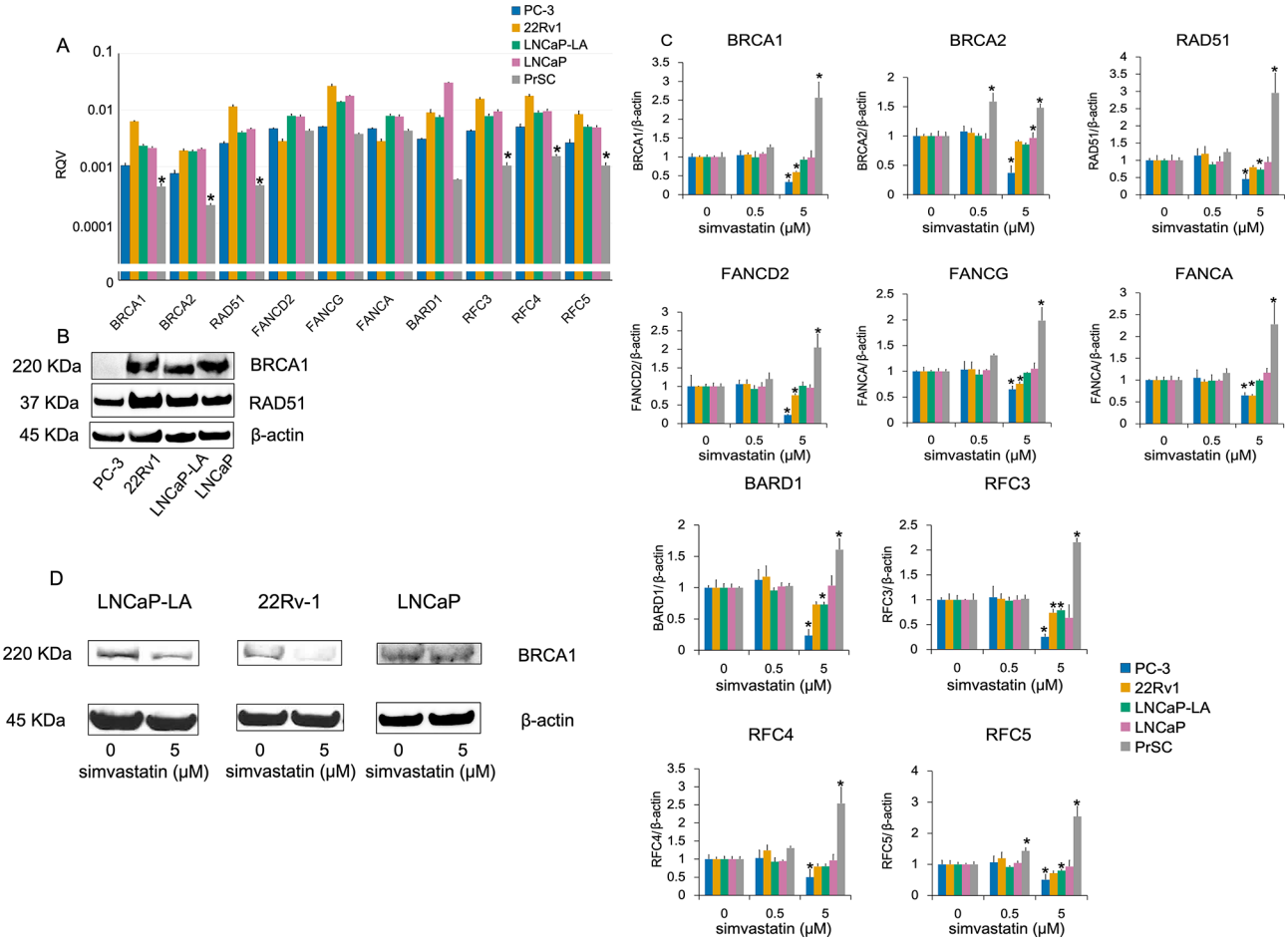

Fig. 1 Effect of simvastatin on DNA repair gene expression in prostate cancer and prostate stromal cells (PrSC). PC-3, 22Rv1, LNCaP-LA, LNCaP, and PrSC cells were incubated with the medium containing 10% FBS for 24 h, and the medium was switched to the indicated concentration of simvastatin in the medium containing 10% FBS. After 48 h, the total RNA (**A**, **C**) and total protein (**B**, **D**) were collected. (**A**) Comparison of mRNA expression levels of each gene in each cell without simvastatin. mRNA expression of BRCA1, BRCA2, RAD51, FANCD2, FANCG, FANCA, BARD1, RFC3, RFC4, and RFC5 was evaluated by performing real-time polymerase chain reaction tests, and the relative quantitative volume (RQV) was calculated by comparing the expression of β-actin. Values are expressed as mean ± standard deviation (SD) ($n = 3$). *$P < 0.05$ vs. the other cells. (**B**) Comparison of protein levels of BRCA1 and RAD51 in each cell without simvastatin. The protein expression of BRCA1 and RAD51 was evaluated by western blotting. A representative experiment is shown, which was repeated three times with similar results. (**C**) Comparison of mRNA expression levels of each gene in each cell after treatment of simvastatin. mRNA expression of BRCA1, BRCA2, RAD51, FANCD2, FANCG, FANCA, BARD1, RFC3, RFC4, and RFC5 was evaluated by performing real-time polymerase chain reaction tests, and the relative quantitative volume (RQV) was calculated by comparing the expression of β-actin. Values are expressed as mean ± standard deviation (SD) ($n = 3$). *$P < 0.05$ vs. 0 μM of each cell. (**D**) Comparison of BRCA1 protein levels in each cell after treatment of simvastatin. The protein expression of BRCA1 was evaluated by western blotting. A representative experiment is shown, which was repeated three times with similar results

The effect of BRCA1 and BRCA2 Inhibition for olaparib-induced Inhibition of cell proliferation in androgen-independent prostate cancer cells

To further determine whether the decreased expression of DNA repair genes affects olaparib-induced inhibition of cell viability, BRCA1 and BRCA2 expression was reduced by transfection with small interfering RNA (siRNA). After siRNA transfection, BRCA1 and BRCA2 mRNA expression were inhibited in both PC-3 and LNCaP-LA cells (Fig. 2A). The protein expression of BRCA1 was reduced by siRNA in LNCaP-LA cells (Fig. 2B). The reduction in BRCA1 and BRCA2

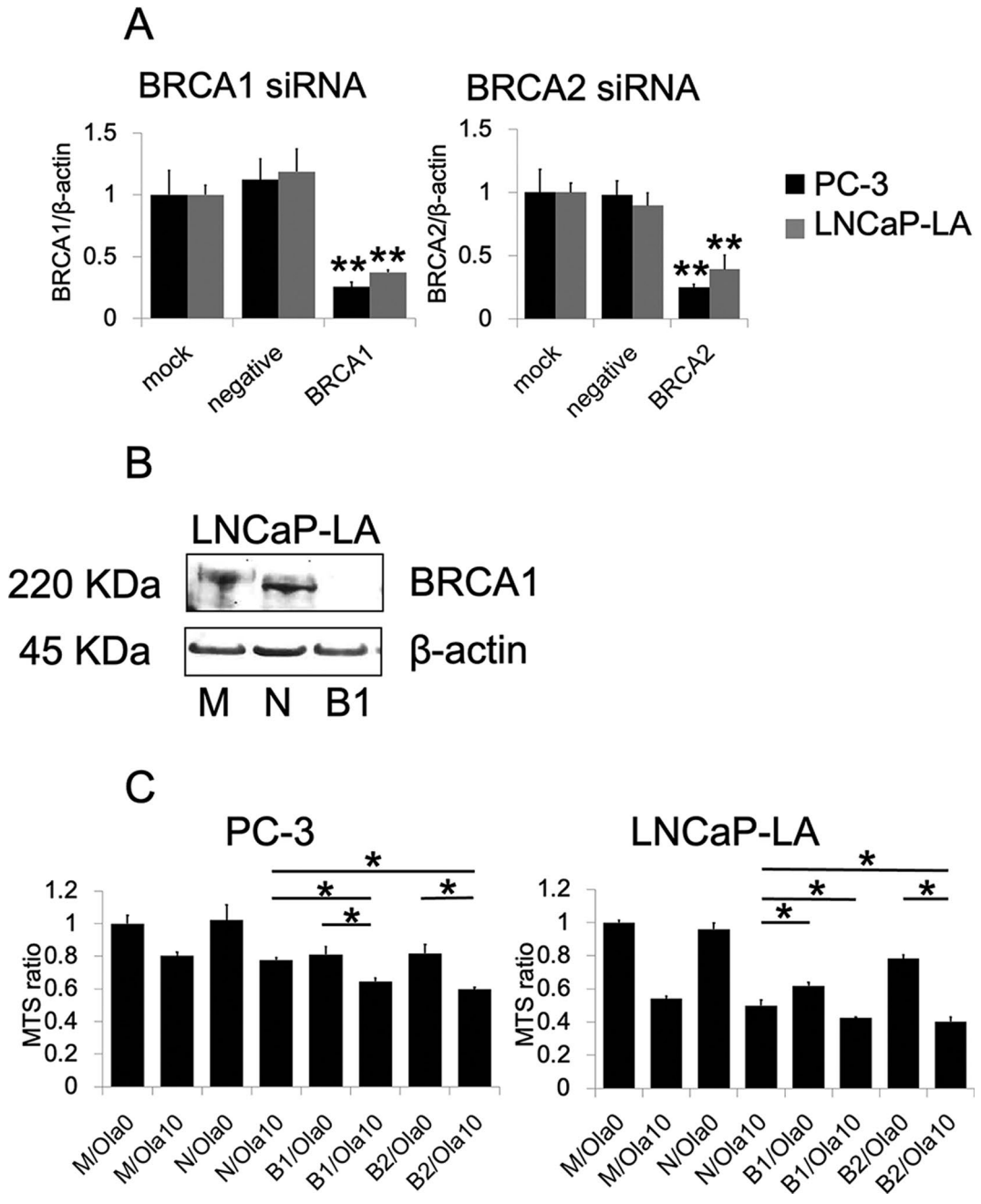

Fig. 2 Effect of BRCA1 and BRCA2 siRNA on androgen-independent prostate cancer cells. **A** and **B**. Effect of siRNA on BRCA1 and BRCA2 expression in PC-3 and LNCaP-LA cells. Mock transfected cells (M) or cells transfected with BRCA1 (B1) or BRCA2 (B2) siRNA or negative control siRNA (N) were incubated for 48 h before harvesting for real-time polymerase chain reaction (**A**) and western blotting (**B**). Values are expressed as means ± standard deviations (SD) ($n=3$). $^{**}P<0.01$ vs. negative (N). **C** Following transfection, PC-3, and LNCaP-LA cells were incubated in the medium. After 48 h, the cells were cultured in a medium with or without olaparib (10 μM). After 72 h (PC-3 cells) and 96 h (LNCaP-LA cells), the number of viable cells was evaluated using an MTS assay. Values are expressed as mean ± SD ($n=4$). $^{*}p<0.05$. Ola; olaparib

expression following siRNA transfection enhanced the cytocidal effects of olaparib in both PC-3 and LNCaP-LA cells (Fig. 2C). There were significant differences between olaparib in the presence of BRCA1 or BRCA2 siRNA and other groups in both cells.

The combined effects of simvastatin and Olaparib in cell proliferation and apoptosis in androgen-independent prostate cancer cells

We evaluated the combined effects of simvastatin and olaparib on cell proliferation in androgen-independent prostate cancer and PrSC cells. Treatment with either simvastatin or olaparib alone inhibited cell proliferation, and the combination of both drugs further enhanced the inhibition of cell proliferation in androgen-independent cells but not in PrSC cells (Fig. 3A and B). Moreover, treatment with olaparib alone inhibited cell proliferation, whereas the combination of simvastatin and olaparib did not further enhance the inhibition of LNCaP cell proliferation (Fig. 3A).

Combined effects of simvastatin and Olaparib in tumor growth in an androgen-independent 22Rv1 xenograft mouse model in vivo

To determine whether the combination of simvastatin and olaparib affected tumor growth in vivo, we injected nude mice with 22Rv1 cells. There were no deceased mice during treatment. Neither simvastatin nor olaparib affected body weight. The combination of simvastatin and olaparib significantly inhibited tumor growth when compared with the other groups (Fig. 4A). The expression of BRCA1 mRNA tended to decrease following treatment with simvastatin and olaparib, although no significant difference was observed (Fig. 4B).

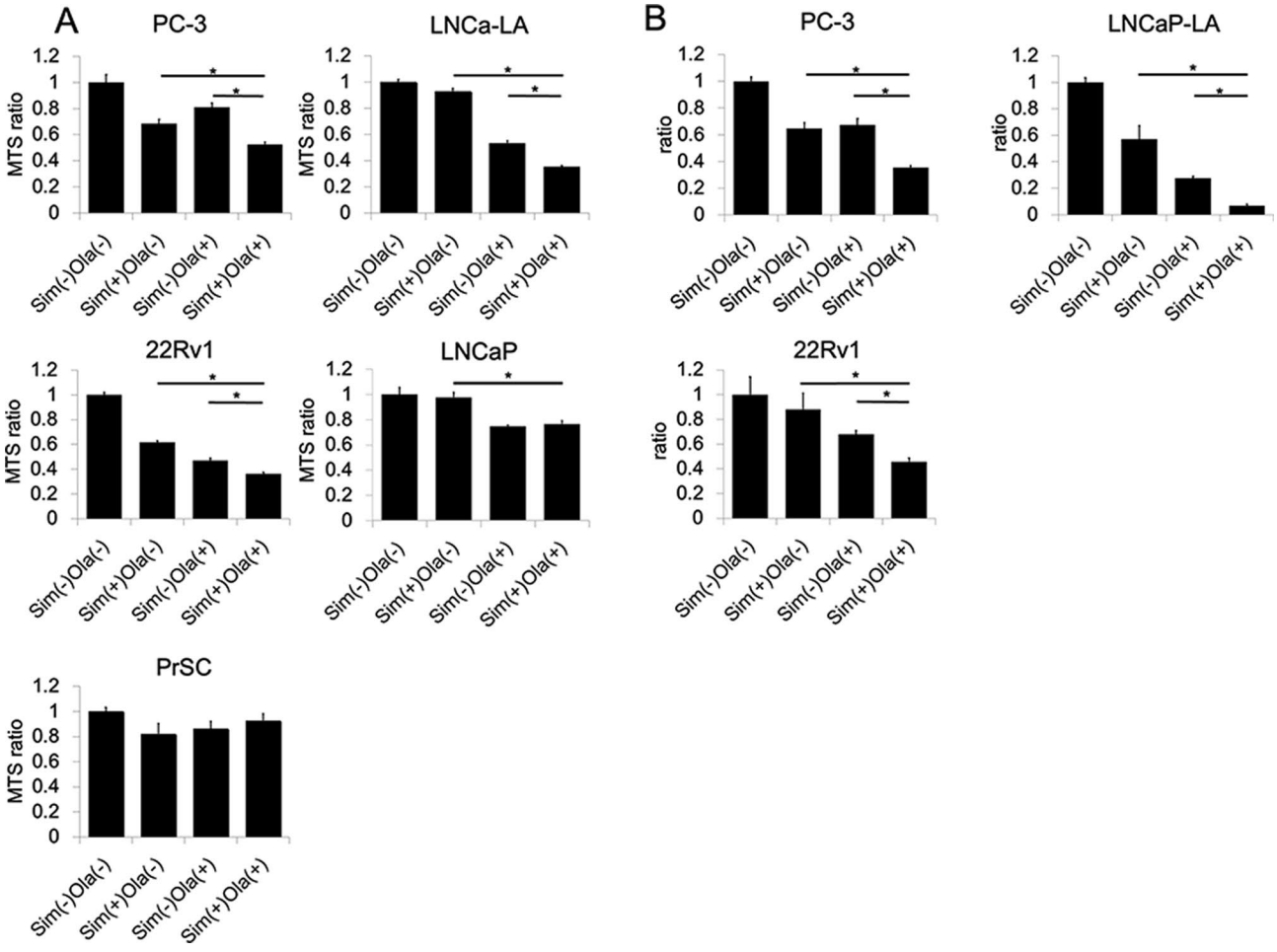

Fig. 3 Effect of combination therapy with simvastatin and olaparib on androgen-independent prostate cancer cells. The cells were then incubated in a medium. After 48 h, the cells were cultured in a medium with or without simvastatin (PC-3, 2 μM; other cells, 5 μM) and olaparib (10 μM). After 72 h for PC-3, LNCaP, and PrSC and 120 h for LNCaP-LA and 22Rv1 cells, the number of viable cells was evaluated using MTS assay (**A**) and cell counts (**B**). Values are expressed as mean ± standard deviation (SD) (A; $n = 5$, B; $n = 4$). *$p < 0.05$. Sim; simvastatin, Ola; olaparib

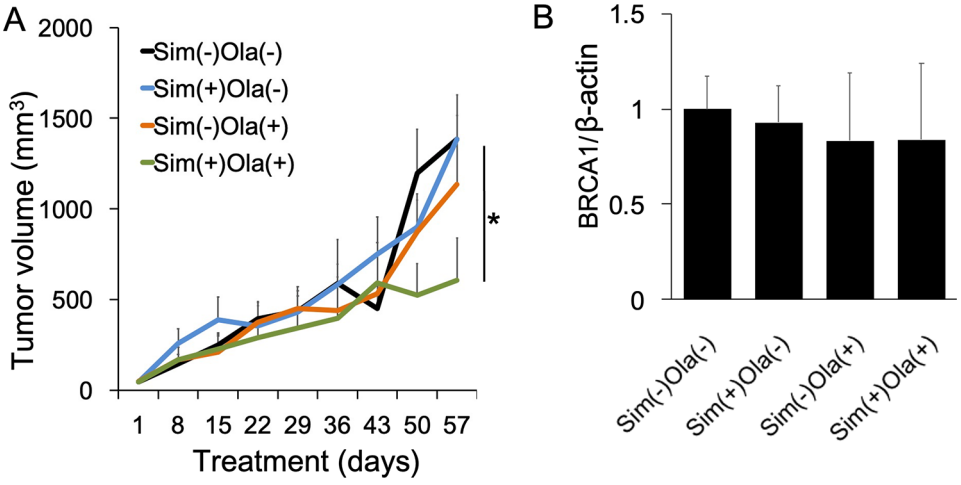

Fig. 4 Effect of combination therapy with simvastatin and olaparib on tumor growth and BRCA1 expression in vivo in a 22Rv1 xenograft model. Graphic view of mean tumor volumes in 22Rv1 xenografts. Mice were injected with 22Rv1 cells (3×10^6 per site) and subsequently orally treated with simvastatin (100 mg/kg once daily) with or without olaparib (50 mg/kg once daily). Line graphs, mean ($n = 5$); bars, standard deviation (SD); *$p < 0.05$ vs. the other group. BRCA1 mRNA expression in xenograft tissue was analyzed via quantitative real-time PCR (B). Values are expressed as mean ± SD ($n = 5$). Sim; simvastatin, Ola; olaparib

The effects of the combination therapy of simvastatin and Olaparib on cell proliferation in taxane-resistant prostate cancer cells

Taxanes are one of the treatments for CRPC [22, 23]. However, in some cases of CRPC, taxanes become ineffective, and it is very difficult to find another treatment for repair. Therefore, we examined whether combination therapy with statins and olaparib is effective for taxane-resistant prostate cancer cells. First, we evaluated the effect of taxane resistance on DNA repair genes in 22Rv1 cells. The mRNA levels of BRCA1, BRCA2, and FANCA were increased significantly in 22Rv1-CR cells but not those of RAD51 and BARD1 (Fig. 5A). There was no significant difference in RAD51, BARD1, and FANCA protein expression between 22Rv1 and 22Rv1-CR cells. Moreover, BRCA1 protein expression was increased in 22Rv1-CR cells (Fig. 5B). In 22Rv1-CR cells, simvastatin also decreased the expression of BRCA1, BRCA2, and FANCA mRNA (Fig. 5C), as well as BRCA1 and FANCA proteins (Fig. 5D). The combination of simvastatin and olaparib further enhanced the inhibition of cell proliferation compared with treatment with either drug alone (Fig. 5E and F).

The effects of combination therapy of simvastatin and Olaparib on ΓH2AX expression in androgen-independent prostate cancer cells

γH2AX, which is a phosphorylated form of H2AX, is reported to be one of the accurate markers of DNA damage [24]. The peaks of γH2AX expression after drug treatments occurred after 48 h in PC-3 cells and 96 h in LNCaP-LA, 22Rv1, and 22Rv1-CR cells (Additional file 1). The combination of simvastatin and olaparib further enhanced the expression of γH2AX proteins compared with monotherapy treatment with either drug (Fig. 6).

Discussion

The main findings from this study are as follows: (1) simvastatin decreased the expression of some DNA repair genes, (2) the combination of simvastatin and olaparib further enhanced the inhibition of cell proliferation compared with the treatment with either drug alone in vitro and in vivo, and (3) the combination of simvastatin and olaparib further enhanced the expression of γH2AX proteins compared with the treatment with either drug alone in androgen-independent and taxane-resistant prostate cancer cells. Overall, these results suggest that the combination of PARP inhibitors and statins could be a new treatment option for CRPC and taxane-resistant prostate cancer.

Statins have received much attention as antitumor agents, in addition to their role in controlling hyperlipidemia. The number of reports on the relationship between statins and prostate cancer is increasing. A large retrospective cohort study showed that statins reduced the risk of aggressive prostate cancer and improved the mortality of prostate cancer [5, 6]. In addition, the use of statins in patients with advanced prostate cancer receiving androgen deprivation therapy was reported to be associated with better all-cause survival and cancer-specific survival [25]. In the future, randomized controlled trials on the effects of statins on prostate cancer are needed. Concerning the mechanism of the therapeutic effects of statins on prostate cancer, our previous study showed that the mechanisms included decreasing the expression of insulin-like growth factor 1 receptor [10],

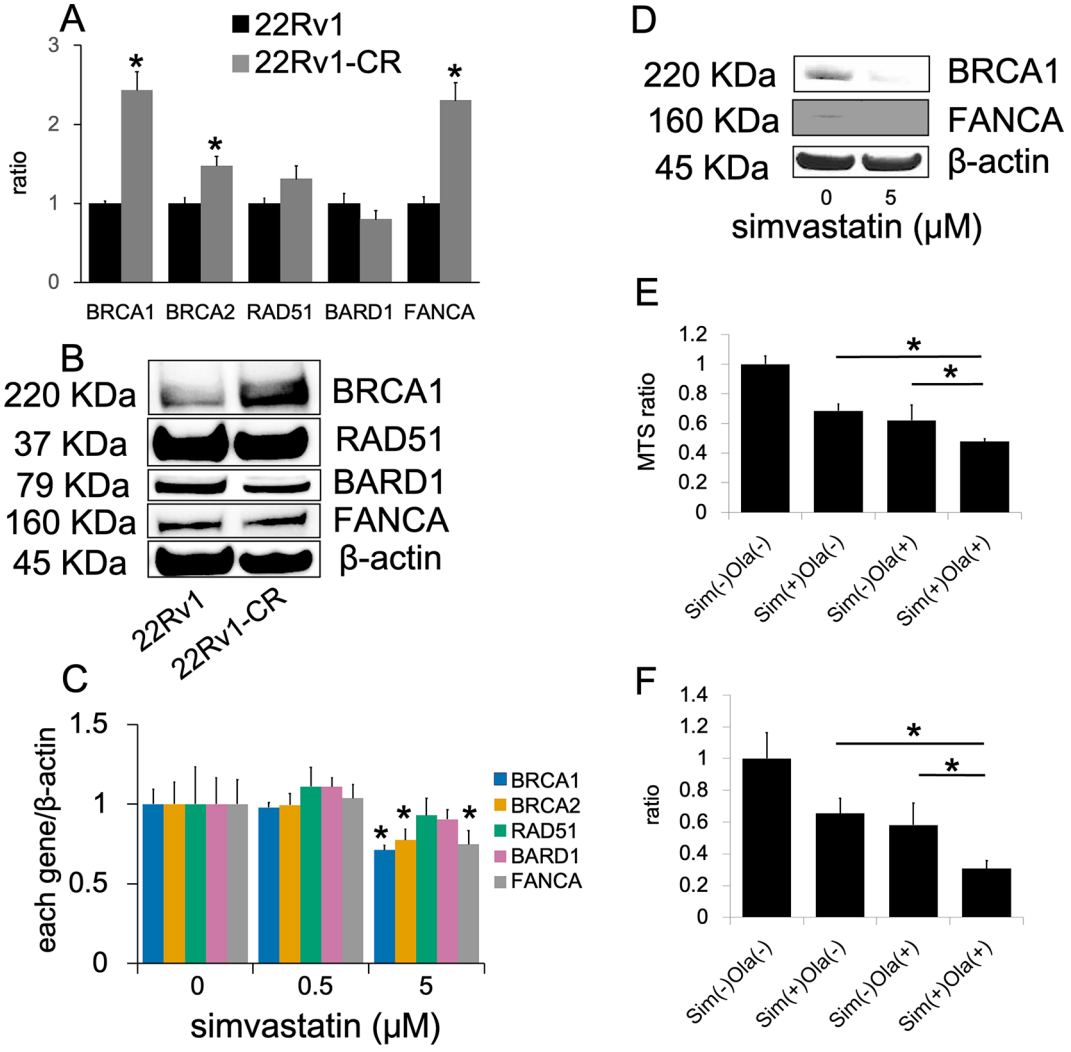

Fig. 5 Effect of combination therapy with simvastatin and olaparib on taxane-resistant prostate cancer cells. **A** and **B**. Comparison of DNA repair gene expression in 22Rv1 and 22Rv1-CR cells. **A**. mRNA expression of BRCA1, BRCA2, RAD51, BARD1, and FANCA was evaluated by RT-PCR, and the relative quantitative volume was calculated by comparing the expression of β-actin. Values are expressed as means ± standard deviations (SD) ($n = 3$). *$P < 0.05$ vs. 22Rv1 cells. **B**. Protein expression of BRCA1, RAD51, BARD1, and FANCA was evaluated using western blotting. A representative experiment is shown, which was repeated three times with similar results. **C** and **D**. Cells were incubated in medium containing various concentrations of simvastatin. After 48 h, the total RNA (**C**) and total protein (**D**) were collected. **A**. mRNA expression of BRCA1, BRCA2, RAD51, BARD1, and FANCA was evaluated by RT-PCR, and the relative quantitative volume was calculated by comparing the expression of β-actin. Values are expressed as mean ± standard deviation (SD) ($n = 3$). *$P < 0.05$ vs. 0 μM of each gene. **D**. Protein expression of BRCA1 and FANCA was evaluated by western blotting. A representative experiment is shown, which was repeated three times with similar results. E and F. Cells were incubated in the medium. After 48 h, the cells were cultured in a medium containing olaparib (10 μM) with or without simvastatin (5 μM). After 96 h, the number of viable cells was evaluated using the MTS assay (**E**) and cell counts (**F**). *$P < 0.05$. Sim; simvastatin, Ola; olaparib

decreasing the expression of ABCA1 [11], and increasing the expression of Annexin A 10 [12]. In addition, there are a variety of other mechanisms, such as the inhibition of cholesterol synthesis [26], decreasing the expression of androgen receptors [27], and inhibition of the mTOR pathway [28]. These data suggest that several other mechanisms may be recognized in the future.

PARP inhibitors have been approved for the treatment of breast, ovarian, and pancreatic cancers with BRCA gene mutations [29–31]. The antitumor effects of PARP inhibitors require defective functioning of DNA repair genes, which is known as synthetic lethality [32]. Concerning prostate cancers, olaparib has been reported to have a curative effect in CRPC patients with defects in DNA repair genes [15]. However, the ratio of DNA repair gene aberrations is not very high in prostate cancer patients [33, 34]. In this study, statins decreased the expression of DNA repair genes in androgen-independent and taxane-resistant prostate cancer cells but not in androgen-dependent prostate cancer and normal human prostate cells, indicating that statins can lead to "BRCAness" pharmaceutically in CRPC patients, and

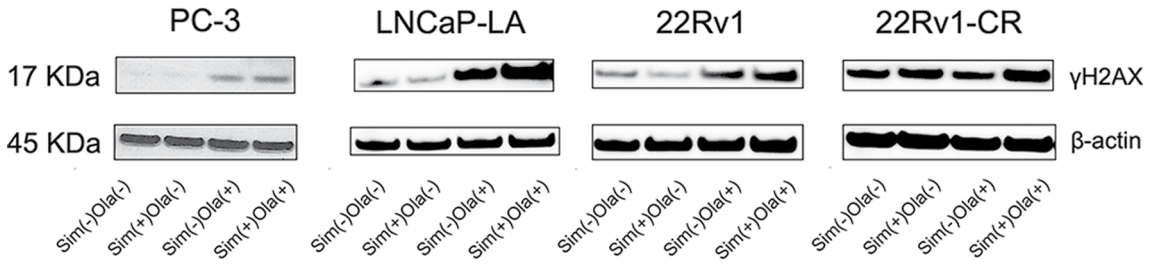

Fig. 6 Effect of combination therapy with simvastatin and olaparib on γH2AX expression in androgen-independent or taxane-resistant prostate cancer cells. Cells were incubated in a medium. After 48 h, cells were cultured in a medium with or without simvastatin (PC-3; 2 μM, other cells; 5 μM) and olaparib (10 μM). Proteins were collected after 48 h for PC-3 and LNCaP-LA cells and 96 h for 22Rv1 and 22Rv1-CR cells. Protein expression of γH2AX was evaluated using western blotting. A representative experiment is shown, which was repeated three times with similar results

PARP inhibitors can provide therapeutic effects in CPRC patients without BRCA mutations. The inhibition of BRCA1, BRCA2, and other DNA repair genes by statins contributes to the sensitivity of PARP inhibitors, which inhibit the DNA damage response in CRPC patients.

Enzalutamide is a novel androgen receptor inhibitor that has been used extensively for CRPC patients [1, 35]. It has been reported that enzalutamide inhibits the expression of DNA repair genes, such as BRCA1, RAD51AP1, RAD51C, RAD54L, and RMI2, in VCAP prostate cancer cells, and the combination of enzalutamide and olaparib synergistically suppresses prostate cancer cell growth [17] in a manner similar to that of our study. In this study, simvastatin decreased the expression of DNA repair genes in AR-deficient PC-3 cells. Clinically, CRPC is heterogeneous, and the androgen dependency and expression of androgen receptors depend on each prostate cancer cell [36]. Therefore, the effects of olaparib on CRPC seem to increase owing to the combined use of enzalutamide and statins.

In addition, this study showed that the γH2AX expression was increased after the combined treatment of simvastatin and olaparib, which indicated that the combination of simvastatin and olaparib inhibits the repair of DNA double-strand breaks and has synthetic lethality in castration-resistant and taxane-resistant prostate cancer cells. Many substances induce DNA damage, such as ultraviolet radiation, X-ray radiation, benzpyrene, platinum, mitomycin, tobacco smoke, and radical oxygen. Clinically, platinum-based chemotherapy is usually used for urothelial cancer and testicular tumors but not for CRPC. There are no randomized control trials showing statistically significant differences in overall survival between platinum-based chemotherapy and other chemotherapies in CRPC [37]. In ovarian cancer, "BRCAness" is reported to be one of the factors causing high sensitivity to PARP inhibitors and platinum-based chemotherapy [38, 39]. Although the difference in the induction of γH2AX expression between olaparib alone and with simvastatin is not substantial in our results, ensuring "BRCAness" by statin treatment also seems to give CRPC cells a high sensitivity to PARP inhibitors and platinum-based chemotherapy. Further studies on the combination therapy of statins, PARP inhibitors, and platinum-based chemotherapy for CRPC are needed.

This study has several limitations. First, the protein expression of BRCA2 was not detected by western blotting in all cells. We used some commercial antibodies, but these did not work well. Second, the concentration of simvastatin was higher than the clinical therapeutic doses [40], although the concentration of olaparib was clinically achievable [41]. Clinically relevant dosages of simvastatin do not affect the mRNA expression of DNA repair genes. Therefore, the possibility that the effect of simvastatin observed in this study was not physiological but due to toxicity cannot be ruled out. Moreover, with regard to animal experiments, the dosage of the drug was determined based on previous reports [42, 43]. Although a concomitant effect was observed at this volume, the potential efficacy of a lower volume cannot be excluded, necessitating further investigation. Third, we could not detect BRCA1 protein but mRNA expression in PC-3 cells. It has been reported that BRCA1 protein expression in PC-3 cells was detected using western blotting [44]; therefore, it appears that BRCA1 protein is expressed in PC-3 cells. The discrepancy could be due to differences in antibody sensitivity, for example, since we used the BRCA1 antibody and a previous report [44] used the Ser1524-phosphorylated-BRCA1 antibody. Fourth, we studied one cell line in an in vivo model; therefore, it would be instructive to conduct additional in vivo models. Fifth, we evaluated DNA damage only by assessing γH2AX, and further investigation is required. Sixth, the BALB/c/nu mice we used in vivo lacked immune function of the thymus gland, which may have affected the inhibitory effect of the drugs on prostate cancer growth.

Conclusions

Simvastatin alters the expression of various genes associated with DNA repair in castration-resistant and taxane-resistant prostate cancer cells. The combination of PARP inhibitors and drugs that decrease the expression of DNA

repair genes can potentially inhibit the growth of castration-resistant and taxane-resistant prostate cancers.

Abbreviations

BARD1	BRCA1 Associated RING Domain 1
BRCA1	Breast Cancer 1
BRCA2	Breast Cancer 2
CS-FBS	charcoal-stripped FBS
CRPC	castration-resistant prostate cancer
FANCA	Fanconi Anemia Complementation Group A
FANCD2	Fanconi Anemia Complementation Group D2
FANCG	Fanconi Anemia Complementation Group G
FBS	fetal bovine serum
MTS	3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2 H-tetrazolium
PARP	poly(ADP-ribose) polymerase
PrSC	prostate stromal cells
RAD51	RAD51 Recombinase
RFC	Replication Factor C Subunit

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-13895-6.

Supplementary Material 1: Additional file 1 The combined effect of simvastatin and olaparib on γH2AX expression in androgen-independent and taxane-resistant prostate cancer cells. Cells were cultured in a medium containing simvastatin (PC-3; 2 μM, other cells; 5 μM) and olaparib (10 μM). After the indicated times, the proteins were collected. Protein expression of γH2AX was evaluated using western blotting

Acknowledgements

We thank Ms. Atsuko Oyama and Ms. Hayumi Oyama for their technical assistance.

Author contributions

YSe perfomed project development, planned and completed experiments, data analysis and manuscript writing. DO, AO, HN, YM and SA performed the experiments. TM and HK contributed to the establishment of drug-resistant cell lines. HM and YSh contributed to project developments. KS supervised the work. All authors read and approved the final manuscript.

Funding

This work was supported by JSPS KAKENHI Grant Number JP19K09684.

Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethical approval and consent to participate

All animal experiments were approved by the Animal Care and Experimentation Committee, Gunma University, Showa Campus, Japan, and were conducted according to the guidelines of this committee.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Urology, Gunma University Graduate School of Medicine, 3-9-22 Showa-machi, Maebashi, Gunma 371-8511, Japan

Received: 7 December 2024 / Accepted: 10 March 2025
Published online: 21 March 2025

References

1. Beer TM, Armstrong AJ, Rathkopf DE, Loriot Y, Sternberg CN, Higano CS, et al. Enzalutamide in metastatic prostate cancer before chemotherapy. N Engl J Med. 2014;371(5):424–33. https://doi.org/10.1056/NEJMoa1405095.
2. Ryan CJ, Smith MR, Fizazi K, Rathkopf DE, Miller K, de Bono JS, et al. Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naive men with metastatic castration-resistant prostate cancer (COU-AA-302): final overall survival analysis of a randomised, double-blind, placebo-controlled phase 3 study. Lancet Oncol. 2015;16(2):152–60. https://doi.org/10.1016/S1470-2045(14)71205-7.
3. Smith MR, Saad F, Chowdhury S, Fizazi K, Sternberg CN, Cella D, et al. Apalutamide treatment and metastasis-free survival in prostate cancer. N Engl J Med. 2018;378(15):1408–18. https://doi.org/10.1056/NEJMoa1715546.
4. Fizazi K, Shore N, Tammela TL, Ulys A, Vjaters E, Polyakov S, et al. Darolutamide in nonmetastatic, castration-resistant prostate cancer. N Engl J Med. 2019;380(13):1235–46. https://doi.org/10.1056/NEJMoa1815671.
5. Van Rompay MI, Solomon KR, Nickel JC, Ranganathan G, Kantoff PW, McKinlay JB. Prostate cancer incidence and mortality among men using Statins and non-statin lipid-lowering medications. Eur J Cancer. 2019;112:118–26. https://doi.org/10.1016/j.ejca.2019.01.008.
6. Raval AD, Thakker D, Negi H, Vyas A, Kaur H, Salkini MW. Association between Statins and clinical outcomes among men with prostate cancer: a systematic review and meta-analysis. Prostate Cancer Prostatic Dis. 2016;19(2):151–62. https://doi.org/10.1038/pcan.2016.1.
7. Furuya Y, Sekine Y, Kato H, Miyazawa Y, Koike H, Suzuki K. Low-density lipoprotein receptors play an important role in the Inhibition of prostate cancer cell proliferation by Statins. Prostate Int. 2016;4(2):56–60. https://doi.org/10.1016/j.prnil.2016.03.001.
8. Zhuang L, Kim J, Adam RM, Solomon KR, Freeman MR. Cholesterol targeting alters lipid raft composition and cell survival in prostate cancer cells and xenografts. J Clin Invest. 2005;115(4):959–68. https://doi.org/10.1172/JCI21404.
9. Papadopoulos G, Delakas D, Nakopoulou L, Kassimatis T. Statins and prostate cancer: molecular and clinical aspects. Eur J Cancer. 2011;47(6):819–30. https://doi.org/10.1016/j.ejca.2011.01.018.
10. Sekine Y, Furuya Y, Nishii M, Koike H, Matsui H, Suzuki K. Simvastatin inhibits the proliferation of human prostate cancer PC-3 cells via down-regulation of the insulin-like growth factor 1 receptor. Biochem Biophys Res Commun. 2008;372(2):356–61. https://doi.org/10.1016/j.bbrc.2008.05.051.
11. Sekine Y, Demosky SJ, Stonik JA, Furuya Y, Koike H, Suzuki K. High-density lipoprotein induces proliferation and migration of human prostate androgen-independent cancer cells by an ABCA1-dependent mechanism. Mol Cancer Res. 2008;8(9):1284–94. https://doi.org/10.1158/1541-7786.MCR-08-0110.
12. Miyazawa Y, Sekine Y, Kato H, Furuya Y, Koike H, Suzuki K. Simvastatin up-regulates Annexin A10 that can inhibit the proliferation, migration, and invasion in androgen-independent human prostate cancer cells. Prostate. 2017;77(4):337–49. https://doi.org/10.1002/pros.23267.
13. Ellisen LW, Farmer H, McCabe N, Lord CJ, Ashworth A, Yu VP. PARP inhibitors in cancer therapy: promise, progress, and puzzles. Cancer Cell. 2011;19(2):165–7. https://doi.org/10.1016/j.ccr.2011.01.010.
14. Lord CJ, Ashworth A. The DNA damage response and cancer therapy. Nature. 2012;481(7381):287–94. https://doi.org/10.1038/nature10760.
15. Mateo J, Carreira S, Sandhu S, de Bono J, Olmos D, Reid AH. DNA-repair defects and Olaparib in metastatic prostate cancer. N Engl J Med. 2015;373(18):1697–708. https://doi.org/10.1056/NEJMoa1506859.
16. Mateo J, Porta N, Bianchini D, McGovern UB, Elliott T, Sandhu S. Olaparib in patients with metastatic castration-resistant prostate cancer with DNA repair gene aberrations (TOPARP-B): a multicenter, open-label, randomised, phase 2 trial. Lancet Oncol. 2020;21(1):162–74. https://doi.org/10.1016/S1470-2045(19)30684-9.
17. Li L, Karanika S, Yang G, Wang J, Bajpai R, Magi-Galluzzi C, et al. Androgen receptor inhibitor–induced BRCAness and PARP Inhibition are synthetically lethal for castration-resistant prostate cancer. Sci Signal. 2017;10(480):eaam7479. https://doi.org/10.1126/scisignal.aam7479.
18. Oka D, Sekine Y, Tsuji Y, Furuya Y, Koike H, Suzuki K. Simvastatin enhances the radiosensitivity of androgen-independent prostate cancer cells via Inhibition of RAD51 expression. Anticancer Res. 2024;44(1):93–8. https://doi.org/10.21873/anticanres.13181.
19. Miyao T, Koike H, Sekine Y, Ohtsu A, Oka D, Suzuki K. YM155 reverses Cabazitaxel resistance in Castration-resistant prostate cancer by reducing survivin expression. Anticancer Res. 2020;40(9):5091–5. https://doi.org/10.21873/anticanres.14512.

20. Suzuki K, Koike H, Matsui H, Sekine Y, Furuya Y, Kato H. Genistein, a soy isoflavone, induces glutathione peroxidase in the human prostate cancer cell lines LNCaP and PC-3. Int J Cancer. 2002;99(6):846–52. https://doi.org/10.1002/ijc.10422.
21. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods. 2001;25(4):402–8. https://doi.org/10.1006/meth.2001.1262.
22. Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, et al. Docetaxel plus prednisone or Mitoxantrone plus prednisone for advanced prostate cancer. N Engl J Med. 2004;351(15):1502–12. https://doi.org/10.1056/NEJMoa040720.
23. de Wit R, de Bono J, Sternberg CN, Chi KN, Omlin A, Sartor AO, et al. Cabazitaxel versus abiraterone or enzalutamide in metastatic prostate cancer. N Engl J Med. 2019;381(26):2506–18. https://doi.org/10.1056/NEJMoa1911206.
24. Mah LJ, El-Osta A, Karagiannis TC. gammaH2AX: a sensitive molecular marker of DNA damage and repair. Leukemia. 2010;24(4):679–86. https://doi.org/10.1038/leu.2010.6.
25. Yang H, Pang L, Hu X, Wang S, Li C, Sun H. The effect of Statins on advanced prostate cancer patients with androgen deprivation therapy or Abiraterone/enzalutamide: A systematic review and meta-analysis. J Clin Pharm Ther. 2020;45(3):488–95. https://doi.org/10.1111/jcpt.13058.
26. Rao S, Porter DC, Chen X, Herliczek T, Lowe M, Keyomarsi K. Lovastatin-mediated G1 arrest is through Inhibition of the proteasome, independent of hydroxymethyl glutaryl-CoA reductase. Proc Natl Acad Sci USA. 1996;96(14):7797–802. https://doi.org/10.1073/pnas.96.14.7797.
27. Yokomizo A, Shiota M, Kashiwagi E, Kuroiwa K, Naito S, Inoue T. Statins reduce the androgen sensitivity and cell proliferation by decreasing the androgen receptor protein in prostate cancer cells. Prostate. 2011;71(3):298–304. https://doi.org/10.1002/pros.21246.
28. Kong Y, Cheng L, Mao F, Zhang J, Liu F, Song Y. Inhibition of cholesterol biosynthesis overcomes enzalutamide resistance in castration-resistant prostate cancer (CRPC). J Biol Chem. 2018;293(37):14328–41. https://doi.org/10.1074/jbc.RA118.004335.
29. Robson M, Im SA, Senkus E, Olaparib I, Villanueva-Vázquez R, Yearley J, et al. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. N Engl J Med. 2017;377(6):523–33. https://doi.org/10.1056/NEJMoa1706450.
30. Pujade-Lauraine E, Ledermann JA, Selle F, Paluch-Shimon S, Cibula D, Colombo N, et al. Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Oncol. 2017;18(9):1274–84. https://doi.org/10.1016/S1470-2045(17)30469-2.
31. Golan T, Hammel P, Reni M, O'Reilly EM, Macarulla T, Mehta A, et al. Maintenance Olaparib for germline BRCA-mutated metastatic pancreatic cancer. N Engl J Med. 2019;381(4):317–27. https://doi.org/10.1056/NEJMoa1903387.
32. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Knights C, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature. 2005;434(7035):917–21. https://doi.org/10.1038/nature03445.
33. Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, et al. The mutational landscape of lethal castration-resistant prostate cancer. Nature. 2012;487(7406):239–43. https://doi.org/10.1038/nature11125.
34. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell. 2010;18(1):11–22. https://doi.org/10.1016/j.ccr.2010.05.026.
35. Derleth CL, Yu EY. Targeted therapy in the treatment of castration-resistant prostate cancer. Oncol (Williston Park). 2013;27(7):620–8.
36. Nelson PS. Molecular States underlying androgen receptor activation: a framework for therapeutics targeting androgen signaling in prostate cancer. J Clin Oncol. 2012;30(6):644–6. https://doi.org/10.1200/JCO.2011.39.2210.
37. Leaf F, Garcia-Perdomo HA. Effectiveness of platinum-based chemotherapy in patients with metastatic prostate cancer: systematic review and meta-analysis. Clin Genitourin Cancer. 2019;17(3):e627–44. https://doi.org/10.1016/j.clgc.2019.04.007.
38. Pennington KP, Walsh T, Harrell MI, Rendi MH, Thornton TA, Norquist BM, et al. Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas. Clin Cancer Res. 2014;20(3):764–75. https://doi.org/10.1158/1078-0432.CCR-13-2287.
39. Mukhopadhyay A, Plummer ER, Elattar A, Sandhu SK, Sundar S, Ganesan TS, et al. Clinicopathological features of homologous recombination-deficient epithelial ovarian cancers: sensitivity to PARP inhibitors, platinum, and survival. Cancer Res. 2012;72(22):5675–82. https://doi.org/10.1158/0008-5472.CAN-12-0470.
40. Murtola TJ, Pennanen P, Syvälä H, Bläuer M, Ylikomi T, Tammela TL. Effects of Simvastatin, acetylsalicylic acid, and Rosiglitazone on proliferation of normal and cancerous prostate epithelial cells at therapeutic concentrations. Prostate. 2009;69(9):1017–23. https://doi.org/10.1002/pros.20944.
41. Thelen KM, Rentsch KM, Gutteck U, Heverin M, Olin H, Andersson U, et al. Brain cholesterol synthesis in mice is affected by high dose of Simvastatin but not of Pravastatin. J Pharmacol Exp Ther. 2006;316(3):1146–52. https://doi.org/10.1124/jpet.105.094136.
42. Senra JM, Telfer BA, Cherry KE, McCrudden CM, Hirst DG, O`Connor MJ, et al. Inhibition of PARP-1 by Olaparib (AZD2281) increases the radiosensitivity of a lung tumor xenograft. Mol Cancer Ther. 2011;10(10):1949–58. https://doi.org/10.1158/1535-7163.MCT-11-0278.
43. Faraoni I, Consalvo MI, Aloisio F, Graziani G, Surace CD. Cytotoxicity and differentiating effect of the poly(ADP-ribose) polymerase inhibitor Olaparib in myelodysplastic syndromes. Cancers (Basel). 2019;11(9):1373. https://doi.org/10.3390/cancers11091373.
44. Hu Z, Kong F, Si M, Zhang S, Li C, Zhao X. Riccardin D exerts its antitumor activity by inducing DNA damage in PC-3 prostate cancer cells in vitro and in vivo. PLoS ONE. 2013;8(9):e74387. https://doi.org/10.1371/journal.pone.0074387.

Publisher's note