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DRG2 levels in prostate cancer cell lines predict response to PARP inhibitor during docetaxel treatment

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Purpose: Developmentally regulated GTP-binding protein 2 (DRG2) regulates microtubule dynamics and G2/M arrest during docetaxel treatment. Poly ADP-ribose polymerase (PARP) acts as an important repair system for DNA damage caused by docetaxel treatment. This study investigated whether DRG2 expression affects response to PARP inhibitors (olaparib) using prostate cancer cell lines PC3, DU145, LNCaP-FGC, and LNCaP-LN3.

Materials and Methods: The cell viability and DRG2 expression levels were assessed using colorimetric-based cell viability assay and western blot. Cells were transfected with DRG2 siRNA, and pcDNA6/V5-DRG2 was used to overexpress DRG2. Flow cytometry was applied for cell cycle assay and apoptosis analysis using the Annexing V cell death assay.

Results: The expression of DRG2 was highest in LNCaP-LN3 and lowest in DU145 cells. Expressions of p53 in PC3, DU145, and the two LNCaP cell lines were null-type, high-expression, and medium-expression, respectively. In PC3 (DRG2 high, p53 null) cells, docetaxel increased G2/M arrest without apoptosis; however, subsequent treatment with olaparib promoted apoptosis. In DU145 and LNCaP-FGC (DRG2 low), docetaxel increased sub-G1 but not G2/M arrest and induced apoptosis, whereas olaparib had no additional effect. In LNCaP-LN3 (DRG2 high, p53 wild-type), docetaxel increased sub-G1 and G2/M arrest, furthermore olaparib enhanced cell death. Docetaxel and olaparib combination treatment had a slight effect on DRG2 knockdown PC3, but increased apoptosis in DRG2-overexpressed DU145 cells.

Conclusions: DRG2 and p53 expressions play an important role in prostate cancer cell lines treated with docetaxel, and DRG2 levels can predict the response to PARP inhibitors.

Keywords: Docetaxel; PARP inhibitor; Prostate cancer

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INTRODUCTION

Prostate cancer has the highest incidence rate among cancer malignancies, accounting for 29% of cancers in men in the United States in 2023 [1]. The incidence of prostate cancer has also increased in Korea due to westernized eat-

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ing habits and an increase in average life expectancy [2]. There are two types of prostate cancer: hormone sensitive prostate cancer (HSPC) and castration-resistant prostate cancer (CRPC), which is hormone-refractory. HSPC can be treated by suppressing associated hormones, whereas CRPC is treated with chemotherapy such as docetaxel [3]. Docetaxel was approved by the U.S. Food and Drug Administration as the first-line treatment for CRPC in 2004 [4]. However, the average survival period of patients treated with docetaxel is 59.1 months, and the 5-year survival rate is as low as 49% [5]. Hence, elucidating the underlying mechanisms to surmount resistance to docetaxel and enhance its efficacy in the treatment of prostate cancer is imperative.

Developmentally regulated GTP-binding proteins (DRGs), a subfamily of the GTPase superfamily [6], consists of two closely related proteins, DRG1 and DRG2 [7]. DRG2 affects cell cycle proliferation and apoptosis in cancer cells [8-10]. DRG2 interacts with Rab5 on endosomes and is required for Rab5 inactivation on endosomes and for recycling of transferrin (Tfn) to the plasma membrane [11]. Recently, it was shown that DRG2 knockdown induces Golgi fragmentation [12] and mitochondrial dysfunction [13], decreases the stability of Rac1-positive membrane tubules in cancer cells [14], and suppresses VEGF-A production in melanoma cells, leading to inhibition of tumor angiogenesis [15]. In addition, DRG2 deficiency enhances DNA damage and senescence induced by oxidative stress [16]. Together, these data demonstrate that DRG2 is an important regulator of signal pathways for cell growth, differentiation, and/or vesicle trafficking.

Recently, new drug classes have been approved for CRPC treatment, one of which is poly ADP-ribose polymerase (PARP) inhibitors [4]. However, many patients who received PARP inhibitor treatment have acquired resistance [17]. In addition, the use of PARP inhibitors is limited by the fact that they are effective against breast cancer susceptibility gene (BRCA) mutations, which exist in only about 20% of prostate cancer patients. These PARP inhibitors are effective in cells, which have undergone G2/M phase arrest [4]. Moreover, we previously confirmed that cells undergo G2/M arrest due to the expression of the DRG2, caused by docetaxel treatment [18]. As G2/M arrest was observed after docetaxel treatment in cells expressing DRG2, we hypothesized that treatment with a PARP inhibitor would be effective.

This study aimed to determine the effect of DRG2 expression on the effectiveness of PARP inhibitors. We hypothesized that G2/M arrest caused by DRG2 expression results in cell death via PARP inhibitors.

MATERIALS AND METHODS

1. Drugs

Docetaxel (Sanofi-Aventis U.S. LLC), olaparib (Selleck Chemicals), and etoposide (Boryung Pharmaceutical Co., Ltd.) were used at the indicated concentrations.

2. Cell culture

The prostate cancer cell lines PC3, DU145, LNCaP-FGC, and LNCaP-LN3 were obtained from the Korean Cell Line Bank, and their status of DRG2, p53, and BRCA1 are listed in Table 1. All cell lines were cultured in RPMI-1640 medium (Welgene) supplemented with 10% fetal bovine serum (Invitrogen) and 1% penicillin/streptomycin (Invitrogen), at 37°C in a humidified chamber containing 5% CO_2.

3. Cell viability assay

Cell viability was analyzed at the indicated times using a D-Plus™ CCK cell viability assay kit (Dongin Biotech) according to the manufacturer's instructions. Absorbance was measured at 450 nm (OD450) for each well using a Wallac Vector 1420 Multilabel Counter (EG&G Wallac).

4. Western blot analysis and siRNA transfection

Total protein was extracted using radioimmunoprecipitation assay (RIPA) buffer containing protease and phosphatase inhibitors (Thermo Fisher Scientific), and the protein concentration was determined using a Bradford protein assay kit (Bio-Rad Laboratories). Proteins were separated

Table 1. Cell lines and status of DRG2, p53, and BRCA1/2

Cell line	Tissue type	KCLB/ATCC number	Characteristic
PC3	Prostate cancer	80020/CRL-1435	DRG2 high, p53 null, BRCA1 WT
LNCaP-LN3	Prostate cancer	80018/000	DRG2 high, p53 medium, BRCA1 WT
LNCaP-FGC	Prostate cancer	21740/CRL-1740	DRG2 low, p53 medium, BRCA1 WT
DU145	Prostate cancer	30081/HTB-81	DRG2 low, p53 high, BRCA1 MT

Data resources for status of p53 and BRCA1: https://portals.broadinstitute.org/ccle
KCLB, Korean Cell Line Bank; ATCC, American Type Culture Collection; DRG2, developmentally regulated GTP-binding protein 2; WT, wild-type; MT, mutant.

via electrophoresis on an 8%–12% SDS-polyacrylamide gel and transferred onto a nitrocellulose membrane (Amersham International). Membranes were blocked with 5% bovine serum albumin (BSA; bioWORLD) in Tris-buffered saline with Tween® 20 (TBST) for 1 hour at room temperature. Membranes were subsequently washed with TBST and incubated overnight at 4℃ with the following primary antibodies: DRG2 (14743-1-AP; Proteintech), caspase-3 (#9662; Cell Signaling Technology [CST]), p53 (#2524; CST), cdc2 (#9116; CST), PARP (#9542; CST), Rad 51 (sc-377467; Santa Cruz Biotechnology), and β-actin (sc-47778; Sigma-Aldrich Inc.) diluted in 5% BSA/TBST. The membranes were again washed with TBST and then incubated for 1 hour in a TBST containing the 2,000-fold diluted secondary antibody anti-mouse or anti-rabbit IgG HRP conjugate (Bethyl Laboratories). After washing with TBST, the specific binding of antibodies was detected using an excellent chemiluminescent substrate (ECL) kit (Thermo Fisher Scientific) following the manufacturer's instructions. Small interfering RNAs (siRNAs) targeting human DRG2 (siDRG2; sc-93839), human p53 (sip53; sc-29435), and control siRNA (scRNA; sc-37007) were purchased from Santa Cruz Biotechnology. AMC-HN3 cells (1.5 or 3×10^5) were transfected with each siRNA using Lipofectamine™ RNAiMAX (Invitrogen).

5. Flow cytometry

Cells were harvested via trypsinization, washed in ice-cold phosphate-buffered saline (PBS), fixed in ice-cold 70% ethanol in PBS, centrifuged at 4℃, and resuspended in chilled PBS. Bovine pancreatic RNAase (Sigma-Aldrich Inc.) was added to the fixed cells at a final concentration of 2 μg/mL and the cells were incubated at 37℃ for 30 minutes. Then, 20 μg/mL of propidium iodide (PI; Sigma-Aldrich Inc.) was added to the cells in each group and incubated for 20 minutes at room temperature. The cells were analyzed by flow cytometry (FACSCalibur; BD Biosciences).

6. Immunofluorescence microscopy

The cells were plated on 18-mm coverslips and fixed with 3.7% paraformaldehyde (PFA), permeabilized in 0.1% Triton X-100 in PBS, and blocked in PBS/5% BSA. DRG2 was detected using a DRG2 polyclonal antibody (14743-1-AP; Proteintech) incubated overnight at 4℃, followed by incubation with fluorescent-conjugated secondary antibodies (Molecular Probes). After washing, cells were mounted on glass slides and examined under a DP40 microscope (Olympus).

7. Determination of apoptosis by Annexin V/PI analysis

Human PC3 cells were seeded on a 60-mm dish and incubated with docetaxel (10 nM) and olaparib (10 μM) for 72 hours, washed twice with ice-cold PBS (pH 7.0), and then resuspended in binding buffer (500 μL). Subsequently, fluorescein isothiocyanate (FITC)-Annexin V (5 μL) was added to PI (5 μL) and incubated for 15 minutes at room temperature in the dark. Samples were analyzed using a fluorescence-activated flow cytometer (NovoCyte Quanteon; Agilent).

8. Statistical analysis

All statistical analyses and calculations were performed using Microsoft Excel spreadsheets (Microsoft) and GraphPad Prism version 10 (GraphPad Software). Group differences were determined using the Student's t-test or Mann–Whitney U test. Data are expressed as the mean±standard deviation. All statistical tests were two-sided, and $p<0.05$ was considered statistically significant.

RESULTS

1. Characteristics of prostate cancer cell lines

To determine the effect of p53 and DRG2 expression on the sensitivity to docetaxel and PARP inhibitor, we used four prostate cancer cell lines expressing different p53 and DRG2 level (Fig. 1A, B, Supplementary Fig. 1, Table 1). While DU145 is a BRCA1 mutant (MT) cell line, the others are BRCA1 wild-type (Table 1). All studied prostate cancer cell lines were treated with docetaxel (0–10 μM) and cell viability was determined after 72 hours. The IC50 values were 13.910, 9.042, 7.973, and 5.968 nM in PC3, DU145, LNCaP-FGC, and LNCaP-LN3 cells, respectively (Fig. 1C). Based on these results, the docetaxel treatment concentration was set at 10 nM for subsequent experiments. DU145 cells showed a continuous decrease in cell viability up to 72 hours, whereas PC3 cells showed recovery after 24 hours treatment (Fig. 1D).

2. Cell cycle arrest after docetaxel treatment

The prostate cancer cell lines were treated with 2, 5, or 10 nM docetaxel for 72 hours and the concentration dependent changes were observed using a microscope. Docetaxel treatment led to a dose-dependent reduction in the cell numbers of DU145, LNCaP-FGC, and LNCaP-LN3 cells (Fig. 2A). In PC3 cells, cell cycle analysis revealed a dose dependent manner increase in G2/M arrest, accompanied by a minor elevation in the sub-G1. Whereas, LNCaP-LN3 cells showed dramatic increase in the sub-G1 and G2/M. In DU145 and LNCaP-FGC cells, a notable dose-dependent increase in the

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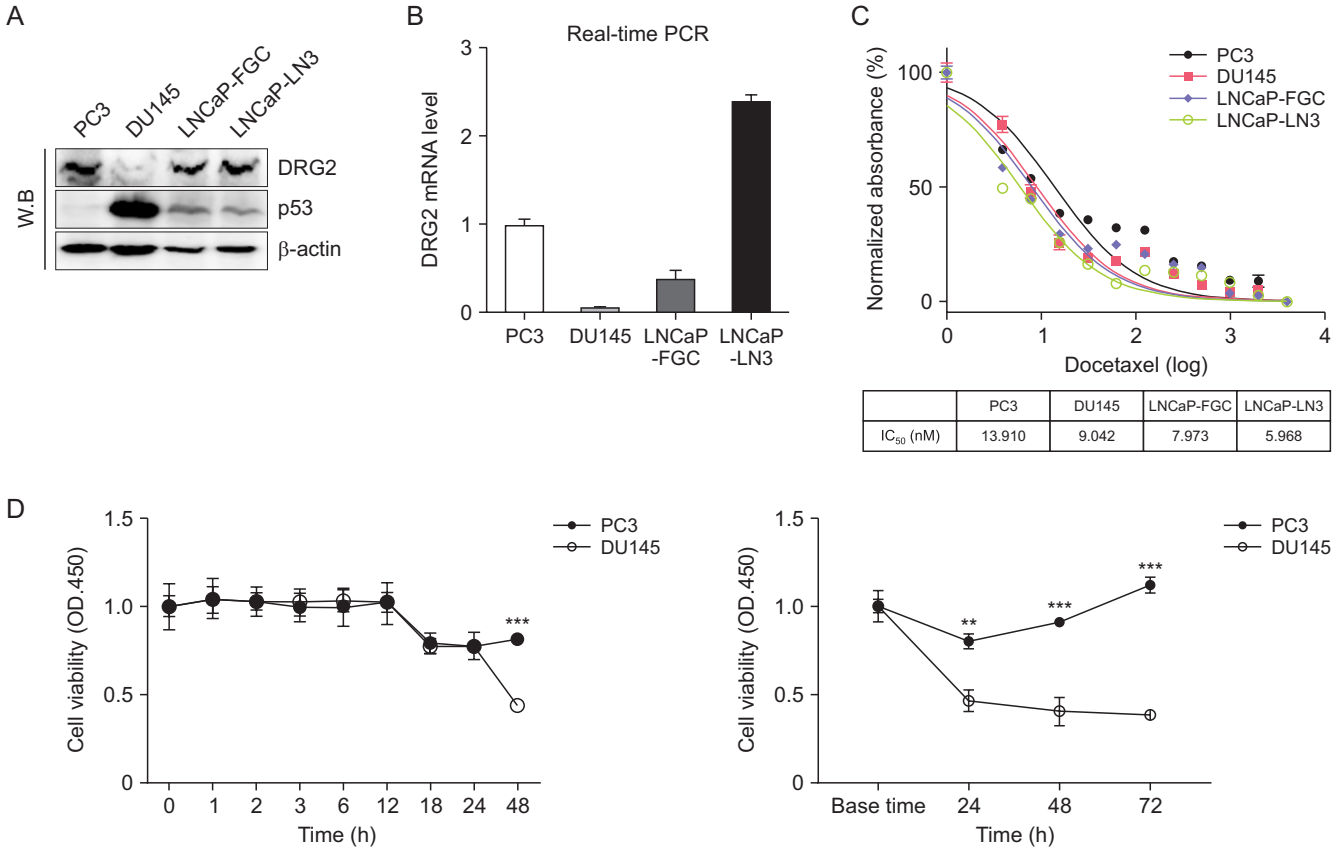

	PC3	DU145	LNCaP-FGC	LNCaP-LN3
IC_{50} (nM)	13.910	9.042	7.973	5.968

Fig. 1. Recovery of prostate cancer cell lines after docetaxel treatment. (A) DRG2 and p53 expressions in prostate cancer cell lines as determined via western blot (W.B) analysis (full length blots; Supplementary Fig. 1). (B) DRG2 expression in prostate cancer cell lines as determined using real-time polymerase chain reaction (PCR). (C) IC50 values of docetaxel on prostate cancer cells were determined using a CCK assay. Cells were exposed to 0–10 μM docetaxel for 72 hours. (D) Cell viability of the prostate cancer cell lines after docetaxel treatment for 72 hours, determined using a CCK assay (during docetaxel treatment [left], after docetaxel treatment [right]). DRG2, developmentally regulated GTP-binding protein 2; OD, optical density. **p<0.01; ***p<0.001.

sub-G1 phase was observed with a minor decrease in the G2/M phase (Fig. 2B).

3. Changes in cell viability based on differences in DRG2 and p53 expressions after docetaxel administration

PC3 cells were treated with 15 μM of APR-246, a p53 activator, followed by docetaxel (10 nM) for 72 hours, and cell viability was assessed. The alteration in cell viability following docetaxel treatment was greater in the presence of APR-246 treatment compared to the absence of treatment (Fig. 3A). The impact of p53 on docetaxel efficacy was assessed through p53 knockdown in DU145 cells. As depicted in Fig. 3B, cell viability significantly decreased upon docetaxel administration for 72 hours; however, this effect was overcome following p53 knockdown using siRNA (sip53) (Fig. 3B). After treatment with 10 nM docetaxel for 72 hours, changes in DRG2 in the cytoplasm and nucleus were confirmed through western blotting. The increased expression level of DRG2 after docetaxel administration was observed only in a PC3 nucleus fraction (Fig. 3C, Supplementary Fig. 2). Following treatment of PC3 cells with docetaxel (10 nM), alterations in the subcellular localization of DRG2 were assessed using immunofluorescence microscopy. In comparison to the control, DRG2, initially distributed throughout the cell, translocated to the nucleus in PC3 cells treated with docetaxel (10 nM) for 72 hours (Fig. 3D). After inducing DRG2 overexpression in DU145 cells, cell viability was assessed following treatment with docetaxel (10 nM) for 72 hours. In DU145-pcDNA6-V5, there was a significant reduction in cell viability, while in DU145-DRG2/pcDNA6-V5, cell viability decrease was overcome compared to DU145-pcDNA6-V5 (Fig. 3E). As illustrated in Fig. 3F, the sub-G1 population increased with a slight elevation in G2/M following the administration of 5 nM docetaxel for 72 hours. However, with DRG2 overexpression, the increment in the sub-G1 was mitigated, and the G2/M exhibited a greater increase than without DRG2 overexpression (Fig. 3F). To assess the impact of DRG2 on PC3 cell

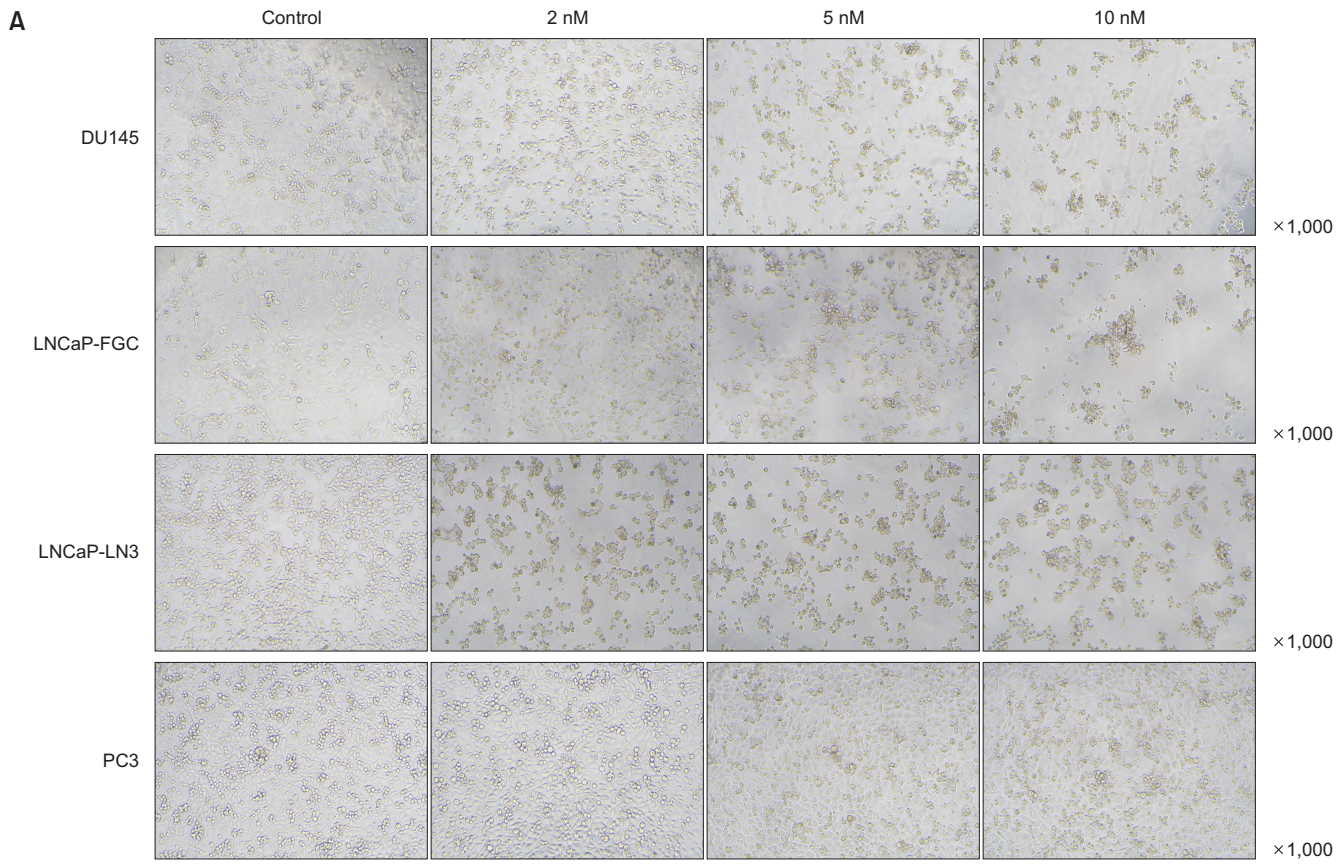

Fig. 2. Changes in the cell cycle of prostate cancer cell lines after docetaxel treatment. (A) Prostate cancer cell lines were monitored using a microscope after docetaxel treatment for 72 hours. (B) Flow cytometry analysis of the docetaxel-treated prostate cancer cells after 72 hours.

(p53 null) viability following docetaxel administration for 72 hours, cell viability was compared under siDRG2 treatment. The result indicated that the presence of DRG2 did not exert a significant effect on cell viability by docetaxel, especially at p53 null condition (Fig. 3G). Under identical DRG2 knockdown condition in PC3 cells, cell cycle analysis revealed a reduction in G2/M arrest and an increase in the sub-G1 phase (Fig. 3H).

4. Relationship between PARP inhibitor and DRG2

The expression level differences of representative homologous recombination (HR) markers, such as PARP and Rad 51, were assessed in PC3 and DU145 cells. PC3 cells exhibited higher expression levels of both proteins compared to DU145 cells (Fig. 4A, Supplementary Fig. 3). When the PARP inhibitor olaparib (10 μM) was applied to PC3 cells, the persistent cell proliferation indicating drug resistance was mitigated (Fig. 4B). The combination treatment effect of docetaxel (5 nM) and olaparib (10 μM) was evaluated through cell viability assay. The results indicated a significant enhancement of drug efficacy with the combination treatment in PC3 cells, while DU145 cells exhibited no significant changes (Fig. 4C). The effects of the combination treatment of docetaxel (10 nM) and olaparib (10 μM) on the cell cycle were evaluated, showing a decrease in the G2/M phase and an increase in the sub-G1 phase in PC3 cells. In contrast, a minor change was observed in G2/M and sub-G1 phase of DU145 cells (Fig. 4D). In PC3 cells, the addition of olaparib significantly increased a PI(+) cell death following docetaxel administration, whereas such an effect was not significant in DU145 cells (Fig. 4E). The influence of DRG2 presence on the combination treatment was examined through siRNA treatment. As depicted in Fig. 4F, the impact of DRG2 knockdown on the combination treatment was not significant, consistent with its effect on docetaxel treatment alone. While DRG2 overexpression led to increased drug resistance, its impact on the combination treatment was not significant in DU145 cells (Fig. 4G).

DISCUSSION

Docetaxel-induced cell death in prostate cancer cells due to docetaxel may occur with or without the formation of giant cells. In the present study, cell death in PC3 cells oc-

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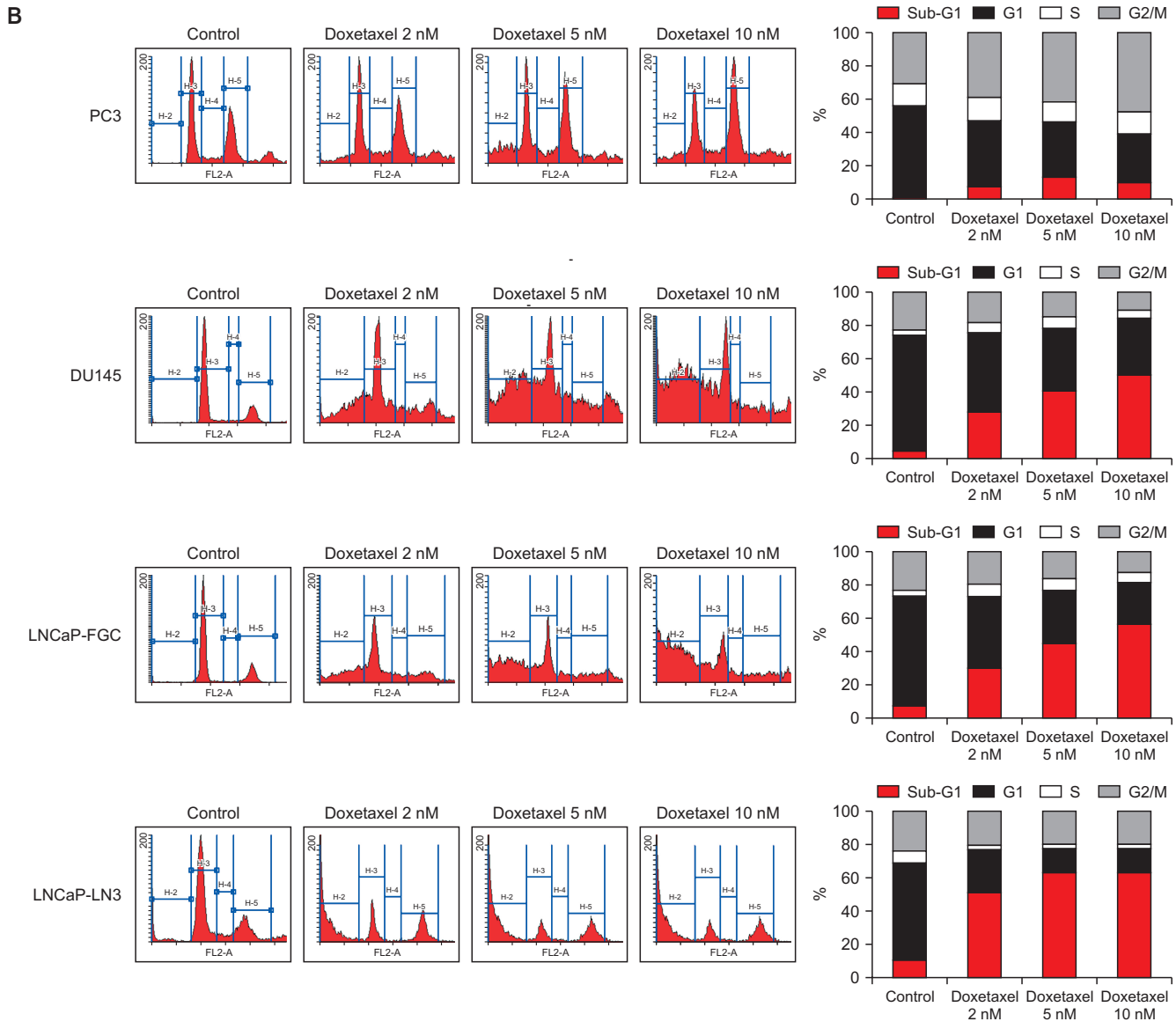

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curred with the formation of giant cells. However, cell death in DU145 cells occurred with almost no giant cell formation (G2/M arrest). Notably, cell death with and without giant cell formation has been termed mitotic catastrophe and apoptosis, respectively [19]. The formation of giant cells is caused by the formation of large cells with multiple micronuclei that appear through mitotic failure [20]. Although many researchers often confuse these two cases during cell death occurs, these two differ fundamentally and occur in different phases of the cell cycle. Lock and Stribinskiene [21] overexpressed Bcl-2 to prevent apoptosis while treating HeLa cells with etoposide and demonstrated increased catastrophic mitosis. Notably, apoptosis is reported to occur at the G1 checkpoint, whereas mitotic catastrophe occurs mainly at the G2 checkpoint [22].

p53 is the mediator of apoptosis at the G1 checkpoint [23]. Further, p53 controls both the G2/M and the G1 cell cycle checkpoints and mediates reversible growth arrest in human fibroblasts [24]. Therefore, when a cell detects DNA damage, apoptosis occurs at the G1 checkpoint in cells expressing p53, but cells lacking p53 do not undergo G1 arrest and pass over. In this study, we observed increased apoptosis in both PC3 and DU145 cells after docetaxel treatment, irrespective of p53 presence. Interestingly, the combination of olaparib and docetaxel significantly increased cell death in PC3 cells (p53 null) but not in DU145 cells (p53 high).

Cells that experienced DNA stress but escaped the G1 checkpoint undergo G2/M arrest [25]. In addition, arrest at the G2/M phase increases in cells with higher expression of DRG2 [18]. Notably, DRG2 translocates to the nucleus in case

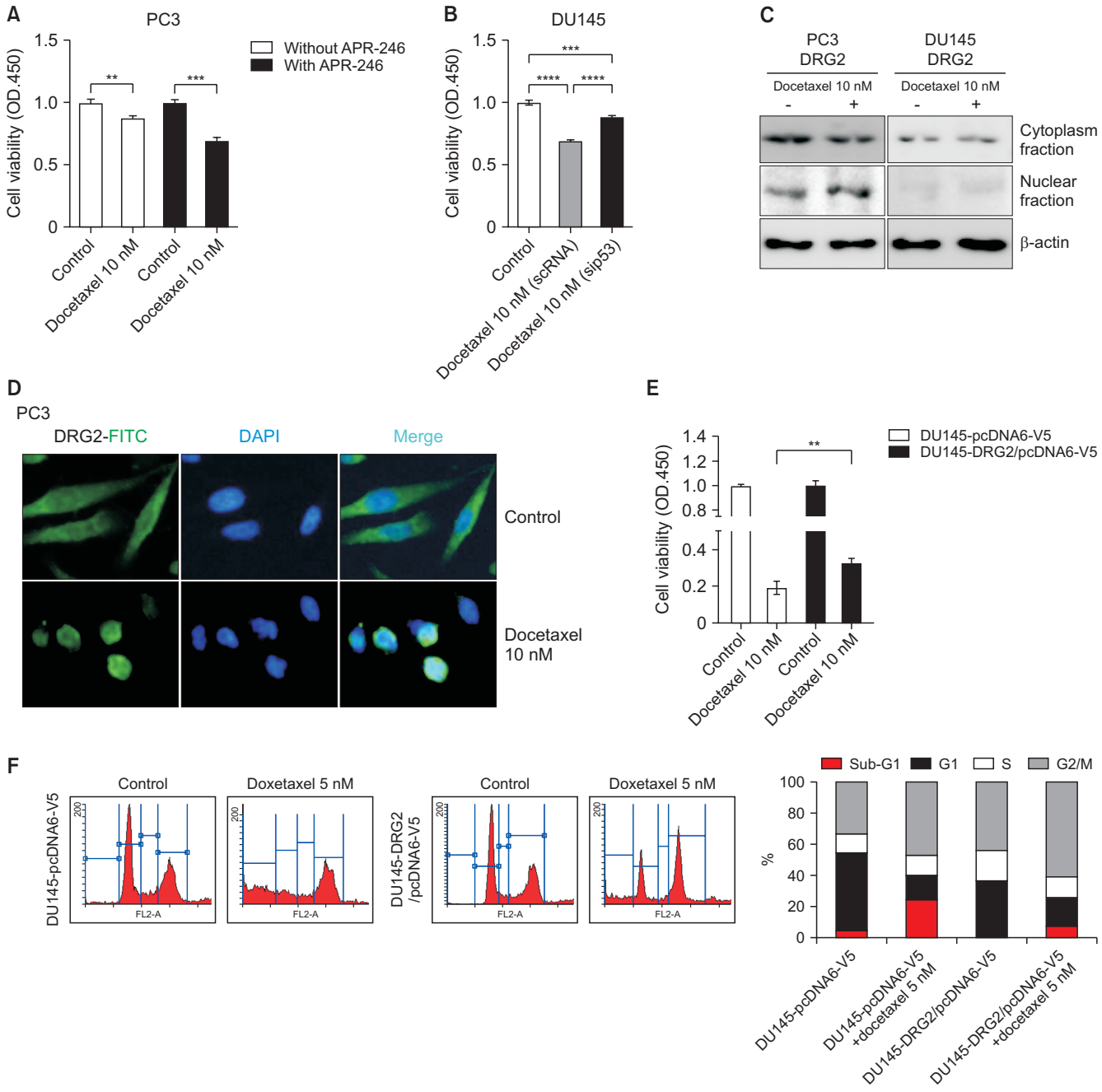

Fig. 3. Differences in cell cycles depending on the presence or absence of DRG2 and p53 expression. (A) Cell viability assay in PC3 cells treated with APR-246 and docetaxel for 72 hours. (B) Cell viability assay in DU145 cells transfected with scRNA or sip53 after docetaxel treatment for 72 hours. (C) Changes in DRG2 expression in the cytoplasm and nuclear extracts after docetaxel treatment for 72 hours analyzed via western blot analysis (full length blots; Supplementary Fig. 2). (D) Immunofluorescence microscopy images of PC3 cells after docetaxel treatment for 72 hours. (E) Cell viability assay in DU145-pcDNA6-V5 and DU145-DRG2/pcDNA6-V5 cells after docetaxel treatment for 72 hours. (F) Flow cytometry analysis showing the difference in cell cycles of DU145-pcDNA6-V5 and DU145-DRG2/pcDNA6-V5 cells after treatment with docetaxel for 72 hours. (G) Cell viability assay in PC3 cells transfected with scRNA or siDRG2 after docetaxel treatment for 72 hours. (H) Flow cytometry analysis showing the difference in cell cycles of PC3 transfected with scRNA or siDRG2 after treatment with docetaxel for 72 hours. Cell viability was determined using the CCK assay. DRG2, developmentally regulated GTP-binding protein 2; OD, optical density; FITC, fluorescein isothiocyanate; DAPI, 4′,6-diamidino-2-phenylindole; ns, no significant. **p<0.01; ***p<0.001; ****p<0.0001.

of DNA damage. Therefore, DRG2 may mediate cell arrest under DNA stress conditions. In our experiments, G2/M arrest increased when docetaxel was administered to PC3 and LNCaP-LN3 cells with high DRG2 expression levels. The decrease in G2/M arrest induced by docetaxel administration in DU145 cells (p53 high) was significantly increased through DRG2 overexpression, emphasizing the pivotal regulatory role of DRG2 in G2/M arrest.

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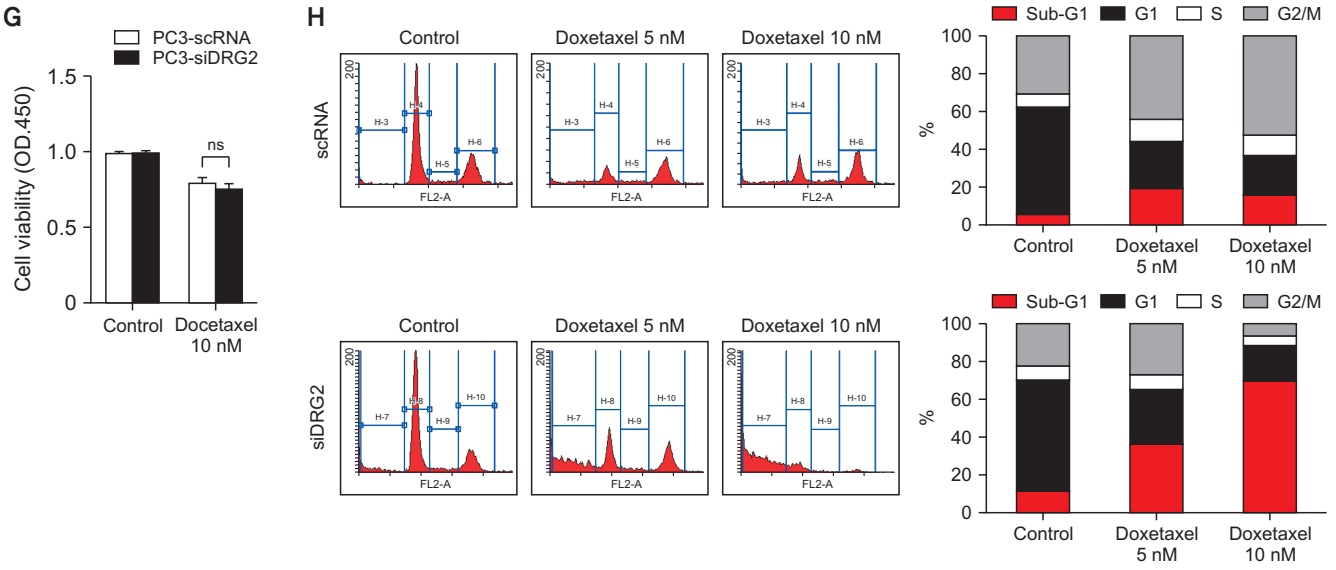

Fig. 3. Continued.

During DNA repair, non-homologous end joint (NHEJ) operates sequentially in the G1, S, and G2/M phases. In contrast, HR has a modest impact on the G1 phase, is most active in the S phase, and diminishes in the G2/M phase. Notably, NHEJ plays a more pivotal role in DNA repair during G2/M than HR [26]. Consequently, NHEJ is expected to be more active if G2/M arrest is prolonged.

PARP, recognized for its involvement in NHEJ activation [27,28], exhibited elevated expression in PC3 cells and lower expression in DU145 cells, as assessed by western blotting. Intriguingly, NHEJ is inhibited in PC3 cells, whereas it remains active in DU145 cells. NHEJ activation in PC3 cells occurs during G2/M arrest. Consequently, PARP assumes a central role, with PARP inhibitors contributing to its suppression [29].

Given the acknowledged significance of DRG2 in G2/M arrest [18], it was anticipated that cells with high DRG2 expression would exhibit an increase in G2/M arrest when subjected to DNA damage. Intriguingly, PC3 cells (DRG2 high) demonstrated augmented G2/M arrest after docetaxel treatment, while DU145 cells (DRG2 low) did not show G2/M arrest. As expected, opposing results were observed with decreased G2/M arrest due to p53 knockdown in PC3 cells and enhanced G2/M arrest following DRG2 overexpression in DU145 cells. Cells with high expression of DRG2 displayed increased G2/M arrest by docetaxel administration, suggesting potential responsiveness to PARP inhibitors. In this study, we found that in cells with high levels of DRG2 (PC3 cells), combination treatment with PARP inhibitor alleviated docetaxel resistance with a decrease in G2/M, thereby indicating an enhancement in the docetaxel effectiveness. BRCA1 mutation is very important for the treatment response when PARP inhibitor monotherapy is used. However, both HR and NHEJ play an important role in DNA repair when DNA stress temporarily increases, such as chemotheraphy or radiation therapy. Therefore, PARP inhibitor can be an effective treatment regardless of BRCA1 mutation in these situations.

Interestingly, we observed that the combination treatment effect was not significant in DU145 cells. One notable difference from PC3 cells was the presence of p53: when p53 was removed from DU145 cells, the effect of docetaxel was reduced. In the presence of p53, DRG2 overexpression drove the reduced effect of docetaxel, while the combination effect with PARP inhibitors was enhanced. Taken together, our findings suggest that docetaxel resistance and overcoming resistance by PARP inhibitor combination therapy is closely related to p53 and DRG2 expression levels, particularly p53 deficiency and DRG2 high expression conditions.

This study has several limitations. The identification of BRCA status, essential for assessing the effectiveness of PARP inhibitors, was not conducted. Previous studies have reported the effect of PARP inhibitors against BRCA 1/2 mutations [30]. Nevertheless, in this study, we consistently observed identical results upon reversing DRG2 in cells, irrespective of BRCA status. Hence, the influence of PARP inhibitors seems to be attributed to DRG2 expression, independent of BRCA expression. As docetaxel is an antimicrotubule agent and DRG2 is involved in microtubule formation, the impact of docetaxel on DRG2 may be attributed to its microtubule activity. However, similar results were obtained with etoposide treatment, which is not an antimicrotubular agent,

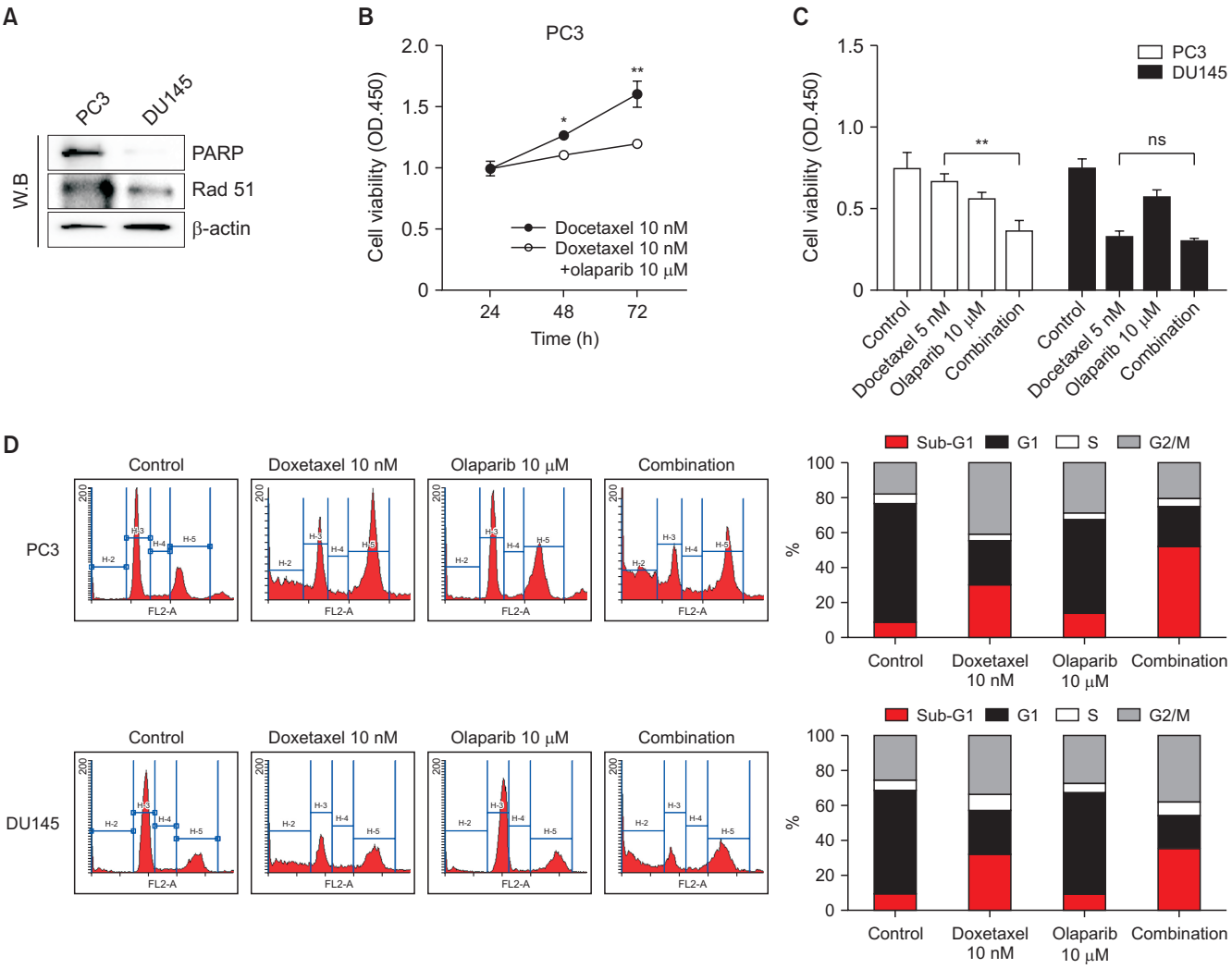

Fig. 4. Changes in cells caused by administration of a PARP inhibitor (olaparib). (A) PARP and Rad 51 expressions in PC3 and DU145 cells as determined via western blot (W.B) analysis (full length blots; Supplementary Fig. 3). (B) Viability of PC3 cells after treatment with docetaxel and olaparib for 72 hours. (C) Viability of PC3 and DU145 cells after treatment with docetaxel and olaparib for 72 hours. (D) Flow cytometry analysis showing differences in the cell cycle of PC3 and DU145 cells after treatment with docetaxel and olaparib for 72 hours. (E) Flow cytometric analysis of PC3 and DU145 cells after treatment with docetaxel and olaparib for 72 hours. Cells were stained with Annexin V fluorescein and propidium iodide. (F) Viability of PC3 cells transfected with scRNA or siDRG2 after treatment with docetaxel and olaparib for 72 hours. (G) Viability of DU145-pcDNA6-V5 and DU145-DRG2/pcDNA6-V5 cells treated with docetaxel and olaparib for 72 hours. Cell viability was determined using the CCK assay. PARP, poly ADP-ribose polymerase; OD, optical density; ns, no significant; FITC, fluorescein isothiocyanate. *p<0.05; **p<0.01; ***p<0.001.

indicating that microtubule activity is not involved (Supplementary Fig. 4). Therefore, understanding the relationship between DRG2 and PARP inhibitors is crucial.

CONCLUSIONS

DRG2 and p53 expressions play an important role in eliciting the response to docetaxel treatment. In prostate cancer cell lines treated with docetaxel, p53 expression affects apoptosis at the G1 checkpoint, whereas DRG2 expression affects G2/M arrest at the G2 checkpoint. G2/M arrest occurring in DRG2-expressing prostate cancer cell lines in turn affects the response to PARP inhibitors. Therefore, DRG2 expression levels in prostate cancer cell lines can predict the response to PARP inhibitors.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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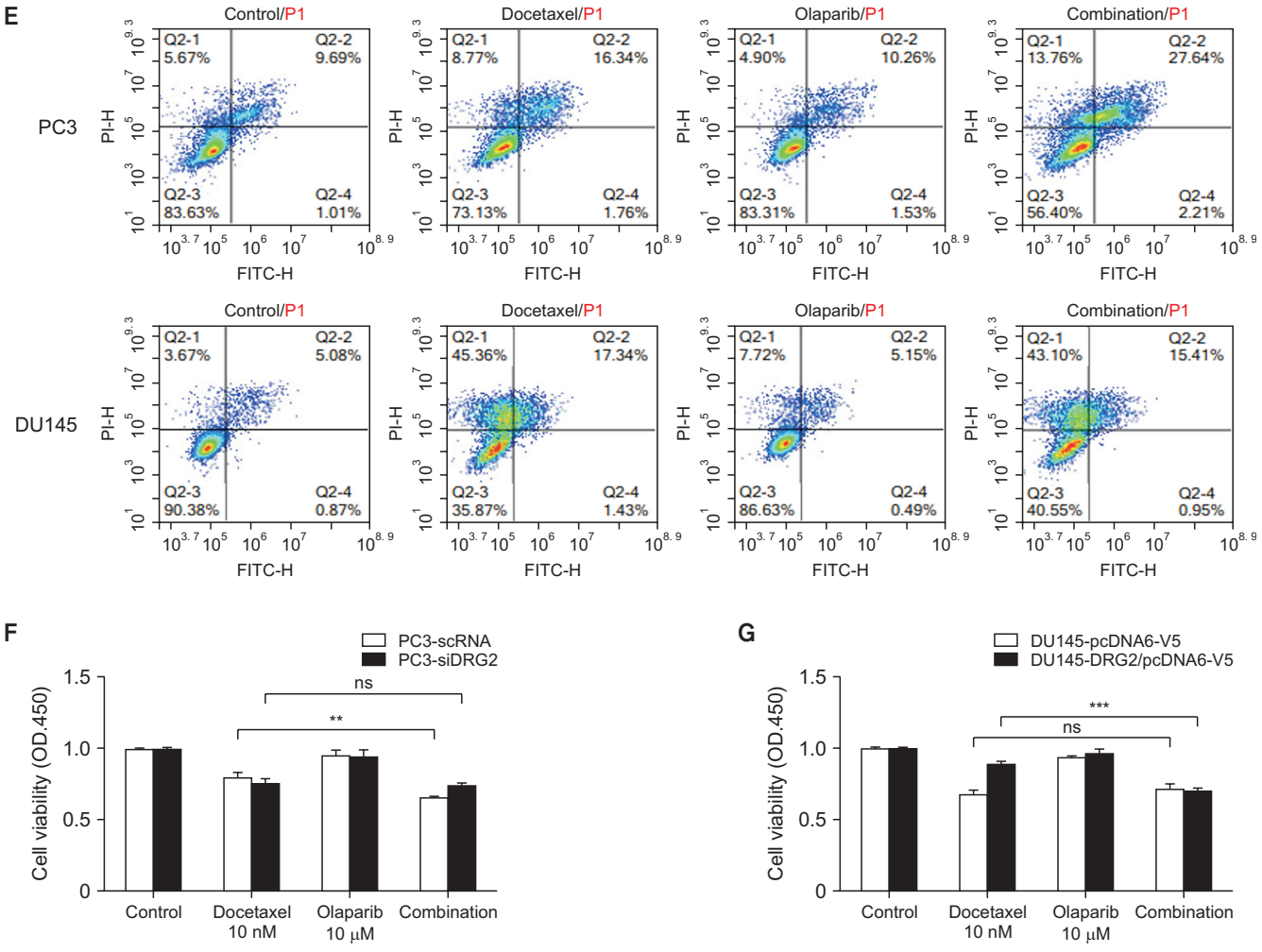

Fig. 4. Continued.

AUTHORS' CONTRIBUTIONS

Research conception and design: Sungchan Park, Won Hyeok Lee, and Seong Cheol Kim. Data acquisition: Jeong Min Lee and Won Hyeok Lee. Statistical analysis: Jeong Min Lee and Won Hyeok Lee. Data analysis and interpretation: Sungchan Park, Won Hyeok Lee, Jeong Min Lee, Sang Hun Lee, and Seong Cheol Kim. Drafting of the manuscript: Jeong Min Lee. Critical revision of the manuscript: Jeong Min Lee, Hyuk Nam Kwon, Seung Hyeon Cho, and Seong Cheol Kim. Obtaining funding: Seong Cheol Kim. Administrative, technical, or material support: Hyuk Nam Kwon and Jeong Woo Park. Supervision: Jeong Woo Park, Sungchan Park, and Seong Cheol Kim. Approval of the final manuscript: all authors.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.4111/icu.20240263.

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