Focal adhesion kinase inhibitor defactinib enhances olaparib-induced suppression of BRCA-proficient breast cancer cell proliferation

HISAKO ONO[1,2], KEIKO TANIGUCHI[1], SHUSUKE YASUDA[1], MAMIKO SUKENO[1], TOMOYA MATSUI[3], YASUTO NAOI[3], MANO HORINAKA[1] and TOSHIYUKI SAKAI[1]

[1]Department of Drug Discovery Medicine, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan; [2]Department of Medical Oncology, Japanese Red Cross Kyoto Daini Hospital, Kyoto 602-8026, Japan; [3]Department of Endocrine and Breast Surgery, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan

Received December 10, 2025; Accepted July 17, 2026

DOI: 10.3892/ol.2026.15868

Abstract. Poly ADP-ribose polymerase (PARP) inhibitors exhibit high activity for carcinomas with homologous recombination (HR) deficiency, such as *BRCA1/2* mutations. PARP inhibitors are primarily administered to patients with breast cancer with *BRCA* germline mutations; therefore, the use of these inhibitors for breast cancer has been limited. The present study was undertaken with the aim of proposing a new treatment for BRCA-proficient breast cancer. This study investigated drugs with good safety profiles to enhance the growth inhibitory effects of the most commonly used PARP inhibitor, olaparib, in BRCA-proficient MDA-MB-231 cells. The focal adhesion kinase (FAK) inhibitor defactinib, which suppressed BRCA expression at the mRNA level, was identified as a candidate by screening for BRCA down-regulators. Defactinib also enhanced olaparib-induced inhibition in other BRCA-proficient breast cancer cell proliferation. Furthermore, apoptosis was more strongly induced by the combination of olaparib and defactinib than by either agent alone. To elucidate the molecular mechanisms underlying the enhanced induction of apoptosis, a Western blot analysis of apoptosis-related proteins indicated the contribution of the downregulated expression of X-linked inhibitor of apoptosis protein. This indicates the efficiency of the combination treatment of the PARP inhibitor olaparib and the FAK inhibitor defactinib for breast cancer cells, which may be useful for BRCA-proficient breast cancer or carcinomas that acquire resistance to PARP inhibitors.

Correspondence to: Dr Hisako Ono, Department of Drug Discovery Medicine, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo, Kyoto 602-8566, Japan
E-mail: hisako-o@koto.kpu-m.ac.jp

Abbreviations: FAK, focal adhesion kinase; HR, homologous recombination; PARP, poly ADP-ribose polymerase

Key words: PARP inhibitor, olaparib, FAK inhibitor, defactinib, BRCA, HR deficiency

Introduction

In cancer therapy, the efficacy of drugs in standard treatment widely varies among individuals. Poly ADP-ribose polymerase (PARP) inhibitors play an essential role in personalized medicine; however, their use in the treatment of breast cancer has been limited to carcinoma with BRCA deficiency, including germline variants (1-3). Approximately 6-7% of breast cancer patients harboring germline or somatic *BRCA* mutations may benefit from PARP inhibitors (3-5).

The tumor suppressor genes *BRCA1* and *BRCA2* are involved in the repair of DNA. BRCA deficiency is the main factor causing homologous recombination (HR) deficiency. Alleles may be inactivated by several mechanisms, such as germline or somatic variants and epigenetic down-regulation. PARP inhibitors exhibit anti-tumor activity against carcinomas with HR deficiency, including BRCA deficiency (6).

While PARP inhibitors have been widely used to treat cancer patients, the acquisition of resistance remains a challenge. HR recovery, such as *BRCA* reversions, called back mutations, has been examined (7,8), and the re-induction of HR deficiency may increase the success of a rechallenge with PARP inhibitors. Moreover, the induction of HR deficiency by some agents may be useful in the treatment of BRCA-proficient tumors with combination therapy using PARP inhibitors (9,10).

The use of PARP inhibitors in breast cancer is limited to patients with BRCA-deficient tumors, including *BRCA* germline mutations, which are established biomarkers of response to PARP inhibitors. Therefore, the present study was undertaken to identify drugs that sensitize BRCA-proficient breast tumors to PARP inhibitors.

Materials and methods

Reagents. Olaparib, talazoparib, defactinib, and GSK2256098 were purchased from Selleck Chemicals. These agents were dissolved in dimethyl sulfoxide (DMSO) and stored at -20˚C.

Cell culture. The human breast cancer cell lines MCF-7 and MDA-MB-231 were obtained as cell lines of NCI-60 from the NCI Developmental Therapeutics Program, and MDA-MB-436 was purchased from the American Type Culture Collection. MCF-7 and MDA-MB-436 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS), 4 mM glutamine, 50 units/ml penicillin, and 100 μg/ml streptomycin, while MDA-MB-231 cells were cultured in RPMI 1640 medium with 10% FBS, 2 mM glutamine, 50 units/ml penicillin, and 100 μg/ml streptomycin. All cell cultures were incubated at 37˚C in 5% CO_2.

Cell viability assay. Cell viability was evaluated using Cell Counting Kit-8 (CCK-8) (Dojindo) as previously reported (11). The CCK-8 solution was added to the medium of some reagent-treated breast cancer cells. The absorbance (450 nm) of the medium was determined using SpectraMax iD3 (Molecular Devices) after 4 h of incubation. The percentage of cell viability was measured relative to DMSO-treated cells. Viable cell numbers were determined using a trypan blue dye exclusion assay. Cells were seeded in triplicate wells and allowed to attach overnight. The following day, cells were treated with the indicated single agents or drug combination. Viable cell numbers were measured at the indicated time points using an EVE automated cell counter (NanoEnTek).

Cell cycle analysis. Cells were washed with phosphate-buffered saline (PBS) and suspended in PBS containing 0.1% Triton X-100, 150 μg/ml RNase A and 50 μg/ml propidium iodide to stain the nuclei. The DNA content in stained nuclei was analyzed by FACSCalibur (BD Biosciences), as previously reported (11,12), or the BD Accuri C6 Plus flow cytometer (BD Biosciences).

Analyses of apoptosis. Apoptosis was detected using the Annexin V-FITC Apoptosis Detection Kit (Nacalai Tesque) according to the manufacturer's instructions. After cells were cultured with agents for 72 h, they were collected, washed with phosphate-buffered saline, and suspended in a binding buffer. Cells were then stained with annexin V-FITC and propidium iodide. After a 15-min incubation, stained cells were examined using the BD Accuri C6 Plus flow cytometer. Data were analyzed using FlowJo software (BD Biosciences).

Quantitative RT-PCR. Total cellular RNA was extracted from cells using Sepasol-RNA I Super G (Nacalai Tesque), and complementary DNA (cDNA) was synthesized from total RNA with High-Capacity cDNA Reverse Transcription kit (Applied Biosystems) as previously reported (12). A quantitative RT-PCR analysis was conducted using QuantStudio3 (Applied Biosystems) with cDNA and TaqMan probes (Thermo Fisher Scientific) to BRCA1 (Hs01556193_m1), BRCA2 (Hs00609073_m1), and GAPDH (Hs02758991_g1).

Western blot analysis. Cells were lysed with RIPA buffer (150 mM NaCl, 50 mM Tris-HCl pH 8.0, 1% NP-40, 0.5% sodium deoxycholate, and 0.1% SDS) supplemented with a 1% protease inhibitor cocktail mix and 1% phosphatase inhibitor cocktail mix (Nacalai Tesque). Lysates were mixed with 5×sample buffer (125 mM Tris-HCl, pH 6.8, 20% glycerol, 10% 2-mercaptoethanol, 4% SDS, and 0.04% bromophenol blue) and boiled at 95˚C for 5 min. An equal amount of the protein extract was separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Membranes were blocked with 5% fat-free dry milk in TBS with 0.1% Tween-20 and then incubated with the indicated primary and secondary antibodies in Signal Enhancer HIKARI (Nacalai Tesque). Proteins were visualized by the Immobilon Western Chemiluminescent HRP Substrate (Millipore) or Chemi-Lumi One L (Nacalai Tesque). Chemiluminescence was detected using the ChemiDoc Touch imaging system (Bio-Rad Laboratories) as previously reported (13). All immunoblot experiments were independently conducted at least three times. The amount of protein bands was quantified with the Image Lab Software (Bio-Rad Laboratories). The following rabbit monoclonal antibodies were obtained from Cell Signaling Technology (CST): PARP (#9542), Bcl-2 (#3498), Bcl-xL (#2769), Bim (#2933), p-Histone H3 Ser10 (#9701), Histone H3 (#4499), p21 (#2947), p-CDK1 Thr15 (#9111), p-ERK (#9101), total ERK (#9102), p-AKT (#4060), and total AKT (#4691). The rabbit monoclonal antibody against survivin was purchased from R&D Systems (#AF886). Mouse monoclonal antibodies against the following proteins were used: BAX (#610982, BD Biosciences), XIAP (#MAB822, R&D Systems), CDK1 (#9116, CST), Cyclin B (#4135, CST) and β-actin (#A5441, Sigma-Aldrich).

Screening for BRCA down-regulators. We screened more than 950 drugs, including those contained in the FDA-approved drug kit purchased from Selleck Chemicals. We checked the package insert for the drugs included and avoided those that are harmful for humans before the start of screening. We screened drugs at a concentration of 10 μM to inhibit the viability of BRCA-proficient MDA-MB-231 cells using the CCK-8 assay. We then identified agents that down-regulated BRCA1 and BRCA2 expression at the mRNA level using quantitative RT-PCR.

Combination index. Combination index values were analyzed using CalcuSyn software (Biosoft) as previously reported (12). Synergism is defined as more than the expected additive effect with a combination index <1.0.

Statistical analysis. *In vitro* data are represented as the mean ± standard deviation (SD) in triplicate or quadruplicate. Samples were considered to be significantly different at $P<0.05$. All statistical analyses were performed using one-way ANOVA followed by Bonferroni or Dunnett's multiple comparison test.

Results

Olaparib inhibits the proliferation of breast cancer cells with G_2/M-phase arrest and sub-G_1 induction. We examined the effects of the PARP inhibitor olaparib in various breast cancer cell lines. We used human BRCA-proficient

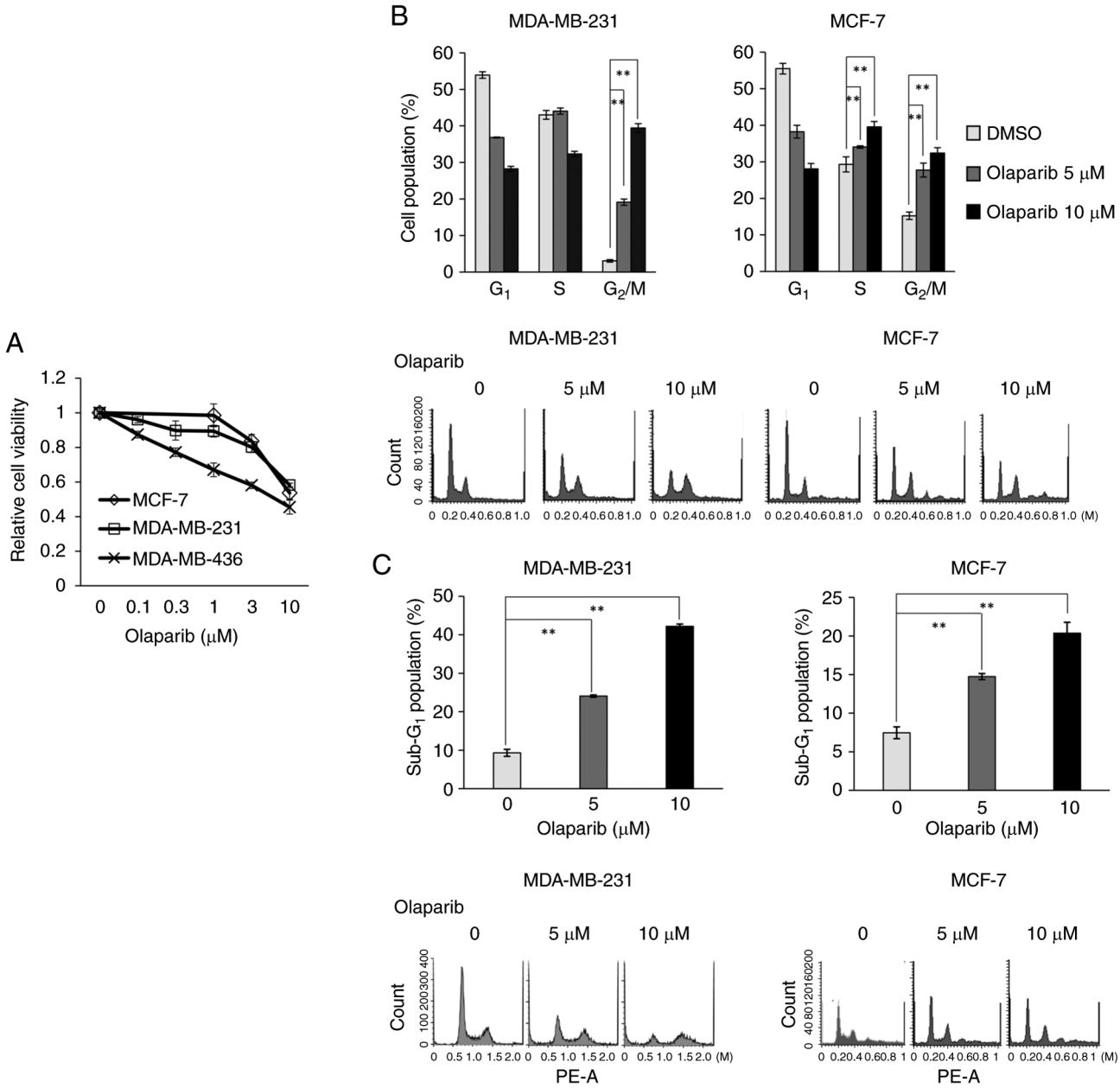

Figure 1. Olaparib inhibits the proliferation of breast cancer cells with G_2/M-phase arrest and sub-G_1 induction. (A) BRCA-proficient MCF-7 and MDA-MB-231 cells and BRCA-deficient MDA-MB-436 breast cancer cells were treated with the indicated concentrations (0-10 μM) of olaparib for 72 h. The inhibition of cell viability was evaluated using Cell Counting Kit-8. The relative cell viability of each treatment was calculated by comparisons with DMSO samples. (B) Cell cycle populations treated with olaparib were analyzed using FACSCalibur. Cell populations in MDA-MB-231 cells treated for 48 h and in MCF-7 cells treated for 24 h. (C) Sub-G_1 cells were analyzed by the BD Accuri C6 Plus flow cytometer in MDA-MB-231 cells and by FACSCalibur in MCF-7 cells. The sub-G_1 population in cells treated with olaparib for 72 h. **P<0.01.

MDA-MB-231 and MCF-7 cells and BRCA-deficient MDA-MB-436 cells. As shown in Fig. 1A, olaparib exerted inhibitory effects on the proliferation of breast cancer cells in a manner that was dependent on BRCA deficiency. The results presented in Fig. 1A suggest that additional agents enhancing olaparib sensitivity for BRCA-proficient breast cancer are needed. We then examined the inhibitory effects of olaparib on the cell cycle in BRCA-proficient MDA-MB-231 and MCF-7 cells. The results obtained showed that olaparib induced apparent G_2/M-phase arrest in both cell lines (Fig. 1B). Moreover, olaparib increased the percentage of sub-G_1 cells, as shown in Fig. 1C, which implies the induction of apoptosis.

The FAK inhibitor defactinib enhances inhibitory effects of olaparib on BRCA-proficient breast cancer cells with the transcriptional down-regulation of BRCA expression. To identify a novel strategy for BRCA-proficient breast cancer, we searched candidate drugs for combination therapy with olaparib by the screening for BRCA down-regulators. After our investigation, we found that cell proliferation was more strongly inhibited by the combination of olaparib and the FAK inhibitor defactinib than by either agent alone in MDA-MB-231 and MCF-7 cells, and the combined effects observed in these cells were synergistic, as indicated by the combination index (CI) (Fig. 2A and B). Several molecular-targeting agents were previously shown to effectively enhance HR deficiency, and

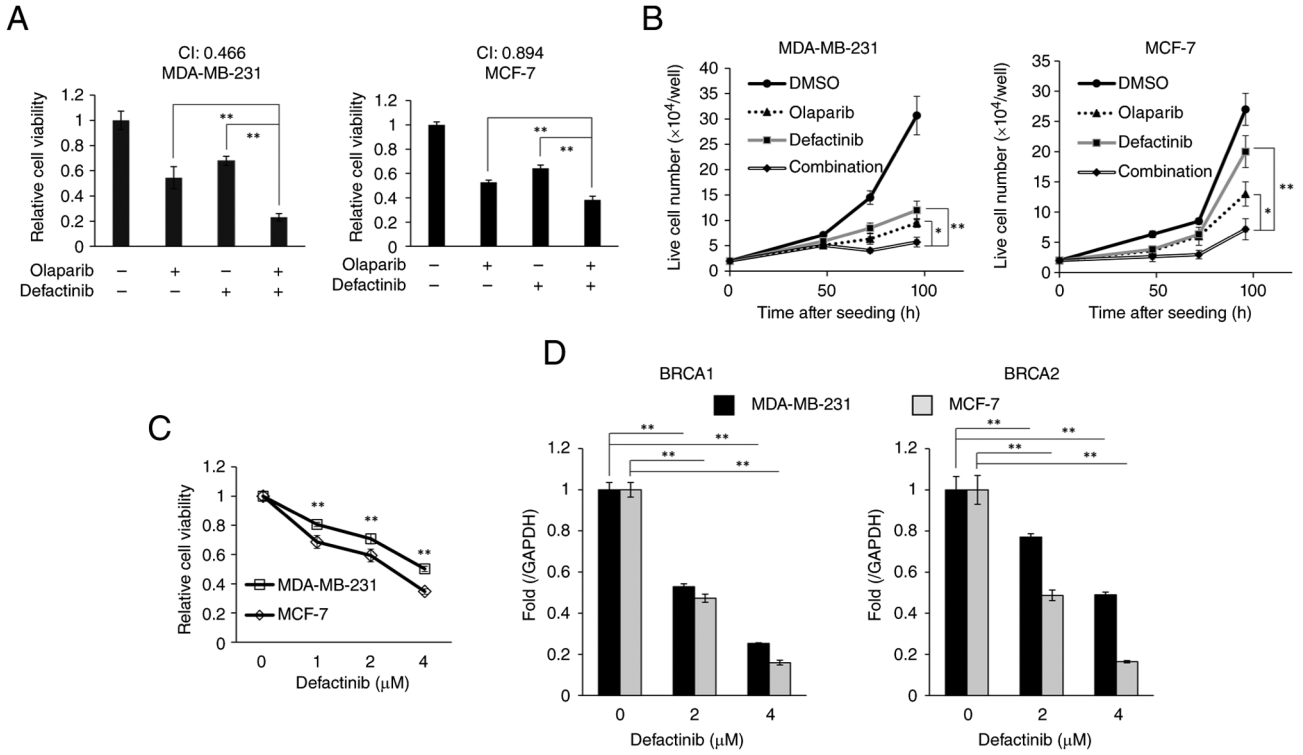

Figure 2. FAK inhibitor defactinib enhances inhibitory effects of olaparib and downregulates BRCA1/2 expression transcriptionally in BRCA-proficient breast cancer cells. (A and B) Efficacy of the combination treatment of olaparib and defactinib in BRCA-proficient MDA-MB-231 and MCF-7 cells was investigated using (A) CCK-8 and (B) trypan blue dye exclusion test. (A) MDA-MB-231 cells were treated for 72 h with DMSO or 20 μM olaparib and/or 2 μM defactinib, while MCF-7 cells were treated with DMSO or 10 μM olaparib and/or 1 μM defactinib. The relative cell viability with the combination treatment was calculated by comparisons with the single treatment. Each combination index was indicated above the columns. (B) Viable cell numbers following treatment with the indicated concentrations of the drugs as described in (A). Cells were treated 24 h after seeding, and viable cells were counted by trypan blue dye exclusion using an EVE automated cell counter. (C) MDA-MB-231 and MCF-7 cells were treated with DMSO or the indicated concentrations of defactinib. The inhibition of cell viability for 72 h was evaluated using CCK-8. The relative cell viability with each treatment was calculated by comparisons with DMSO samples. (D) BRCA1 and BRCA2 expression at the mRNA level was examined in MDA-MB-231 cells treated with the indicated concentrations of defactinib for 48 h and in MCF-7 cells for 24 h analyzed by reverse transcription-quantitative PCR. The expression in each treatment was calculated by comparisons with DMSO samples. *P<0.05 and **P<0.01. CCK-8, Cell Counting Kit-8; FAK, focal adhesion kinase.

inhibited the cell growth when administered in combination with PARP inhibitors (9,10). Defactinib dose-dependently inhibited the proliferation of MDA-MB-231 and MCF-7 cells (Fig. 2C), and also down-regulated BRCA1 and BRCA2 mRNA expression in these cells (Fig. 2D).

Defactinib induces apoptosis more potently in combination with olaparib. Since defactinib enhanced the inhibitory effects of olaparib in BRCA-proficient breast cancer cells, we investigated the mechanisms of action of this combination. The results of a FACS analysis showed that the percentage of MDA-MB-231 cells treated with combination treatment did not increase in the G_2/M phase over time compared with each agent alone, whereas the percentage of sub-G_1 cells was significantly increased (Figs. 3A and S1A). On the other hand, the percentage of MCF-7 cells in the G_2/M phase as well as that in the sub-G_1 phase increased significantly higher with the combination than with either agent alone (Figs. 3B and S1B). We then performed a detailed apoptosis analysis of both cell lines. The induction of apoptosis in MDA-MB-231 cells by the combination was significantly stronger than that by either agent alone, while that induced in MCF-7 cells by the combination was significantly stronger than that by defactinib alone, but only slightly stronger than that by olaparib alone (Fig. 3C). Our results showed only modest differences in apoptotic induction in MDA-MB-231 and MCF-7 cells. To verify whether the differences in phenotypes between the cell lines might be due to the differences in the signaling pathways involved, we confirmed the different dependences on RAS/RAF/ERK (MAPK) or PI3K/AKT pathway (Fig. S2).

The combination of olaparib and defactinib inhibits the proliferation of BRCA-proficient breast cancer cells with suppressing XIAP. To clarify the molecular mechanisms underlying the enhanced induction of apoptosis by the combination treatment, we examined the expression of various apoptosis-related proteins in MDA-MB-231 cells with clear increase in apoptosis, which representative data shown in Fig. 4 and densitometry bar graphs and statistical comparisons in Supplementary Fig. 3. We investigated the cleavage of PARP with enhanced apoptosis, which increased in the combination treatment (Figs. 4A and S3). Based on previous findings on the effects of olaparib (14), we investigated the expression of Bcl-2 family proteins; however, they did not contribute fully to the enhanced induction of apoptosis by the combination treatment (Fig. 4B and C). The expression of XIAP and survivin, as major anti-apoptotic proteins, was also assessed (Fig. 4D and A). The results obtained showed that reduction

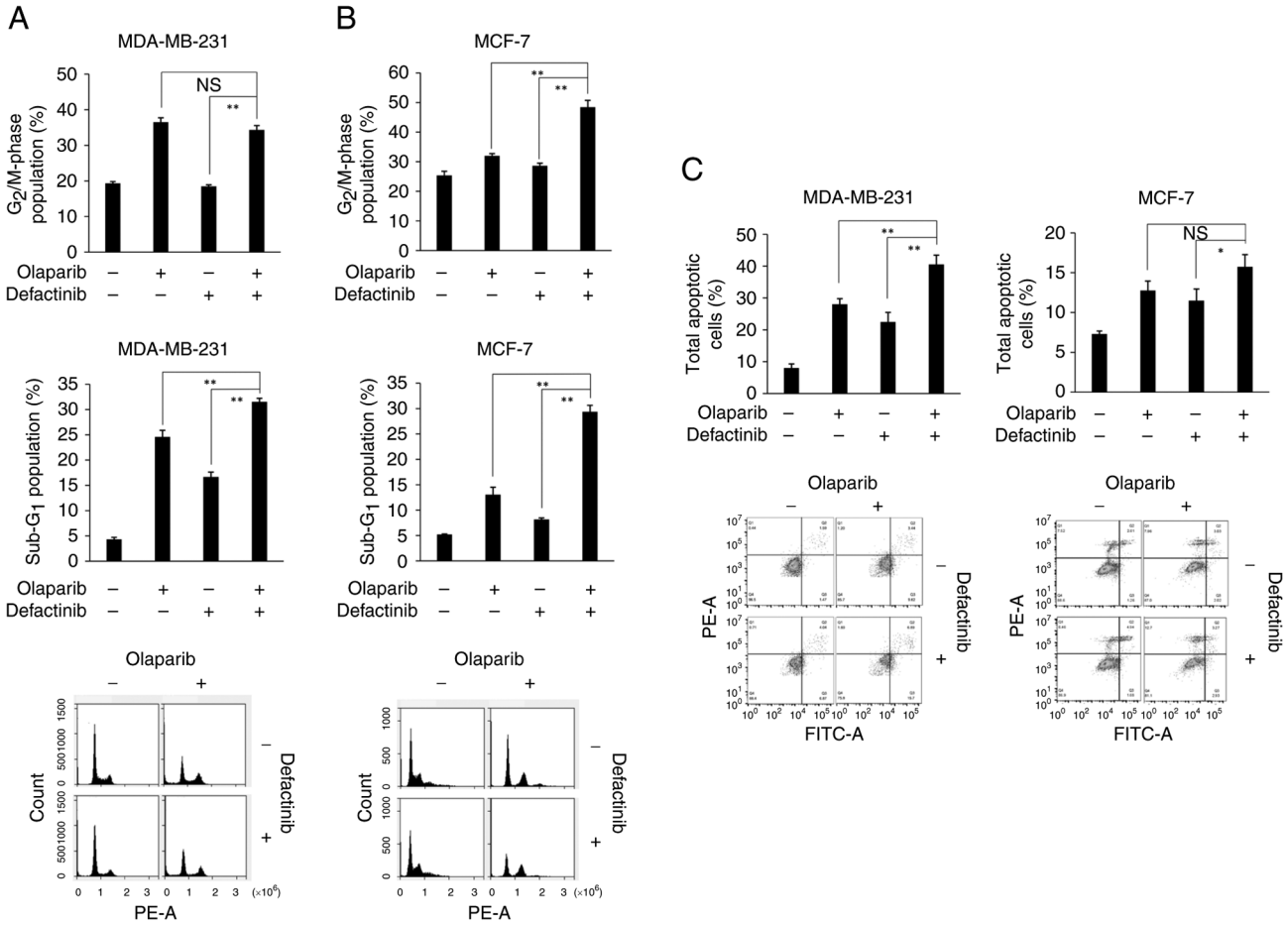

Figure 3. Combination treatment of olaparib and defactinib inhibits the proliferation of BRCA-proficient breast cancer cells with apoptosis. G_2/M and sub-G_1 populations in (A) MDA-MB-231 and (B) MCF-7 cells with the combination treatment relative to each single treatment were analyzed using the BD Accuri C6 Plus flow cytometer. MDA-MB-231 cells were treated for 72 h with DMSO or 20 μM olaparib and/or 2 μM defactinib, while MCF-7 cells were treated for 72 h with DMSO or 10 μM olaparib and/or 1 μM defactinib. (C) The percentage of total apoptotic cells was also analyzed by flow cytometry. These cells were treated for 72 h with DMSO or the indicated concentrations of the drugs as described in (A) and (B). *P<0.05 and **P<0.01. NS, not significant.

in the expression of XIAP was significantly greater with the combination than with olaparib alone (Figs. 4D and S3). We next also evaluated the expression of cell cycle regulatory proteins using MCF-7 cells, which representative data shown in Fig. 4E and densitometry bar graphs and statistical comparisons in Fig. S4. The apparent accumulation of only MCF-7 cells treated with the combination in the G_2/M phase was observed by FACS analysis (Figs. S1B and 3B), while the reduction in histone H3 Ser10 phosphorylation indicated impaired mitotic entry; however, the absence of CDK1 Tyr15 phosphorylation, p21 induction, and Cyclin B accumulation suggests that canonical G_2 checkpoint response was not robustly activated.

The synergistic effects on the combination of PARP inhibitors and FAK inhibitors. To provide a scientific explanation for combining PARP and FAK inhibitors, we expanded the analysis shown in Fig. 5. We used MDA-MB-231 and MCF-7 cells and included talazoparib and GSK2256098 known as a potent FAK inhibitor. We investigated the combination effect of talazoparib and defactinib, and this combination treatment also showed the synergistic effects in both cells. However, the efficacy of PARP inhibitors and GSK2256098 was shown in only MDA-MB-231 cells. We also investigated the inhibition of cell proliferation, and BRCA1 and BRCA2 mRNA expression in these cells treated with GSK2256098 (Fig. S5).

Discussion

Focal adhesion kinase (FAK) is a cytoplasmic non-receptor tyrosine kinase that regulates cell proliferation, migration, and survival through transmembrane receptors, such as integrins, growth factors, and cytokine receptors (15-18). The aberrant activation or overexpression of FAK has been reported in multiple cancer types, including breast cancer, supporting its potential as a therapeutic target (19-21). Defactinib, an oral and selective FAK inhibitor, is currently one of the most clinically advanced agents. It has recently gained regulatory approval in combination therapy with the novel RAF/MEK inhibitor avutometinib also known as CKI27, CH5126766, RO5126766, or VS-6766 derived from our established cell-based assay (22,23) for recurrent low-grade serous ovarian cancer with KRAS mutations (24,25), and has also been investigated in other types of cancers, such as non-small cell lung cancer and melanoma (26-28). Based on the development of defactinib, FAK inhibitors are expected to have translational relevance when used in combination strategies. Considering our findings that the combination treatment

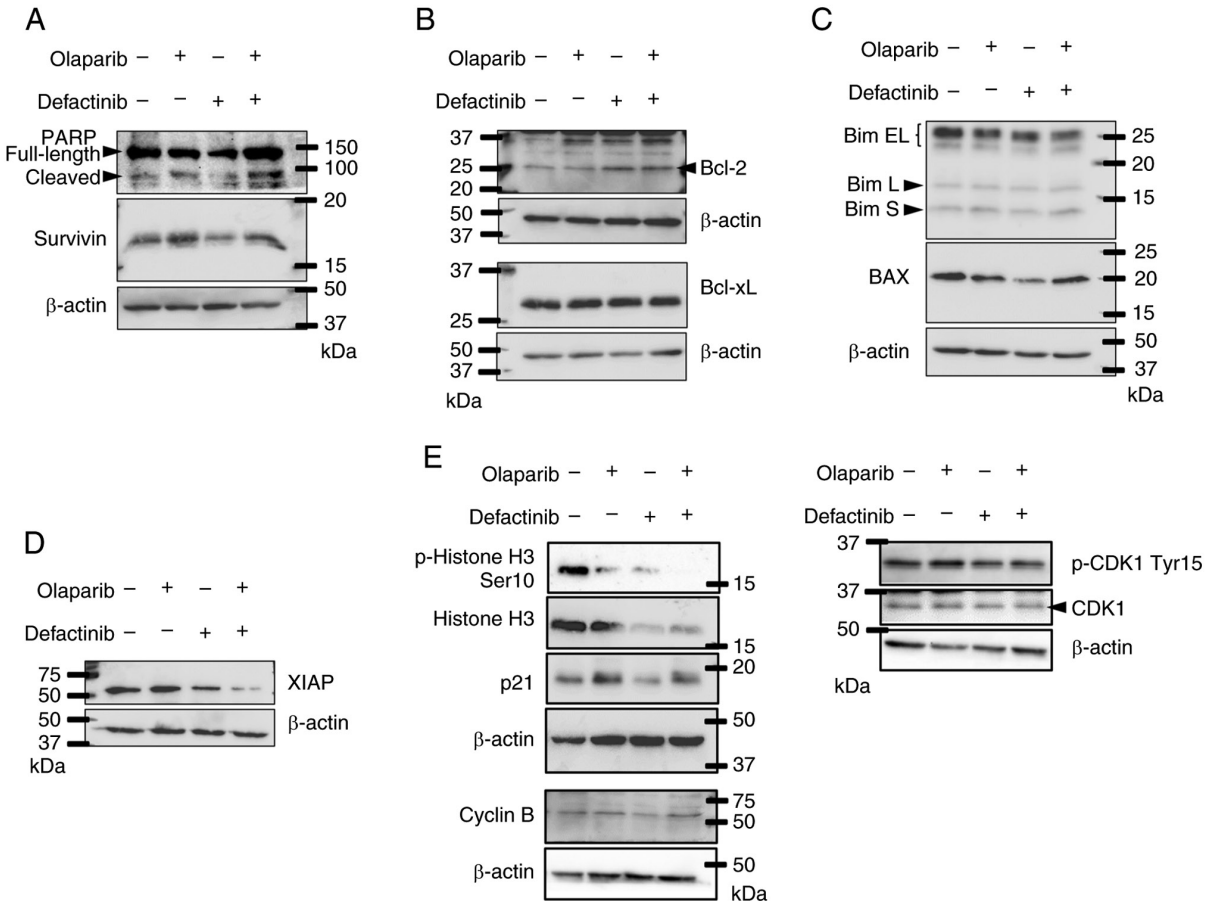

Figure 4. Defactinib induces apoptosis more strongly in combination with olaparib with suppressing XIAP. Western blotting data for apoptosis-related proteins in MDA-MB-231 cells treated with DMSO or olaparib and/or defactinib for 48 h. Cells were treated with DMSO or 20 μM olaparib and/or 2 μM defactinib. The expression of the cleavage of (A) PARP, (B and C) several Bcl-2 family proteins and also (D) XIAP and (A) survivin as major anti-apoptotic proteins. (E) Western blotting data for cell cycle-related proteins in MCF-7 cells treated with DMSO or olaparib and/or defactinib for 24 h. Cells were treated with DMSO or 10 μM olaparib and/or 1 μM defactinib. The molecular weight markers are indicated in the figures. XIAP, X-linked inhibitor of apoptosis protein; PARP, poly ADP-ribose polymerase.

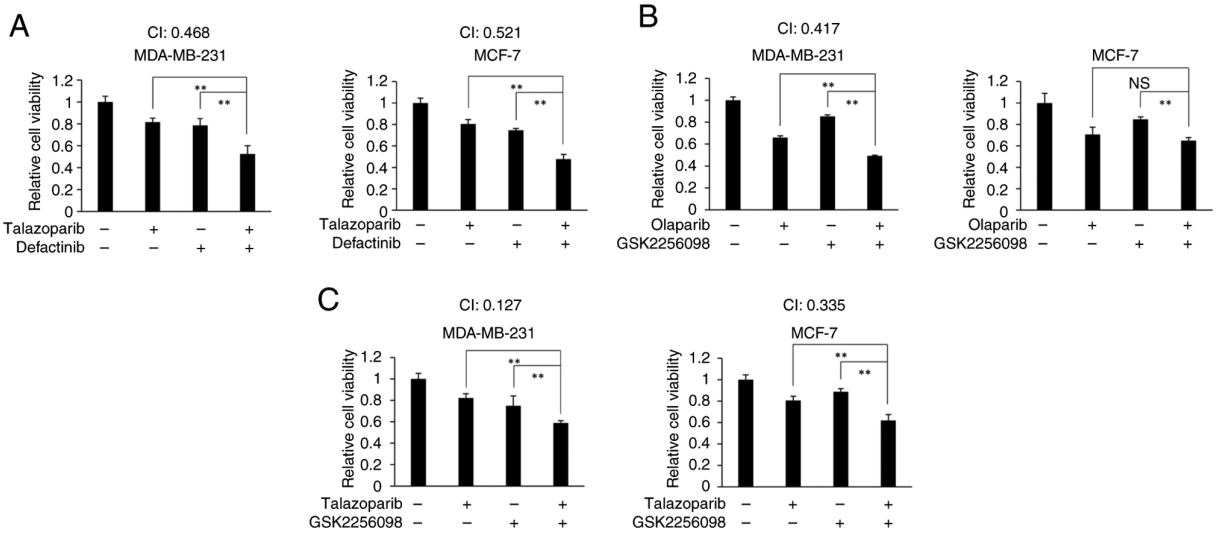

Figure 5. Combination treatment of a PARP inhibitor and FAK inhibitor. (A) The efficacy of the combination treatment of talazoparib and defactinib in MDA-MB-231 and MCF-7 cells was investigated using Cell Counting Kit-8. Cells were treated with DMSO or 5 nM talazoparib and/or 1 μM defactinib. (B) Similar to that in (A), the efficacy of the combination treatment of olaparib and GSK2256098 in cells was investigated. MDA-MB-231 cells were treated for 72 h with DMSO or 10 μM olaparib and/or 0.5 μM GSK2256098, while MCF-7 cells were treated with DMSO or 10 μM olaparib and/or 20 μM GSK2256098. (C) The efficacy of the combination treatment of talazoparib and GSK2256098 was also investigated. MDA-MB-231 cells were treated for 72 h with DMSO or 10 nM talazoparib and/or 0.5 μM GSK2256098, while MCF-7 cells were treated with DMSO or 5 nM talazoparib and/or 5 μM GSK2256098. The relative cell viability with the combination treatment was calculated by comparisons with the single treatment. The combination index of significant effects is shown above the columns. **P<0.01. NS, not significant; FAK, focal adhesion kinase; PARP, poly ADP-ribose polymerase.

of olaparib or talazoparib and defactinib exerted synergistic effects (Figs. 2A and 5A), defactinib is expected to enhance PARP inhibitors-induced suppression of BRCA-proficient breast cancer cell proliferation.

The concentrations of olaparib (10-20 μM) used in our vitro experiments exceed the clinically achievable plasma exposure rage reported for the approved dose of 300 mg twice daily (600 mg/day). However, these concentrations are consistent with those used in previous preclinical studies and were selected to define the maximal pharmacologic and mechanistic effects of olaparib *in vitro* (10). In contrast, the concentrations of talazoparib (5-10 nM) used in this study are within the clinically achievable plasma range associated with the approved dose of 1mg once daily. Defactinib has demonstrated relevant activity in the low μM range, consistent with the reported IC50 values in NSCLC cell lines (27) and our findings in breast cancer cells. Importantly, the efficacy of combination was observed in our study at low μM concentrations of defactinib. Furthermore, the translational relevance of this therapeutic strategy is supported by the success of recent clinical approval of a combination therapy containing defactinib (24,25). Nevertheless, further studies using clinically relevant exposures and *in vivo* models are needed to confirm the translational relevance of our findings.

In the present study, we demonstrated that the pharmacological inhibition of FAK suppressed BRCA1 and BRCA2 expression at the mRNA level (Figs. 2D and S5B), suggesting a transcriptional mechanism contributing to HR impairment. Although the direct molecular link between BRCA transcription and FAK signaling remains to be fully elucidated, the present results are consistent with previous findings indicating that FAK plays a role in the regulation of the DNA damage response and also that its inhibition compromises the capacity for DNA repair and increases sensitivity to genotoxic stress (29). In addition, FAK has been reported to regulate downstream signaling pathways, such as the RAS or PI3K/AKT pathway, which have been implicated in the maintenance of BRCA expression and HR proficiency (9,19). Collectively, these studies support a model in which FAK inhibition disrupts DNA damage response signaling and may lead to the transcriptional down-regulation of BRCA expression, thereby contributing to a BRCAness-like state and providing a potential mechanistic rationale for enhanced sensitization to PARP inhibitors.

Olaparib has been shown to inhibit cancer growth through apoptosis in various types of cancer (10,14,30,31). Therefore, we hypothesized that the addition of defactinib may enhance this effect by the further induction of apoptosis. However, the present results showed only modest differences in apoptotic induction in MDA-MB-231 and MCF-7 cells (Fig. 3C). Targeting MAPK (RAS/RAF/ERK) or PI3K/AKT signaling pathway has known to induce apoptosis (32,33) and can be therapeutic focus in a large fraction of breast cancer (19,34-36). Given the previously reported interactions between FAK and these signaling (19,37), differences in cascade dependency shown in Fig. S2 may underlie the distinct apoptotic response to the combination treatment.

Olaparib was the only PARP inhibitor available in our clinical setting at the initiation of this study and its indication for breast cancer was limited to patients with *BRCA* germline mutations. More recently, the use of olaparib has expanded to breast cancer with somatic *BRCA* mutations and talazoparib has also been approved for breast cancer with germline *BRCA* mutations in our country (2). Somatic *BRCA*-mutated breast cancer has been shown to respond to PARP inhibitors as well as germline *BRCA*-mutated cases; however, somatic *BRCA* mutations are rare in breast cancer, thereby limiting the number of patients eligible for PARP inhibitors (3-5). Given that PARP inhibitors exhibit anti-tumor activity against carcinomas with HR deficiency beyond *BRCA* mutations (6), it is important to expand their use in the treatment of breast cancer. We also believe that proposing a new treatment strategy using PARP inhibitors for BRCA-proficient breast cancer would be useful.

PARP inhibitors are widely used in the treatment of ovarian cancer with HR deficiency or platinum sensitivity (38-42). However, resistance to PARP inhibitors is a major clinical challenge in long-time use. Several mechanisms of resistance have been shown, including the restoration of HR through *BRCA* reversion mutations and changes in DNA damage response signaling (7,8); however, studies addressing PARP inhibitor resistance in breast cancer remain limited. The present results may be noteworthy because we suggest that defactinib not only enhances the initial sensitivity to PARP inhibitors, but also has the potential to re-induce the effectiveness of olaparib in resistant tumors or delay resistance. Moreover, combination strategies involving PARP inhibitors in ovarian cancer have already exhibited clinical benefits, and combination therapy using olaparib and the VEGF inhibitor bevacizumab has been administered as a first-line treatment (39). Ongoing clinical trials on combination therapies using olaparib have been examined with molecular-targeting agents (43,44). The present results extend this concept by proposing FAK inhibition as a novel approach for combination therapy, which may be applicable not only to BRCA-proficient breast cancer, but also to PARP inhibitor-resistant ovarian cancer.

There are a number of limitations that need to be addressed. The present results are based primarily on *in vitro* models. Furthermore, although we demonstrated the transcriptional down-regulation of BRCA expression, the precise molecular mechanisms linking FAK inhibition and HR deficiency require further investigation. In addition, this study did not directly evaluate the acquired reverse mutation by the treatment with PARP inhibitors; therefore, the ability of defactinib to recover established resistance needs to be confirmed in further studies.

In conclusion, this is the first study to demonstrate that combination treatment of PARP inhibitors and the FAK inhibitor defactinib exerted synergistic effects in inhibiting the proliferation of BRCA-proficient breast cancer cells. The consistent synergy observed with olaparib and talazoparib highlights the potential of this strategy. These results provide a rationale for further clinical investigations of this combination to expand the therapeutic scope and overcome resistance to PARP inhibitors.

Acknowledgements

We thank Ms. Emi Nishimoto (Department of Drug Discovery Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan) for assistance with the additional experiments conducted during manuscript revision.

Funding

This study was supported by JSPS KAKENHI (grant no. 20K18906).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

HO and TS conceptualized the study. HO and MH designed the study. HO and TS acquired funding for the study. HO, KT, SY, MS, TM, YN and MH acquired and analyzed the data. HO, MH and TS confirmed the authenticity of all the raw data. HO wrote the original draft and HO, KT, SY, YN, MH and TS edited the manuscript. TS supervised the research. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Robson M, Im SA, Senkus E, Xu B, Domchek SM, Masuda N, Delaloge S, Li W, Tung N, Armstrong A, *et al*: Olaparib for metastatic breast cancer in patient with a germline BRCA mutation. N Engl J Med 377: 523-533, 2017.
2. Litton JK, Rugo HS, Ettl J, Hurvitz SA, Gonçalves A, Lee KH, Fehrenbacher L, Yerushalmi R, Mina LA, Martin M, *et al*: Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. N Engl J Med 379: 53-763, 2018.
3. Tung NM, Robson ME, Ventz S, Santa-Maria CA, Nanda R, Marcom PK, Shah PD, Ballinger TJ, Yang ES, Vinayak S, *et al*: TBCRC 048: Phase II Study of olaparib for metastatic breast cancer and mutations in homologous recombination-related genes. J Clin Oncol 38: 4274-4282, 2020.
4. Momozawa Y, Iwasaki Y, Parsons MT, Kamatani Y, Takahashi A, Tamura C, Katagiri T, Yoshida T, Nakamura S, Sugano K, *et al*: Germline pathogenic variants of 11 breast cancer genes in 7,051 Japanese patients and 11,241 controls. Nat Commun 9: 4083, 2018.
5. Winter C, Nilsson MP, Olsson E, George AM, Chen Y, Kvist A, Törngren T, Vallon-Christersson J, Hegardt C, Häkkinen J, *et al*: Targeted sequencing of BRCA1 and BRCA2 across a large unselected breast cancer cohort suggests that one-third of mutations are somatic. Ann Oncol 27: 1532-1538, 2016.
6. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C, *et al*: Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 434: 917-921, 2005.
7. Edwards SL, Brough R, Lord CJ, Natrajan R, Vatcheva R, Levine DA, Boyd J, Reis-Filho JS and Ashworth A: Resistance to therapy caused by intragenic deletion in BRCA2. Nature 451: 1111-1115, 2008.
8. Lin KK, Harrell MI, Oza AM, Oaknin A, Ray-Coquard I, Tinker AV, Helman E, Radke MR, Say C, Vo LT, *et al*: BRCA reversion mutations in circulating tumor DNA predict primary and acquired resistance to the PARP inhibitor rucaparib in high-grade ovarian carcinoma. Cancer Discov 9: 210-219, 2019.
9. Ibrahim YH, García-García C, Serra V, He L, Torres-Lockhart K, Prat A, Anton P, Cozar P, Guzmán M, Grueso J, *et al*: PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient triple-negative breast cancer to PARP inhibition. Cancer Discov 2: 1036-1047, 2012.
10. Min A, Im SA, Kim DK, Song SH, Kim HJ, Lee KH, Kim TY, Han SW, Oh DY, Kim TY, *et al*: Histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA), enhances anti-tumor effects of the poly (ADP-ribose) polymerase (PARP) inhibitor olaparib in triple-negative breast cancer cells. Breast Cancer Res 17: 33, 2015.
11. Ono H, Sowa Y, Horinaka M, Iizumi Y, Watanabe M, Morita M, Nishimoto E, Taguchi T and Sakai T: The histone deacetylase inhibitor OBP-801 and eribulin synergistically inhibit the growth of triple-negative breast cancer cells with the suppression of survivin, Bcl-xL, and the MAPK pathway. Breast Cancer Res Treat 171: 43-52, 2018.
12. Ono H, Horinaka M, Sukeno M, Morita M, Yasuda S, Nishimoto E, Konishi E and Sakai T: Novel RAF/MEK inhibitor CH5126766/VS-6766 has efficacy in combination with eribulin for the treatment of triple-negative breast cancer. Cancer Sci 112: 4166-4175, 2021.
13. Saito Y, Taniguchi K, Ii H, Horinaka M, Kageyama S, Nakata S, Ukimura O and Sakai T: Identification of c-Met as a novel target of γ-glutamylcyclotransferase. Sci Rep 24: 11922, 2023.
14. Wu C, Peng S, Pilié PG, Geng C, Park S, Manyam GC, Lu Y, Yang G, Tang Z, Kondraganti S, *et al*: PARP and CDK4/6 inhibitor combination therapy induces apoptosis and suppresses neuroendocrine differentiation in prostate cancer. Mol Cancer Ther 20: 1680-1691, 2021.
15. Hanks SK, Calalb MB, Harper MC and Patel SK: Focal adhesion protein-tyrosine kinase phosphorylated in response to cell attachment to fibronectin. Proc Natl Acad Sci USA 89: 8487-8491, 1992.
16. Schaller MD, Borgman CA, Cobb BS, Vines RR, Reynolds AB and Parsons JT: pp125FAK a structurally distinctive protein-tyrosine kinase associated with focal adhesions. Proc Natl Acad Sci USA 89: 5192-5196, 1992.
17. Mitra SK and Schlaepfer DD: Integrin-regulated FAK-Src signaling in normal and cancer cells. Curr Opin Cell Biol 18: 516-523, 2006.
18. Hauck CR, Hsia DA and Schlaepfer DD: The focal adhesion kinase-a regulator of cell migration and invasion. IUBMB Life 53: 115-119, 2002.
19. Pylayeva Y, Gillen KM, Gerald W, Beggs HE, Reichardt LF and Giancotti FG: Ras- and PI3K-dependent breast tumorigenesis in mice and humans requires focal adhesion kinase signaling. J Clin Invest 119: 252-266, 2009.
20. Aboubakar Nana F, Vanderputten M and Ocak S: Role of focal adhesion kinase in small-cell lung cancer and its potential as a therapeutic target. Cancers (Basel) 29: 1683, 2019.
21. Murphy JM, Jeong K, Ahn EE and Lim SS: FAK inhibition suppresses breast cancer progression via DNA methylation-mediated DAB2 gene reactivation. BioRxiv 10: 624992, 2024.
22. Ishii N, Harada N, Joseph EW, Ohara K, Miura T, Sakamoto H, Matsuda Y, Tomii Y, Tachibana-Kondo Y, Iikura H, *et al*: Enhanced inhibition of ERK signaling by a novel allosteric MEK inhibitor, CH5126766, that suppresses feedback reactivation of RAF activity. Cancer Res 1: 4050-4060, 2013.
23. Sakai T: 'RB-reactivator screening' as a novel cell-based assay for discoveries of molecular targeting agents including the first-in-class MEK inhibitor trametinib (trade name: Mekinist). Pharmacol Ther 236: 108234, 2022.
24. McNamara B, Demirkiran C, Hartwich TMP, Bellone S, Manavella D, Mutlu L, Greenman M, Zipponi M, Yang-Hartwich Y, Yang K, *et al*: Preclinical efficacy of RAF/MEK clamp avutometinib in combination with FAK inhibition in low grade serous ovarian cancer. Gynecol Oncol 183: 133-140, 2024.
25. Grisham R, Monk BJ, Van Nieuwenhuysen E, Moore KN, Fabbro M, O'Malley DM, Oaknin A, Thaker P, Oza AM, Colombo N, *et al*: GOG-3097/ENGOT-ov81/GTG-UK/RAMP 301: A phase 3, randomized trial evaluating avutometinib plus defactinib compared with investigator's choice of treatment in patients with recurrent low grade serous ovarian cancer. Int J Gynecol Cancer 35: 101832, 2025.

26. Capelletto E, Bironzo P, Denis L, Koustenis A, Bungaro M and Novello S: Single agent VS-6766 or VS-6766 plus defactinib in KRAS-mutant non-small-cell lung cancer: The RAMP-202 phase II trial. Future Oncol 18: 1907-1915, 2022.
27. Yoshimura A, Horinaka M, Yaoi T, Ono H, Itoh K, Yamada T, Takayama K and Sakai T: Epithelial-mesenchymal transition status is a remarkable biomarker for the combination treatment with avutometinib and defactinib in KRAS-mutated non-small cell lung cancer. Br J Cancer 131: 361-371, 2024.
28. Almazan J, Turapov T, Kircher DA, Stanley KA, Culver K, Medellin AP, Field MN, Parkman GL, Colman H, Coma S, *et al*: Combined inhibition of focal adhesion kinase and RAF/MEK elicits synergistic inhibition of melanoma growth and reduces metastases. Cell Rep Med 6: 101943, 2025.
29. Tang KJ, Constanzo JD, Venkateswaran N, Melegari M, Ilcheva M, Morales JC, Skoulidis F, Heymach JV, Boothman DA and Scaglioni PP: Focal adhesion kinase regulates the DNA damage response and its inhibition radiosensitizes mutant KRAS lung cancer. Clin Cancer Res 22: 5851-5863, 2016.
30. Shimo T, Kurebayashi J, Kanomata N, Yamashita T, Kozuka Y, Moriya T and Sonoo H: Antitumor and anti-cancer stem cell activity of a poly ADP-ribose polymerase inhibitor olaparib in breast cancer cells. Breast Cancer 21: 75-85, 2014.
31. Xu K, Chen Z, Cui Y, Qin C, He Y and Song X: Combined olaparib and oxaliplatin inhibits tumor proliferation and induces G2/M arrest and gamma-H2AX foci formation in colorectal cancer. Onco Targets Ther 8: 3047-3054, 2015.
32. Yue J and López JM: Understanding MAPK signaling pathways in apoptosis. Int J Mol Sci 21: 2346, 2020.
33. Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y and Greenberg ME: Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 91: 231-241, 1997.
34. Vasudevan KM, Barbie DA, Davies MA, Rabinovsky R, McNear CJ, Kim JJ, Hennessy BT, Tseng H, Pochanard P, Kim SY, *et al*: AKT independent signaling downstream of oncogenic PIK3CA mutations in human cancer. Cancer Cell 16: 21-32, 2009.
35. Forbes S, Clements J, Dawson E, Bamford S, Webb T, Dogan A, Flanagan A, Teague J, Wooster R, Futreal PA and Stratton MR: COSMIC 2005. Br J Cancer 94: 318-322, 2006.
36. Turner NC, Oliveira M, Howell SJ, Dalenc F, Cortes J, Gomez Moreno HL, Hu X, Jhaveri K, Krivorotko P, Loibl S, *et al*: CAPItello-291 study group, Capivasertib in hormone receptor-positive advanced breast cancer. N Engl J Med 38: 2058-2070, 2023.
37. Tamura M, Gu J, Danen EH, Takino T, Miyamoto S and Yamada KM: PTEN interactions with focal adhesion kinase and suppression of the extracellular matrix-dependent phosphatidylinositol 3-kinase/Akt cell survival pathway. J Biol Chem 274: 20693-20703, 1999.
38. Moore K, Colombo N, Scambia G, Kim BG, Oaknin A, Friedlander M, Lisyanskaya A, Floquet A, Leary A, Sonke GS, *et al*: Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. N Engl J Med 379: 2495-2505, 2018.
39. Ray-Coquard I, Pautier P, Pignata S, Pérol D, González-Martín A, Berger R, Fujiwara K, Vergote I, Colombo N, Mäenpää J, *et al*: PAOLA-1 Investigators, Olaparib plus bevacizumab as first-line maintenance in ovarian cancer. N Engl J Med 381: 2416-2428, 2019.
40. González-Martín A, Pothuri B, Vergote I, DePont Christensen R, Graybill W, Mirza MR, McCormick C, Lorusso D, Hoskins P, Freyer G, *et al*: PRIMA/ENGOT-OV26/GOG-3012 Investigators, Niraparib in patients with newly diagnosed advanced ovarian cancer. N Engl J Med 381: 2391-2402, 2019.
41. Coleman RL, Fleming GF, Brady MF, Swisher EM, Steffensen KD, Friedlander M, Okamoto A, Moore KN, Efrat Ben-Baruch N, Werner TL, *et al*: Veliparib with first-line chemotherapy and as maintenance therapy in ovarian cancer. N Engl J Med 381: 2403-2415, 2019.
42. Nakamura K, Hayashi H, Kawano R, Ishikawa M, Aimono E, Mizuno T, Kuroda H, Kojima Y, Niikura N, Kawanishi A, *et al*. BRCA1/2 reversion mutations in a pan-cancer cohort. Cancer Sci 115: 635-647, 2024.
43. Nicum S, McGregor N, Austin R, Collins L, Dutton S, McNeish I, Glasspool R, Hall M, Roux R, Michael A, *et al*: Results of a randomised Phase II trial of olaparib, chemotherapy or olaparib and cediranib in patients with platinum-resistant ovarian cancer. Br J Cancer 130: 941-950, 2024.
44. Konstantinopoulos PA, Gonzalez-Martin A, Cruz FM, Friedlander M, Glasspool R, Lorusso D, Marth C, Monk BJ, Kim JW, Hinson P, *et al*: EPIK-O/ENGOT-OV61: Alpelisib plus olaparib vs cytotoxic chemotherapy in high-grade serous ovarian cancer (phase III study). Future Oncol 18: 3481-3492, 2022.