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Original Article

Procyanidin C1 combined with olaparib induces senescence in BRCA1-proficient breast cancer cells

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Abstract: PARP inhibitors (PARPi) are effective only in BRCA1-mutated tumors, with BRCA1-proficient patients deriving minimal benefit. Here, we demonstrate that PCC1 persistently induces double-strand DNA breaks (DSBs) in BRCA1-proficient breast cancer cells. Critically, PARP inhibition blocks base excision repair (BER), leading to accumulation of DNA damage and direct activation of senescence programs. Compared to monotherapy, the combination of olaparib and PCC1 significantly enhanced cellular senescence and suppressed tumor growth. In sum, we developed a novel therapeutic regimen that extends the utility of PARP inhibitors to BRCA1-proficient breast cancer patients through synergistic co-targeting of DNA damage responses.

Keywords: Procyanidin C1, BRCA1-proficient, PARP inhibitors, cellular senescence, breast cancer

Introduction

Cellular senescence refers to a permanent state of cell cycle arrest that occurs when proliferating cells are subjected to various stresses [1]. Since senescent cells possess unique biological characteristics, such as defects in DNA damage repair, the "one-two punch" sequential cancer therapy strategy has gained increasing attention in the field of oncology. This approach involves first inducing senescence in cancer cells with drugs, and then selectively eliminating these cells by exploiting their unique physiological changes [2]. However, a limitation of this strategy is that typically only a small fraction of cancer cells undergo senescence during conventional chemotherapy or radiotherapy, as the senescence response is only triggered within a specific DNA damage window. Therefore, enhancing the efficiency of senescence induction is a key challenge.

PARP inhibitors can bind competitively with NAD+ to the catalytic domain of PARP-1, inhibiting its enzymatic activity and impairing DNA repair. PARP inhibitors are more effective when homologous recombination repair (HRR) is insufficient, making PARP inhibitors a treatment option for patients with BRCA1 mutant tumors. However, this does not rule out the use of PARP inhibitors in tumors with normal BRCA1 functions. Studies have shown that PARP inhibitors have a synergistic effect in inducing iron death in ovarian cancers with normal BRCA1 functions [3]. In addition, paclitaxel can be used in conjunction with PARP inhibitors in tumors with normal BRCA1 functions [4]. Interestingly, PARP inhibitors also have a role in promoting cellular senescence [5]. In addition, PARP inhibitors have shown synergistic potential in inducing cellular aging when combined with CDK4/6 inhibitors. For example, combination with CDK4/6 inhibitors can effectively

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induce cellular senescence [6], demonstrating the potential of combination therapies.

Procyanidins are the terminal products of the flavonoid biosynthesis pathway and are widely found in the seeds and bark of numerous plants [7]. Among them, PCC1 has been demonstrated to have the ability to selectively eliminate senescent cells [8]. In this study, we found that when PCC1 was combined with the PARP inhibitor olaparib, it did not exhibit senolytic effects; Instead, it significantly promoted cellular senescence synergistically. Mechanistically, PCC1 can promote DNA damage in breast cancer cells, and when combined with PARP inhibitors, it extends the DNA damage window by inhibiting DNA repair through the suppression of PARP enzyme activity. Preclinical data suggested that PCC1 can not only synergize with olaparib to inhibit the proliferation of BRCA1-mutant breast cancer cells but also broaden the application scope of PARP inhibitors, making them effective against BRCA1-proficient breast cancer cells.

In summary, we have identified a novel integrated Chinese and Western medicine regimen, which warrants further investigation and application in clinical settings as a potential cellular senescence-inducing and chemosensitizing strategy.

Material and methods

Cell lines and cell culture

BRCA1-proficient breast cancer cell MDA-MB-231, and BRCA1-mutant breast cancer cell SUM149PT and HCC1937 were obtained from ATCC. MDA-MB-231 and SUM149PT cells were cultured in DMEM (Gibco) supplemented with 10% FBS (Gibco), while HCC1937 cells were cultured in 1640 medium (Gibco) with 10% FBS (Gibco).

RNA interference

Cells were transfected with 100 nM siRNAs (Qingke, China) targeting BRCA1, 53BP1, Snail, or a scrambled (SCR) control sequence (Ruibo, China) using Lipofectamine 3000 (Invitrogen) following the manufacturer's guidelines. The targeting sequences for siRNAs were as follows: BRCA1 siRNA-1: GAGTATGCAAACAGCTATAAT; BRCA1 siRNA-2: TATAAGACCTCTGGCATGAAT; 53BP1 siRNA-1: GATACTTGGTCTTACTGGTTT; 53BP1 siRNA-2: GCCAGGTTCTAGAGGATGA; Snail siRNA-1: CCACTCAGATGTCAAGAAGTA; Snail siRNA-2: GCCTTCAACTGCAAATACT.

Flow cytometry

Cells were stained with annexin-V-FITC and propidium iodide according to the manufacturer's instructions (Beyotime, China) and analyzed using a NovoCyte D3000 flow cytometer (Agilent). For cell cycle analysis, cells were fixed in ice-cold 70% ethanol for at least 12 hours and then stained with propidium iodide in the presence of RNase. DNA content and cell cycle analysis were performed using NovoExpress software.

SA-β-galactosidase detection

Cells grown in 6-well plates were treated with either DMSO control or PCC1 for 6 days. Subsequently, the cells were fixed in 4% PFA and subjected to SA-β-Gal staining using the Cell Senescence SA-β-Gal Staining Kit (C0602, Beyotime).

Transwell assays

Transwell invasion and migration assays were performed using 8 μm chambers (corning) with or without Matrigel (Corning Costar, United States). 1×10^5 cells in 200 μL serum-free medium with PCC1 were added to the upper chamber, while 600 μL of culture medium with 20% FBS was placed in the lower well. After 48 hours of culture, cells on the upper surface of the membrane were removed, and cells on the lower surface were fixed with 4% formaldehyde at 37°C for 15 minutes. Subsequently, they were stained with 0.05% crystal violet for 20 minutes. Transwell assays were repeated for three times and relative quantification analysis was based on grayscale values. Five random fields of view were assessed for each group.

Gelatin zymography

Cells were treated with PCC1 for 48 hours in a serum-free medium. Protein determinations of the conditioned medium were carried out using the BCA assay kit (BOSTER, China) after centrifugation at 400 × g for 5 minutes. MMP activities in the conditioned medium were then assessed using the Zymography Assay Kit

(Xinfan BioTECH, China) following the manufacturer's protocols. Densitometric analysis was performed using ImageJ.

Immunoblotting

Cells were lysed with RIPA buffer (Beyotime, China) containing protease inhibitors from MCE (China), and protein concentrations were quantified using the BCA assay Kit (BOSTER). Proteins (10-20 μg) were separated on SDS-PAGE gels, transferred to PVDF membranes (Millipore), blocked with 5% milk in 0.1% TBS/Tween-20 at room temperature for 2 hours, incubated with primary and secondary antibodies, and visualized using the ECL detection kit (Beyotime, China). The following antibodies were used: γ-H2AX (9718, CST), PARP (AF1657, beyotime), Cleaved PARP (AF1567, beyotime), CASPASE3 (AC030, beyotime), CleavedCASPASE3 (AC033, beyotime), BCL-2 (ab182858, abcom), snail (3879, CST), BRCA1 (D120321, sangon), 53BP1 (biolegend), GAPDH (beyotimeAF1186).

RNA isolation and quantitative real-time PCR (qRT-PCR)

RNA isolation and qRT-PCR were performed as previously described [9]. Briefly, total RNA was extracted using TRIzol (Invitrogen), and PrimeScript RT Master Mix (Takara) was used for two-step real-time RT-PCR analysis on a Roche LightCycler 480. GAPDH was used as a loading control. Primer sequences are provided in Table S1.

Immunofluorescence

Cells were seeded onto coverslips in 24-well plates and grown for 3 days. Cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.25% Triton in phosphate-buffered saline (PBS) for 15 minutes. Slides were blocked for 1 hour in PBS containing 3% bovine serum albumin (BSA). After extensive washing, cells were stained with antibodies targeting γ-H2AX (Cell Signaling, #9718, 1:200), in combination with Cy3-labeled or Alexa Fluor 488-labeled Goat Anti-Rabbit IgG (H+L) (Beyotime), and counterstained with DAPI.

Xenograft experiments

Female NCG mice were purchased from GemPharmatech Co.Ltd (Jiangsu, China). Female balb/c mice were purchased from Guangdong Provincial Medical Laboratory Animal Center. All mice were housed under specific pathogen-free (SPF) conditions in individually ventilated cages, with a controlled environment. Mice were provided with autoclaved standard rodent diet and water ad libitum. All animal procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee of the Institute of Basic Medicine and Oncology, Chinese Academy of Sciences (Approval No. 2024R0004). For in vivo tumor growth assay, mice at 4 weeks of age underwent subcutaneous xenograft breast cancer cells. 1×10^7 MDA-MB-231 cells were suspended in 100 μl PBS with matrigel (1:1) and were subcutaneously transplanted into the right side of the posterior flank of NCG mice. 1×10^6 4T1 cells were suspended in 100 μl PBS and were subcutaneously transplanted into the right side of the posterior flank of balb/c mice. When the tumors reached 100 mm^3 in volume, mice were divided randomly into the DMSO-treated group, PCC1-treated group (20 mg/kg), olaparib- treated group (50 mg/kg), and combo-treated group. Drugs were delivered via i.p. injection biweekly. Meanwhile, tumor growth was examined biweekly with a vernier caliper. Tumor volumes were calculated by using the equation: $V = A \times B2/2$ (mm^3), wherein A is the largest diameter and B is the perpendicular diameter. After 21 days, mice were sacrificed, and tumors were collected and weighed. A part of each tumor was fixed with RNA later (beyotime), and the rest of the tumor was frozen in liquid nitrogen.

At the experimental endpoint or if humane endpoints were reached earlier, mice were euthanized by carbon dioxide (CO_2) inhalation followed by cervical dislocation as a secondary confirmatory method.

Statistical analysis

All the statistical analyses in this study were performed by using Prism 9.0. Data are presented as mean ± SEM. The two-tailed unpaired Student's t-test, one-way analysis of variance (ANOVA) with Tukey's multiple comparisons test, and two-way ANOVA with Sidak's multiple comparisons test were used to evaluate the statistical differences in the treatment. All quantitative data are presented as the mean ±

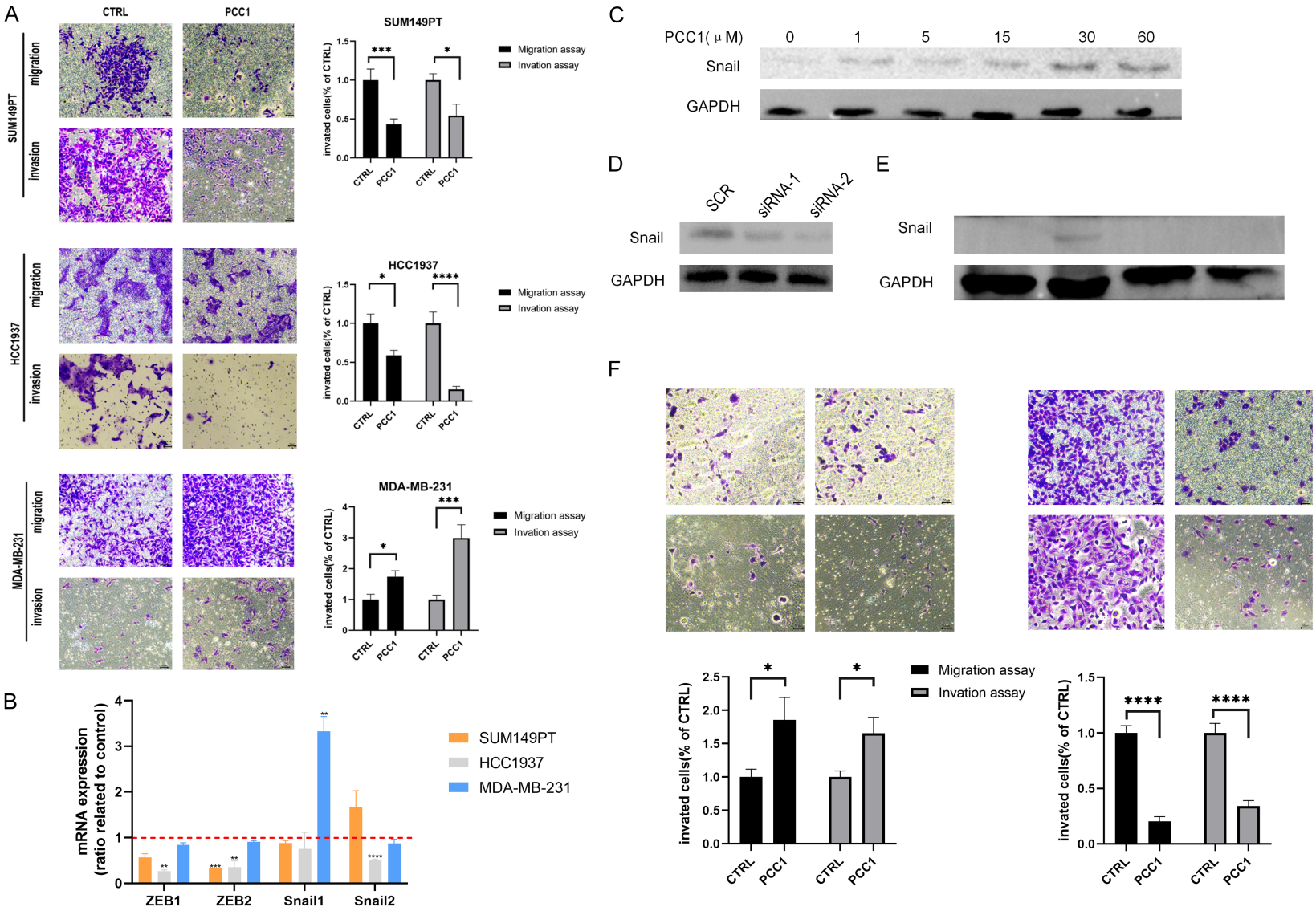


Figure 1. PCC1 upregulates the migratory ability of MDA-MB-231 cells via SNAIL. (A) Migration and invasion capabilities of SUM149PT, HCC1937, and MDA-MB-231 cells were assessed using a Transwell assay after 48 hours of treatment with DMSO or PCC1 (30 μM) (200X). (B) Relative mRNA levels of upstream EMT transcription factors in SUM149PT, HCC1937, and MDA-MB-231 cells treated for 24 hours with DMSO or PCC1 (30 μM) were measured by qPCR. (C)

SNAIL protein expression levels in MDA-MB-231 cells treated with PCC1 at the indicated concentration (30 μM) for 24 hours were detected by Western blot. (D) Immunoblotting of Snail and GAPDH at 72 hours after transfection with the indicated siRNAs in MDA-MB-231 cells. (E, F) Cells transfected with negative control siRNA (SCR) or Snail siRNA (siSnail) for 24 hours were subsequently treated with DMSO or PCC1 (30 μM) for 48 hours. SNAIL protein expression was analyzed by immunoblotting (E), and cellular migration and invasion were assessed using a Transwell assay (200X). (F) Statistical significance was tested using the t-test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

standard deviation (SD) of at least three independent biological replicates.

Results

PCC1 enhances the migratory capacity of MDA-MB-231 cells by upregulating SNAIL

PCC1 has been found to inhibit the proliferation of MDA-MB-231 cells [10]. However, unexpectedly, PCC1 was observed to enhance the migratory and invasive capabilities of MDA-MB-231 cells, an effect not seen in HCC1937 and SUM149PT cells (**Figure 1A**). To investigate the underlying mechanism, we examined the expression of epithelial-mesenchymal transition (EMT) related transcription factors and protein markers. The results revealed that PCC1 promoted the expression of the Snail transcription factor in MDA-MB-231 cells, thereby enhancing their migratory and invasive capacities (**Figure 1B**, **1C**). First, we silenced Snail expression in MDA-MB-231 cells. As shown in **Figure 1D**, siRNA-2 exhibited the most pronounced inhibitory effect. Subsequent knockdown experiments of Snail reversed the migration-promoting effect of PCC1 on MDA-MB-231 cells (**Figure 1E**, **1F**).

PCC1 promotes senescence in MDA-MB-231 cells

PCC1 promotes cytokine-induced senescence, leading to an increased expression of EMT-related genes, particularly Snail [11]. Cellular senescence causes cell cycle arrest, inhibiting tumor cell proliferation while promoting cell migration through the SASP. Therefore, we hypothesize that PCC1 induces senescence in MDA-MB-231 cells. Normal cells exhibit a narrow range of cell volume distribution, whereas senescent cells display an enlarged cell volume phenotype [12]. Additionally, increased cell volume can remodel the proteome, leading to cellular senescence [13]. We characterized the changes in physical parameters of MDA-MB-231 cells using flow cytometry. After 72 hours of PCC1 treatment, the mean FSC of MDA-MB-231 cells increased, indicating an increase in average tumor cell volume (**Figure 2A**). Subsequently, we assessed the proportion of SA-β-gal+ cells in MDA-MB-231 cells exposed to PCC1. The proportion of SA-β-gal+ cells significantly increased after PCC1 treatment (**Figure 2B**), along with the accumulation of mitochondria, lysosomes, and 53BP1 nuclear foci (**Figure 2C**, **2D**), all indicating that PCC1 promotes senescence in MDA-MB-231 cells. Cellular senescence leads to the upregulation of anti-apoptotic gene expression. We found that PCC1 inhibited PARP cleavage in a concentration-dependent manner, thereby inhibiting caspase-3 cleavage and attenuating the caspase cascade (**Figure 2E**). This suggests that PCC1 possesses anti-apoptotic potential. Furthermore, when co-administered with olaparib, PCC1 suppressed olaparib-induced apoptosis (**Figure 7A**).

PCC1 promotes MDA-MB-231 cell migration by regulating the SASP

Cellular senescence can lead to changes in the SASP, including the regulation of the MMP and TIMP families, thereby promoting cell migration. First, MMP2 enzymatic activity was detected using gelatin zymography. In contrast to HCC1937 and SUM149PT (**Figure 3A**), PCC1 promoted MMP2 enzymatic activity levels in MDA-MB-231 cells in a concentration-dependent manner (**Figure 3B**). Additionally, after exposure to PCC1, MDA-MB-231 cells remodeled the TIMP1/TIMP2 ratio, with decreased TIMP1 expression and increased TIMP2 expression, whereas SUM149PT and HCC1937 showed the opposite trend (**Figure 3C**). This suggests that PCC1 promotes the migration and invasion capabilities of MDA-MB-231 cells by remodeling the SASP.

MDA-MB-231 cells can counteract PCC1-induced DNA damage by upregulating DNA damage response

Persistent DNA damage (genotoxic stress) triggers signaling cascades that drive cells into senescence to avoid replicating a damaged

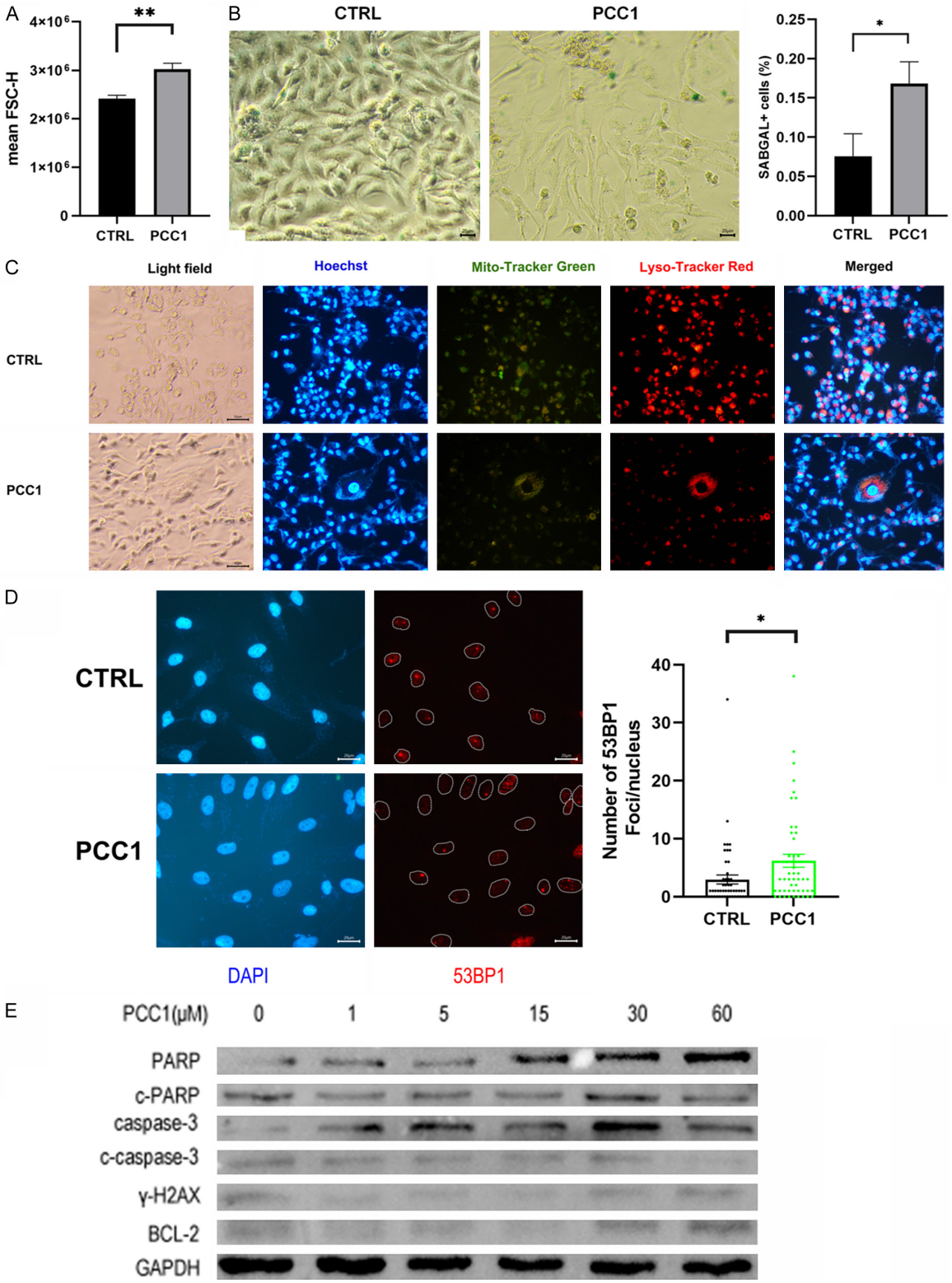


Figure 2. Exposure to PCC1 induces cellular senescence in MDA-MB-231 cells through activation of the DNA damage response. (A) Flow cytometry analysis of mean FSC (Forward Scatter) in MDA-MB-231 cells treated with DMSO control or PCC1 for 72 hours. (B) Detection of cellular senescence in MDA-MB-231 cells treated with DMSO control or PCC1 for 72 hours using β-Galactosidase staining (200X). (C, D) Mitochondria and lysosomes labeled with Mito-Tracker and Lyso-Tracker (200X) (C), and immunofluorescence detection of 53BP1 nuclear foci (400X) (D) in MDA-MB-231 cells treated with DMSO control or PCC1 for 72 hours. (E) Immunoblot analysis after exposure of MDA-MB-231 cells to varying concentrations of PCC1.Statistical significance was tested using the t-test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

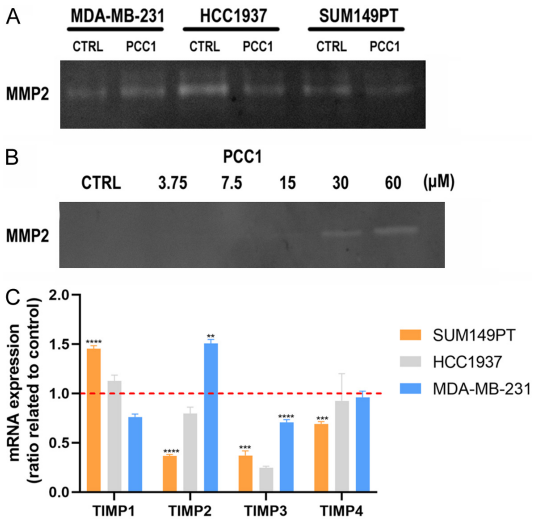


Figure 3. PCC1 affects MMP2 enzymatic activity by modulating the TIMP1/TIMP2 ratio. A. MMP2 enzymatic activity detected by gelatin zymography in MDA-MB-231, HCC1937, and SUM149PT cells treated with DMSO control or 30 μM PCC1 for 48 hours. B. Changes in MMP2 enzymatic activity detected by gelatin zymography in MDA-MB-231 cells treated with PCC1 at the indicated concentrations for 48 hours. C. Relative mRNA levels of TIMPs evaluated by real-time Q-PCR in breast cancer cells treated with DMSO control or 30 μM PCC1 for 2 days. Statistical significance was tested using the t-test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

genome. We first examined the number of γ-H2AX nuclear foci after PCC1 exposure and found that PCC1 consistently induced DNA damage across multiple breast cancer cell lines (**Figure 4A**). Notably, PCC1 simultaneously promoted the transcription of CHK1/ATR and CHK2/ATM in MDA-MB-231 cells, while it had no effect on HCC1937 and SUM149PT cells (**Figure 4B**). In summary, the differential DNA damage repair capacity is one of the reasons for the varying phenotypic responses to PCC1 among different breast cancer cell lines.

BRCA1 gene status is crucial for PCC1-induced senescence in breast cancer cells

ATM and ATR are upstream target genes of BRCA1. When BRCA1 is mutated, it leads to defects in homologous recombination repair, thereby inhibiting the activation of ATM and ATR [14]. To analyze whether the induction of senescence in MDA-MB-231 cells by PCC1 is due to the activation of the homologous recombination repair pathway, we silenced the BRCA1 and 53BP1 genes. BRCA1-siRNA-1 and 53BP1-siRNA-2 showing the highest knockdown efficiency (**Figure 5A**, **5B**), these two siRNAs were selected for all subsequent experiments. We found that silencing BRCA1 reversed the senescence-promoting effect of PCC1, whereas this was not observed with 53BP1 silencing (**Figure 5C**). This suggests that the activation of homologous recombination repair, rather than non-homologous end joining, is responsible for the senescence-promoting effect of PCC1. Moreover, BRCA1 silencing, but not 53BP1 silencing, increased the sensitivity of MDA-MB-231 cells to PCC1 (**Figure 5D**). Additionally, the migration-promoting effect of PCC1 on MDA-MB-231 cells was also reversed by BRCA1 silencing (**Figure 5E**).

PCC1 and olaparib synergistically inhibit breast cancer cell proliferation by promoting DNA damage

Olaparib is a PARP inhibitor that competes with NAD+ to bind to the catalytic domain of PARP-1, thereby inhibiting its enzymatic activity and impairing proper DNA repair [15]. Since tumor cells counteract PCC1-induced DNA damage by upregulating PARP, we subsequently combined PCC1 with Olaparib to observe the efficacy of the combination therapy. PCC1 significantly sensitized the anti-cancer effect of Olaparib, which was effective not only in BRCA1-mutant breast cancer cells HCC1937 but also rendered Olaparib effective against BRCA1-proficient breast cancer cells MDA-MB-231 (**Figure 6A**, **6B**). We further validated the antitumor activity of this combination in two xenograft models (**Figure 6C**). First of all, all treatment groups had no significant effect on the body weight of mice (**Figure 6D**). Further in vivo experiments indicated that monotherapy with either PCC1 or olaparib failed to robustly inhibit the growth of BRCA1-proficient MDA-MB-231 tumors. In contrast, the combination therapy markedly suppressed tumor progression, as evidenced by reduced tumor volume (**Figure 6E**) and a significant decrease in tumor weight (**Figure 6F**, **6G**). Similar combination efficacy was also confirmed in the 4T1 tumor model (**Figure 6H**), where the combined treatment significantly reduced tumor weight compared to single-agent therapies.

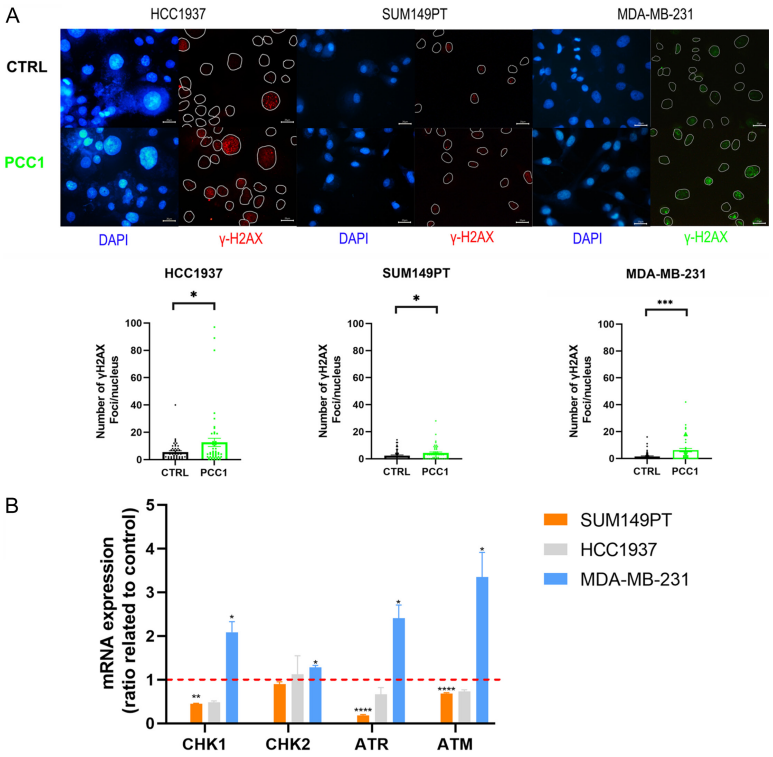


Figure 4. PCC1 targets the activation of the ATM/ATR pathway to promote DNA damage repair in BRCA1-proficient breast cancer cells. A. Quantification of γ-H2AX nuclear foci detected by immunofluorescence in HCC1937, SUM149PT, and MDA-MB-231 cells treated with 30 μM PCC1 or DMSO control for 24 hours (400X). B. Relative mRNA levels of CHK1, CHK2, ATR, and ATM evaluated by real-time Q-PCR in breast cancer cells treated with DMSO control or 30 μM PCC1 for 24 hours.Statistical significance was tested using the t-test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

PCC1 in combination with olaparib induces senescence in BRCA1-proficient breast cancer cells

To investigate the reasons behind the synergistic inhibition of breast cancer cell proliferation by PCC1 and Olaparib, we assessed the impact of PCC1 and Olaparib on cell apoptosis using flow cytometry. Unexpectedly, PCC1 did not enhance Olaparib-induced apoptosis in breast cancer cells; rather, PCC1 alone and in combination with Olaparib exhibited an inhibitory effect on apoptosis (**Figure 7A**, **7B**). Given that cellular senescence confers an anti-apoptotic phenotype, we further tested the synergistic effect of PCC1 and Olaparib on cell senescence. Both PCC1 and Olaparib alone promoted cell senescence; importantly, the combination of PCC1 and Olaparib significantly increased the proportion of senescent cells (**Figure 7C**). This suggests that the sensitizing effect of PCC1 on Olaparib may be due to the induction of cellular senescence. Quercetin is a classical senolytic agent but has also been found to induce tumor cell senescence [16]. To further explore the reasons and mechanisms for the increased proportion of senescent cells with combination therapy, we pretreated cells with Olaparib for 3 days and then observed the sequential effects of Quercetin and PCC1 (**Figure 7D**). The results showed that the Olaparib-PCC1 group had the highest cell senescence induction capability. However, the combination of PCC1 and Oparib did not significantly affect the aging of MDA-MB-231 cells previously treated with Oparib for 3 days, indicating that this combination therapy should be administered simultaneously to induce cell aging. In addition, quercetin did not significantly promote senescence in MDA-MB-231 cells pretreated with Olaparib, whereas sequential treatment with Olaparib followed by PCC1 promoted senescence. This suggests that the prosenescence effect of PCC1 on MDA-MB-231 cells may be specific to PCC1 rather than a general property of antisenescent drugs.

To validate these findings in vivo, we performed IHC for Ki67 and cleaved caspase-3 in 4T1 tumor tissues. Representative images and corresponding histoscores revealed that the combination of PCC1 and olaparib significantly reduced Ki67 expression and increased cleaved caspase-3 levels, indicating suppressed proliferation and enhanced apoptosis in vivo (**Figure 7E**, **7F**). Consistent with the in vitro findings, SA-β-galactosidase staining of 4T1 tumor tissues further confirmed that the combination treatment robustly induced cellular senescence in vivo (**Figure 7G**). Overall, these data suggest that PCC1 synergizes with olaparib to inhibit breast cancer growth by inducing cellular senescence rather than apoptosis, and this effect is effective both in vitro and in vivo.

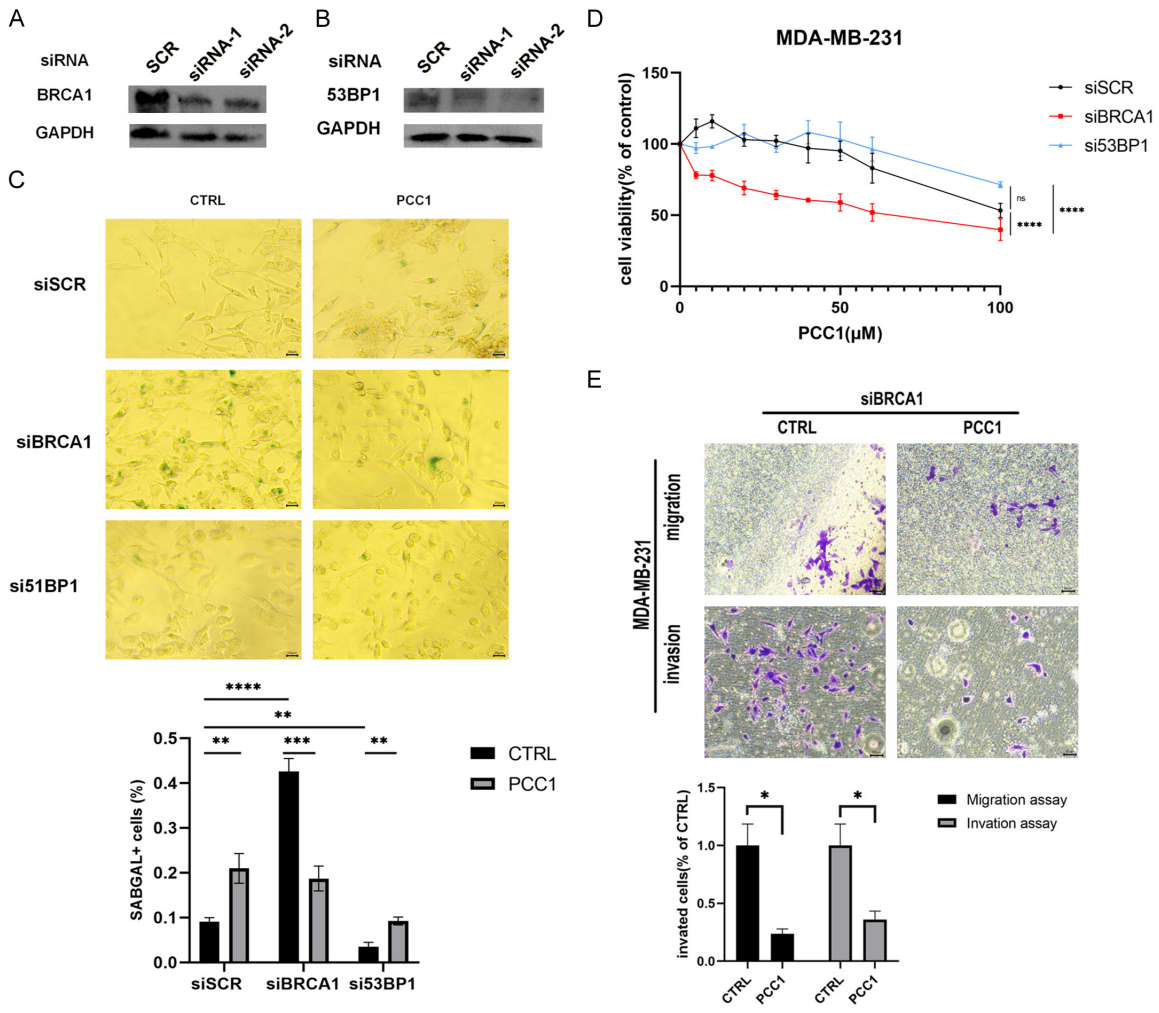


Figure 5. PCC1-induced cellular senescence is dependent on BRCA1 gene status. (A, B) Immunoblotting of BRCA1 (A), 53BP1 (B), and GAPDH at 72 hours after transfection with the indicated siRNAs in MDA-MB-231 cells. (C) Detection of cellular senescence by β-galactosidase staining in MDA-MB-231 cells transfected with negative control siRNA (siSCR), BRCA1 siRNA (siBRCA1), and 53BP1 siRNA (si53BP1) for 24 hours, followed by treatment with DMSO control or 30 μM PCC1 for 72 hours (200X). Data are shown as mean ± s.d. and analysed with two-way ANOVA followed by Tukey's multiple comparisons test. (D) Cell proliferation detected by CCK8 assay in MDA-MB-231 cells transfected with the indicated siRNAs for 24 hours, followed by treatment with complete medium containing PCC1 at the indicated concentrations for 24 hours. Data are shown as mean ± s.d. and analysed with two-way ANOVA followed by Tukey's multiple comparisons test. (E) Detection of cell migration and invasion abilities by transwell assay in MDA-MB-231 cells transfected with BRCA1 siRNA (siBRCA1) for 24 hours, followed by treatment with 30 μM PCC1 or DMSO control for 48 hours (200X). Statistical significance was tested using the t-test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

Discussion

PCC1 is a traditional plant monomer derived from grape seed extract (GSE), which has been found to selectively eliminate senescent cells [8]. However, the DNA damage repair mechanisms of senescent cells and tumor cells are different. Senescent cells tend to repair double-strand breaks via non-homologous end joining (NHEJ) rather than the more precise homologous recombination repair (HRR) [17]. Tumor cells, due to their high genomic instability, often have mutations in DNA damage response proteins or cell cycle regulatory proteins, enhancing or selectively using specific DNA repair pathways. The effects of senolytics on non-senescent tumor cells remain unclear. In this study, we found that PCC1 broadly induces DNA damage in breast cancer cells. For BRCA1-proficient breast cancer cells, PCC1

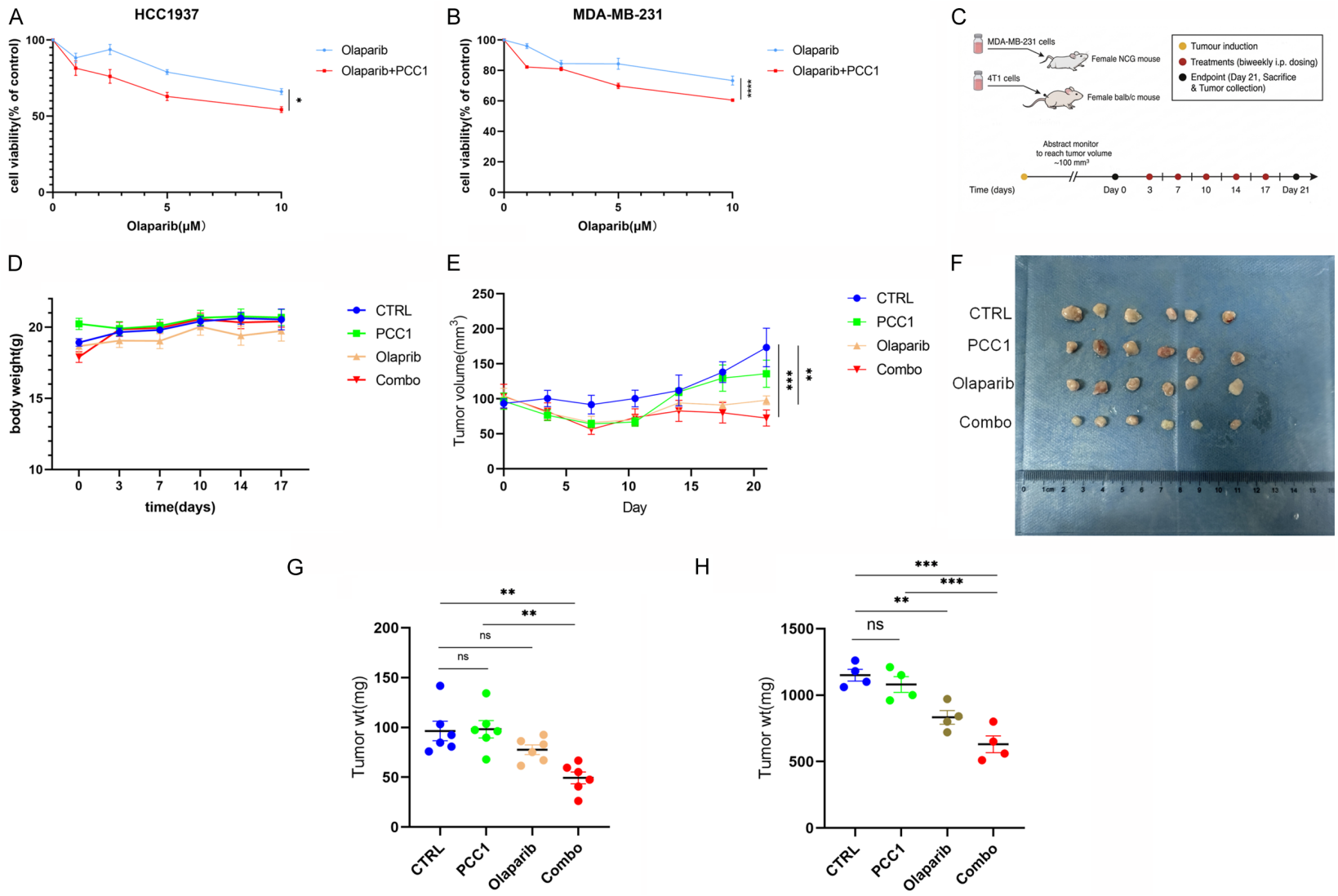
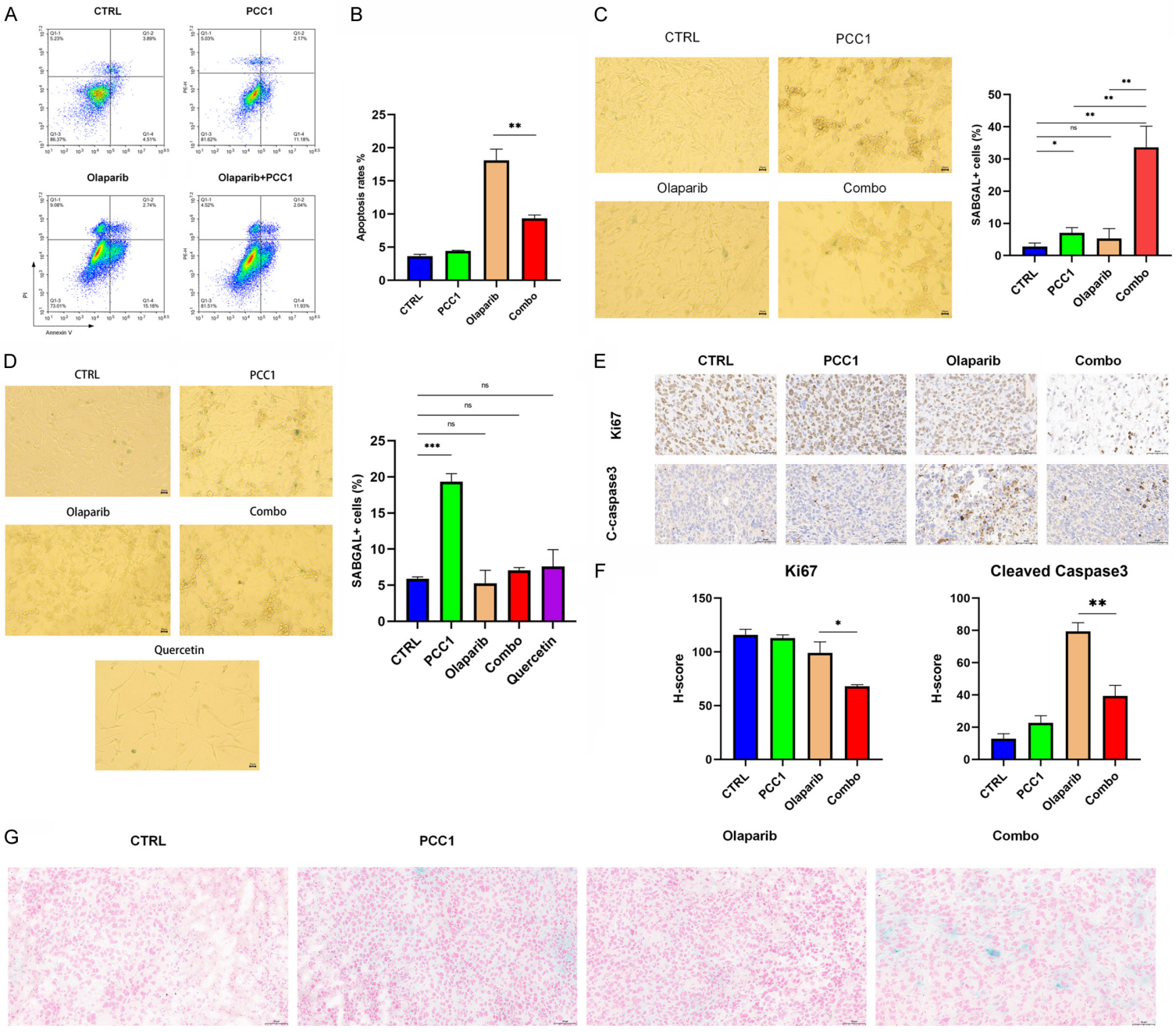


Figure 6. PCC1 synergizes with olaparib to inhibit breast cancer cell proliferation in vitro and in vivo. (A, B) Proliferation of BRCA1-mutant breast cancer cells HCC1937 (A) and BRCA1-proficient breast cancer cells MDA-MB-231 (B) detected by CCK8 assay after treatment with the indicated concentrations of olaparib combined with 30 μM PCC1 for 72 hours. Data are shown as mean ± s.d. and analysed with two-way ANOVA followed by Tukey's multiple comparisons test. (C) Schematic of the experimental schedule of the tumour model. (D) The weight of dissected xenograft tumors in each group. (E) Tumour growth curve of the MDA-MB-231 tumour after the implantation of various treatments. Data are shown as mean ± s.d. and analysed with two-way ANOVA followed by Tukey's multiple comparisons test. (F) Photographs of dissected xenograft tumors from NCG mice after sacrifice. (G, H) Tumour weight of the collected MDA-MB-231 (G) and 4T1 (H) tumour tissues after different treatments. Data are shown as mean ± s.d. and analysed with one-way ANOVA followed by Tukey's multiple comparisons test.


A
CTRL
PCC1
Olaparib
Olaparib+PCC1
PI
Annexin V
B
Apoptosis rates %
CTRL
PCC1
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C
CTRL
PCC1
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SABGAL+ cells (%)
D
CTRL
PCC1
Olaparib
Combo
Quercetin
SABGAL+ cells (%)
E
CTRL
PCC1
Olaparib
Combo
Ki67
C-caspase3
F
Ki67
Cleaved Caspase3
H-score
G
CTRL
PCC1
Olaparib
Combo

Figure 7. PCC1 in combination with olaparib induces senescence in breast cancer cells. (A, B) Apoptosis detected by flow cytometry (A) and the apoptosis rates (B) in MDA-MB-231 cells treated with DMSO control, PCC1 (30 μM), olaparib (5 μM), or their combination (Combo) for 3 days. (C) Senescence detected by β-galactosidase staining in MDA-MB-231 cells treated with DMSO control, PCC1 (30 μM), olaparib (5 μM), or combo for 6 days. (D) Senescence detected by β-galactosidase staining in MDA-MB-231 cells pre-treated with olaparib (5 μM) for 3 days, followed by treatment with DMSO control, PCC1 (30 μM), olaparib (5 μM), Combo, or quercetin (100 μM) for 3 days. (E, F) Representative immunohistochemistry (IHC) image in 4T1 tumour tissues for Ki67 and cleaved caspase3 (400X) (E) and corresponding histoscores (F). (G) Representative images of SA-β-Gal staining in 4T1 tumor tissues after treatment (400X). Data are shown as mean ± s.d. and analysed with one-way ANOVA followed by Tukey's multiple comparisons test.

upregulates the homologous recombination repair pathway to counteract DNA damage, leading to cellular senescence. In contrast, BRCA1-mutant breast cancer cells, which have defects in homologous recombination repair.

PARP inhibitors are anti-cancer drugs that target the DNA damage repair mechanism. They prevent the repair of DNA damage by blocking PARP activity. Therefore, PARP inhibitors are commonly used as sensitizers in chemotherapy or radiotherapy. Importantly, PARP inhibitors can act synergistically with CDK4/6 inhibitors to promote tumor cell senescence, demonstrating their potential in combination therapy. In this study, we found that the PARP inhibitor olaparib, when used in combination with PCC1, can significantly promote senescence and inhibit the proliferation of breast cancer cells with functional BRCA1. However, the underlying mechanism remains unclear and may involve overlapping effects on DNA damage repair.

Quercetin has been confirmed as a drug that clears senescent cells, and it was recently found to induce senescence in tumor cells [16]. Our study also indicates that PCC1 has the ability to induce senescence in tumor cells. This suggests that drugs that clear senescent cells may have the characteristic of inducing tumor cell senescence, possibly due to similar underlying mechanisms, but further investigation is required. In addition, our study shows that the pro-senescence effect of senolytic drugs on tumor cells can be triggered by PARP inhibitors, indicating that under certain conditions, there may be a mutual conversion between senolytic drugs and senescence inducers, a mechanism that warrants further study. Interestingly, the combined use of classic senolytic drugs dasatinib and quercetin accelerates aging in female mice, but has no significant effect on male mice [18].

SASP can regulate the tumor microenvironment. To elucidate the effects of drugs on tumor cell senescence and to exclude the potential impact of immune cells on cell senescence, this study used highly immunodeficient mice. Senescent cancer cells have dual characteristics: they can activate dendritic cells (DCs) and antigen-specific CD8 T cells, thereby promoting anti-tumor immunity. Senescent cells can induce senescence in neighboring cells through SASP and direct cell-cell interactions, thereby limiting the proliferation of pre-malignant or fully malignant cells that have not yet senesced around them [19]. Conversely, SASP can promote tumor growth and metastasis in various types of cancers [20]. In an immune microenvironment, the role of SASP in tumor therapy is complex and further research is needed to determine whether the optimal synergy of PCC1 and PARP inhibitors is achieved through simultaneous administration or sequential administration.

In summary, this study found that PCC1 can synergize with olaparib to promote senescence in BRCA1-proficient breast cancer cells. In addition, PCC1 can expand the application of olaparib and synergistically inhibit the proliferation of breast cancer cells with normal BRCA1 function.

Conclusion

Procyanidin C1 can combined with olaparib to promote the cellular senescence of BRCA1 proficient breast cancer cells, thus playing a role in inhibiting proliferation. Combined regimen can not only act on BRCA1 mutant breast cancer cells, but also expand the use of PARP inhibitors to make them effective for BRCA1 proficient breast cancer.

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Disclosure of conflict of interest

None.

Abbreviations

PCC1, Procyanidin C1; PARP, poly ADP-ribose polymerase; SASP, senescence-associated secretory phenotype; CTRL, control; SCR, scrambled control sequence; FSC, Forward Scatter.

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Table S1. Primer sequences

Gene name	Forward	Reverse
GAPDH	CGGATTTGGTCGTATTGGG	CTGGAAGAT GGTGATGGGATT
Snai1	TCGGAAGCCTAACTACAGCGA	AGATGAGCATTGGCAGCGAG
Snai2	CGAACTGGACACACATACAGTG	CTGAGGATCTCTGGTTGTGGT
ZEB-1	TTACACCTTTGCATACAGAACCC	TTTACGATTACACCCAGACTGC
ZEB-2	GGAGACGAGTCCAGCTAGTGT	CCACTCCACCCTCCCTTATTTC
TIMP1	ACCACCTTATACCAGCGTTATGA	GGTGTAGACGAACCGGATGTC
TIMP2	TGCAGATGTAGTGATCAGGGC	TCTCAGGCCCTTTGAACATC
TIMP3	GCTGGAGGTCAACAAGTACCA	CACAGCCCCGTGTACATCT
TIMP4	TTGGTGCAGAGGGAAAGTCT	GGTACTGTGTAGCAGGTGGTGA
CHK1	ATATGAAGCGTGCCGTAGACT	TGCCTATGTCTGGCTCTATTCTG
CHK2	AAGCTAAATCATCCTTGCATC	AGCTTCTTTCAGGCGTTTATTC
ATM	CAGGGTAGTTTAGTTGAGGTTGACAG	CTATACTGGTGG TCAGTGCCAAAGT
ATR	GCCGCTCCGATCGTGTAC	TTTGTATGCTCTGTGATAACCTTGTTT