



# Developmentally regulated GTP-binding protein 2 levels in prostate cancer cell lines impact docetaxel-induced apoptosis

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**Purpose:** This study aimed to confirm the association between developmentally regulated GTP-binding protein 2 (DRG2) expression and docetaxel-induced apoptosis and to determine whether prostate cancer responses to docetaxel treatment differ with DRG2 expression.

**Materials and Methods:** PC3, DU145, and LNCaP prostate cancer cell lines were used. The MTT assay was used to determine cell viability. Western blotting analysis was performed using anti-DRG2 antibodies. Cells were transfected with 50 nmol DRG2 siRNA using an siRNA transfection reagent for DRG2 knockdown. The cell cycle was analyzed by using flow cytometry, and apoptosis was detected by using the Annexin V cell death assay.

**Results:** DRG2 expression differed in each prostate cancer cell line. Docetaxel reduced DRG2 expression in a dose-dependent manner. Upon DRG2 knockdown in prostate cancer cells, an increase in the sub-G1 phase was observed without a change in the G1 or G2/M phases. When 4 nM docetaxel was administered to DRG2 knockdown prostate cancer cell lines, an increase in the sub-G1 phase was observed without increasing the G2/M phase, which was similar to that in DU145 cells before DRG2 knockdown. In PC3 and DU145 cell lines, DRG2 knockdown increased docetaxel-induced Annexin V (+) apoptosis by 8.7 and 2.7 times, respectively.

**Conclusions:** In prostate cancer cells, DRG2 regulates G2/M arrest after docetaxel treatment. In prostate cancer cells with DRG2 knockdown, apoptosis increases without G2/M arrest in response to docetaxel treatment. These results show that inhibition of DRG2 expression can be useful to enhance docetaxel-induced apoptosis despite low-dose administration in castration-resistant prostate cancer.

**Keywords:** Apoptosis; DRG2 protein; Prostate cancer

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## INTRODUCTION

Prostate cancer treatments have been studied and developed for decades, and use of docetaxel for metastatic castration-resistant prostate cancer (CRPC) has been shown to provide survival benefits [1,2]. Unlike hormone-sensitive prostate cancer, CRPC has a median survival of less than 2 years owing to the adverse effects of therapeutic agents and resistance to them [3]. CRPC has also been reported to demonstrate neuroendocrine or aggressive-variant phenotypes that are resistant to taxane; therefore, new management of taxane-resistant prostate cancer is needed [4-6].

A study in breast cancer cell lines showed that docetaxel induces an alternative form of cell death known as mitotic catastrophe, or cell death occurring during metaphase [7,8]. This event is initially characterized by chromosome mis-segregation followed by aberrant mitosis or imperfect cell division [9]. Nuclear envelopes subsequently arise around single chromosomes or chromosome groups, resulting in large cells with multiple micronuclei that are morphologically distinguishable from apoptotic cells [10]. Both the mitotic catastrophe and apoptosis play an important role in cell death by docetaxel treatment in prostate cancer cells [11]. However, the mitotic catastrophe and stress-induced premature senescence, such as irreversible growth arrest, are also associated with escape from genotoxic insults because these give stemness to cancer cells at the DNA damage checkpoint [12]. Given these points, the mitotic catastrophe observed after docetaxel treatment is a double-edged sword that can cause both cell death and resistance.

Developmentally regulated GTP-binding proteins (DRGs) are a novel evolutionally conserved family of GTP-binding proteins. These proteins harbor five characteristic motifs, G1–G5, that are believed to interact with GTP [7]. Apart from these motifs, the DRGs do not display significant similarity with the well-characterized G-proteins; therefore, they constitute a new subfamily within the superfamily of GTP-binding proteins [13]. There are at least two distinct members, DRG1 and DRG2, that are widely expressed in human and mouse tissues; they show a similar distribution pattern, which suggests similar function [14]. Overexpression of DRG2 increases G2/M phase cells and decreases sensitivity to nocodazole-induced apoptosis in human T cells [15,16]. Thus, the evolutionary conservation of DRGs suggests that they play an essential role in controlling cell growth and differentiation. Many studies have been conducted on DRG2, and it is known to be involved in mitosis by affecting microtubule polymerization [17,18]. In particular, DRG2 is reported to be involved in G2/M progression, and thus seems

to play an important role in the cell response to genotoxic stress. The roles of DRG2 in T cell leukemia, cervical cancer, melanoma, lung cancer, and hepatocellular carcinoma have been reported, but there has been no such study on urological cancers [15-21]. Moreover, it is necessary to confirm the association between DRG2 and prostate cancer, which is treated with the chemotherapeutic agent docetaxel, an anti-microtubule agent.

Therefore, this study aimed to confirm the association between the level of DRG2 expression and docetaxel-induced apoptosis, and to determine whether the response of prostate cancer to docetaxel treatment differs depending on DRG2 expression. We hypothesized that DRG2 plays a key role in G2/M progression, which is an important step for repairing DNA damage after chemotherapy.

## MATERIALS AND METHODS

### 1. Reagent

Docetaxel (Taxotere) was obtained from Aventis Pharmaceuticals (Bridgewater, NJ, USA).

### 2. Antibodies

The antibodies used in this study were the anti-DRG2 antibody (14743-1-AP; Proteintech, Rosemont, IL, USA) and anti-GAPDH antibody (sc-47724; Santa Cruz Biotechnology, Dallas, TX, USA).

### 3. Cell culture

Prostate cancer cell lines PC3, DU145, and LNCaP were obtained from the Korean cell line bank. The PC3 and DU145 cell lines were cultured in RPMI-1640 (WELGENE, Gyeongsan, Korea), and the LNCaP cell line was cultured in Eagle's minimum essential medium (WELGENE), each supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA) and 1% penicillin/streptomycin (Invitrogen). Cells were cultured at 37°C in a humidified chamber containing 5% CO<sub>2</sub>.

### 4. Viability assay (MTT assay)

The cells were seeded into 96-well plates. At the indicated time points, the cells were incubated with 100 µL sterile MTT (0.5 mg/mL; Sigma-Aldrich, St. Louis, MO, USA) for 4 hours at 37°C, and then the medium was removed and replaced with 150 µL dimethyl sulfoxide (DMSO; Sigma-Aldrich). Absorbance was measured at 570 nm, with 655 nm as the reference wavelength. All experiments were performed in triplicate.

## 5. Western blot analysis

Total proteins were extracted using RIPA buffer containing proteases and phosphatase inhibitors (Thermo Fisher Scientific, Waltham, MA, USA), and protein concentration was determined by using the Bradford protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA). Proteins were separated by electrophoresis on a 10% to 13% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane (Amersham International, Little Chalfont, UK). The membranes were blocked with 5% bovine serum albumin (BSA: bioWORLD, Dublin, OH, USA) in Tris-buffered saline with Tween<sup>®</sup>20 (TBST) for 1 hour at room temperature (temperature range, 20°C–25°C). Membranes were subsequently washed with Tris-buffered saline with Tween<sup>®</sup>20 (TBST) and incubated overnight at 4°C with primary antibodies to DRG2 (147431-AP; Proteintech, Rosemont, IL, USA) and GAPDH (sc-47724; Proteintech) diluted in 5% BSA/TBST. Membranes were washed with TBST and then incubated for 1 hour with the secondary antibody (anti-mouse or anti-rabbit IgG HRP conjugate; Bethyl Laboratories, Montgomery, TX, USA) diluted 2,000-fold in TBST. After washing with TBST, the specific binding of antibodies was detected by using an ECL kit (Thermo Fisher Scientific), following the manufacturer's protocol.

## 6. RNA interference

At 24 hours before transfection, cells were plated into 6-well plates ( $1 \times 10^5$  cells/well). Cells were transfected with 50 nmol of DRG2 siRNA (sc-93839; Proteintech) or control siRNA (sc-37007; Proteintech) using the siRNA transfection reagent from Santa Cruz Biotechnology. The cells were then grown for 24 hours prior to western blot analysis.

## 7. Flow cytometry

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

## 8. Annexin V cell death assay

Apoptotic cell death was measured using a fluorescein isothiocyanate (FITC)-conjugated Annexin V/PI assay kit. Briefly, cells were washed with ice-cold PBS, resuspended in

100 µL binding buffer, and stained with 5 µL of FITC-conjugated Annexin V (10 mg/mL) and 10 µL of PI (50 mg/mL). The cells were then incubated for 15 minutes at room temperature in the dark, 400 µL of binding buffer was added, and the cells were analyzed by flow cytometry (FACSCalibur; BD Biosciences).

## 9. Statistical analysis

The expression of DRG2 in each dose of docetaxel was obtained from three independent experiments and expressed as means compared with the expression of GAPDH. Statistical evaluation of the results was performed by ANOVA with Dunnett multiple comparisons test. Prism (GraphPad Software, San Diego, CA, USA) was used to calculate and analyze the statistical differences among three docetaxel dose groups. A p-value of <0.05 was considered to be statistically significant.

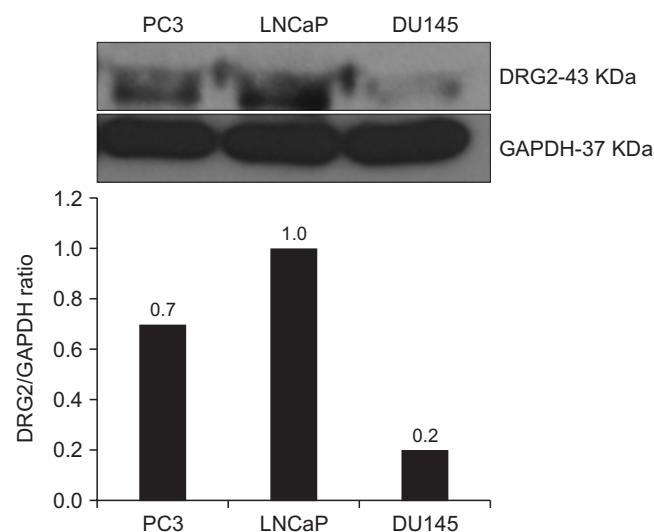
## 10. Ethics statement

This study was approved by the institutional review board and ethics committee of Ulsan University Hospital (IRB No: NON2017-009-004).

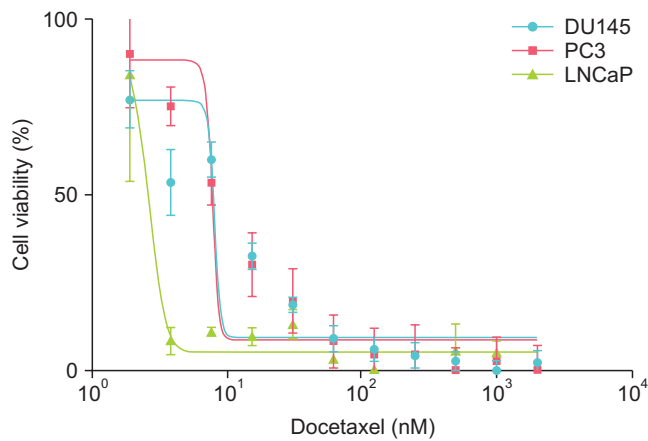
# RESULTS

## 1. DRG2 expression differs in each prostate cancer cell line

A western blot assay was used to compare the DRG2 expression in prostate cancer cell lines. DRG2 expression was compared by using GAPDH as the loading control. DRG2 ex-



**Fig. 1.** Developmentally regulated GTP-binding protein 2 (DRG2) expression in prostate cancer cell lines. A western blot assay was performed with specific antibodies against DRG2 and GAPDH. DRG2 expression in each cell line was expressed based on its ratio with GAPDH.



**Fig. 2.** Cell viability assay in prostate cancer cell lines after docetaxel treatment. Cells were exposed to 0 nM to 2,048 nM docetaxel for 48 hours. Cell viability was determined by using the MTT assay. IC<sub>50</sub> values were measured by using Prism (GraphPad Software, San Diego, CA, USA).

pression was found to differ in each cell line. The expression of DRG2 was the highest in the LNCaP cell line, whereas that in DU145 cells was the least. Compared with GAPDH, the ratio of DRG2 expression was 0.7, 1.0, and 0.2 for the PC3, LNCaP, and DU145 cell lines, respectively (Fig. 1).

## 2. Docetaxel blocks the growth of prostate cancer cells in a dose-dependent manner

After prostate cancer cells were exposed to 0 to 2,048 nM docetaxel for 48 hours, cell viability was measured. Docetaxel reduced the proliferation of all prostate cancer cells. The IC<sub>50</sub> values for each prostate cancer cell line (DU145, PC3, and LNCaP) were 23.32 nM, 19.55 nM and 4.58 nM, respectively (Fig. 2). With reference to the LNCaP cell line, which had the lowest IC<sub>50</sub> among the cell lines, 4 nM and 8 nM were used as the docetaxel doses in this experiment.

## 3. Docetaxel reduces the G1/G2 ratio in prostate cancer cells

Because 4 nM and 8 nM were administered according to the IC<sub>50</sub> of LNCaP cells, docetaxel was unable to block cell growth except in the LNCaP cell line (Fig. 3A). Upon treatment with 4 nM docetaxel, the proportion of cells in G1 decreased but that in the sub-G1 phase increased in all cell lines. By contrast, the proportion of cells in G2/M increased in PC3 and LNCaP cells, but not in DU145 cells (Fig. 3B). In all cell lines, docetaxel treatment suppressed the G1 phase in a dose-dependent manner. In contrast, an increase in the G2/M

phase was observed with dose escalation in PC3 and LNCaP cells, but not in DU145 cells (Fig. 3C). Further, the G1/G2 ratio was reduced by docetaxel in a dose-dependent manner in all cell lines.

## 4. Docetaxel reduces the expression of DRG2

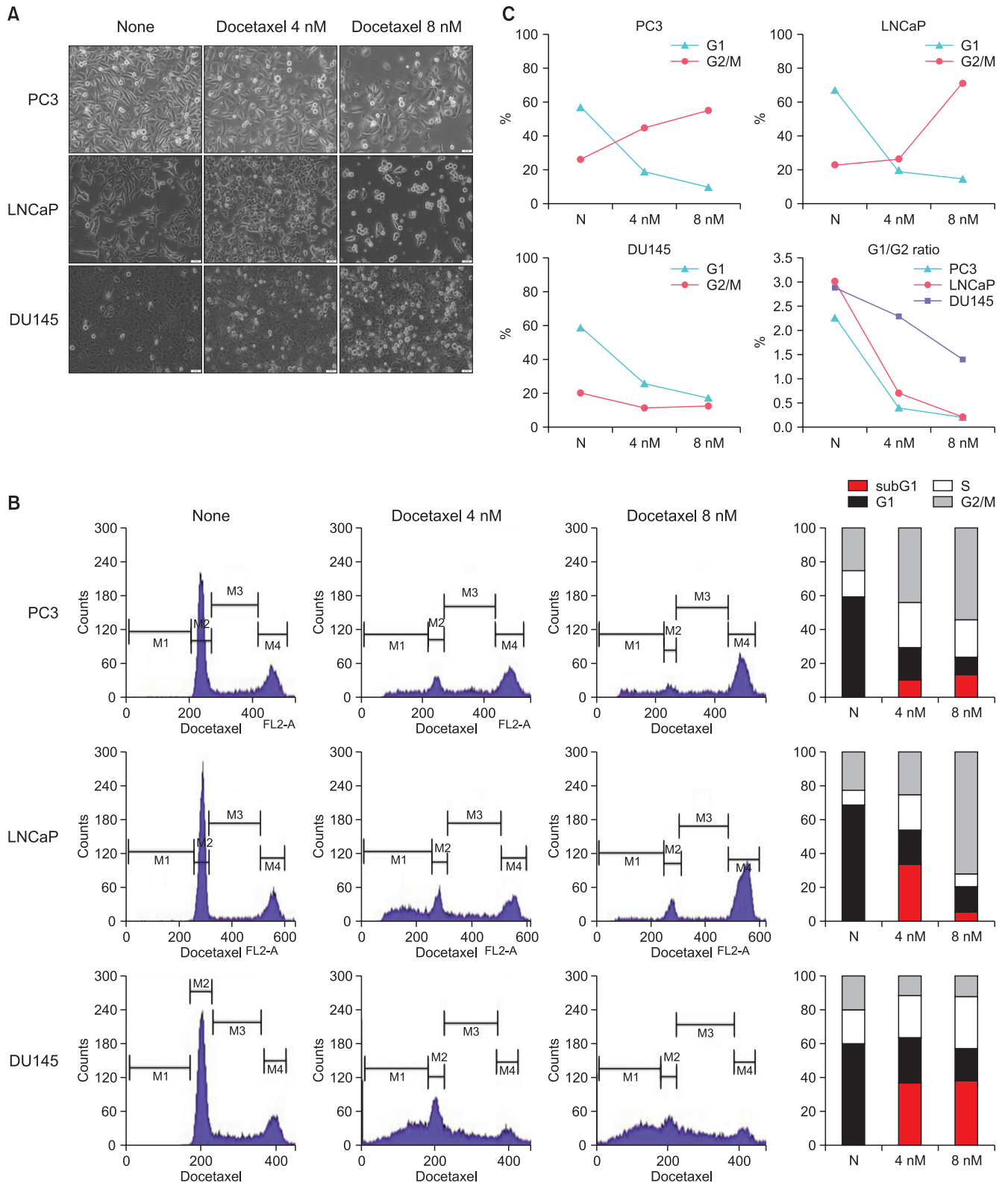
After administering docetaxel at 0 nM, 4 nM, and 8 nM to each prostate cancer cell line, the expression of DRG2 was confirmed by western blot analysis (Fig. 4). When the change in DRG2 expression was analyzed by using the DRG2/GAPDH ratio, docetaxel was found to reduce the level of DRG2 expression in a dose-dependent manner ( $p=0.0427$  in PC3,  $p=0.0147$  in LNCaP, and  $p=0.0326$  in DU145).

## 5. DRG2 knockdown increases the sub-G1 phase

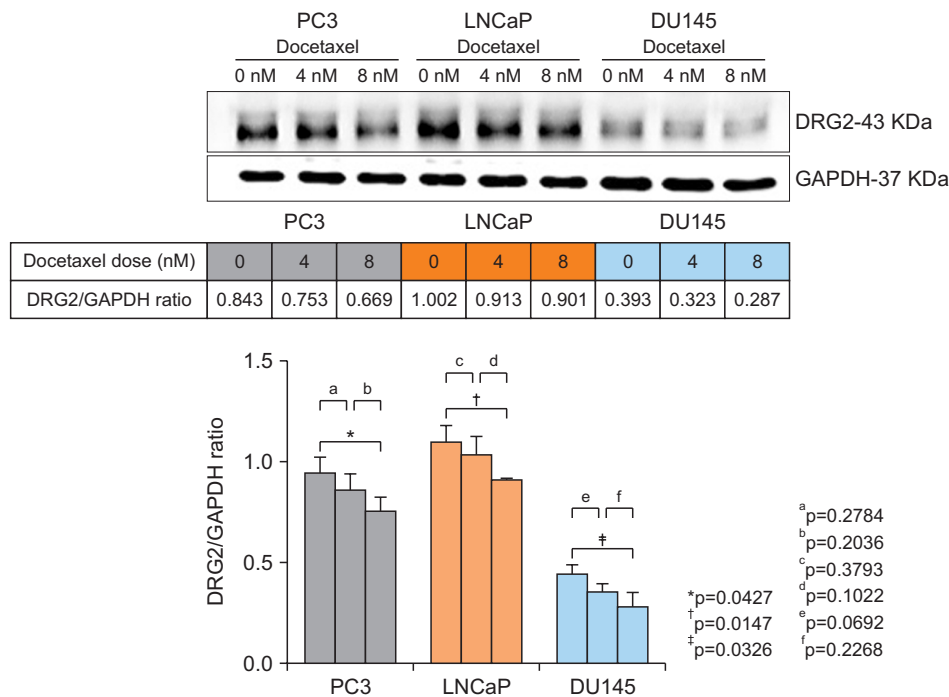
To generate DRG2 knockdown prostate cancer cells, DRG2 siRNA was transfected into each cell line (Fig. 5A). Upon DRG2 knockdown in prostate cancer cells, an increase in the sub-G1 phase without any change in the G1 or G2/M phase was observed. This change was more remarkable in the PC3 and LNCaP cell lines than in the DU145 cell line (Fig. 5B).

## 6. Inhibition of DRG2 with siDRG2 increased docetaxel-induced apoptosis

All prostate cancer cell lines except for DU145 showed a decrease in the G1 phase after treatment with 4 nM docetaxel and a dramatic increase in the G2/M phase. By contrast, DU145 showed an increase in the sub-G1 phase without an increase in the G2/M phase (Fig. 6A). To generate DRG2 knockdown prostate cancer cells, DRG2 siRNA was transfected into each cell line. Upon treatment with 4 nM docetaxel in DRG2 knockdown prostate cancer cell lines, an increase in the sub-G1 phase was observed without an increase in the G2/M phase, which was similar to the pattern observed in the DU145 cell line before DRG2 knockdown (Fig. 6A). DRG2 knockdown had little effect on the change in the G1 phase, but showed the effect of eliminating the increase in the G2/M phase caused by docetaxel treatment (Fig. 6B). Flow cytometry analysis of prostate cancer cells doubly labeled with Annexin V and PI showed that docetaxel treatment induced apoptosis in these cells (Fig. 6C). In the PC3 and DU145 cell lines, which rarely showed apoptosis from 4 nM docetaxel treatment, knockdown of DRG2 increased the docetaxel-induced Annexin V (+) apoptosis by 8.7 and 27 times, respectively (Fig. 6D).



**Fig. 3.** Changes in the cell cycle after docetaxel treatment in prostate cancer cells. (A) Phase-contrast photomicrographs showing the effect of docetaxel on each prostate cancer cell line ( $\times 200$ ). (B) Flow cytometry analysis for cell cycle DNA content in the G0/G1, S, and G2/M phases after treatment of prostate cancer cells with docetaxel for 48 hours. (C) The G1/G2 ratio decreases with increasing dose of docetaxel in prostate cancer cells.



**Fig. 4.** The change in developmentally regulated GTP-binding protein 2 (DRG2) expression after docetaxel treatment. DRG2 expression was analyzed by western blot analysis. The change in DRG2 expression after docetaxel (0 nM, 4 nM, and 8 nM) treatment is shown by the DRG2/GAPDH ratio.

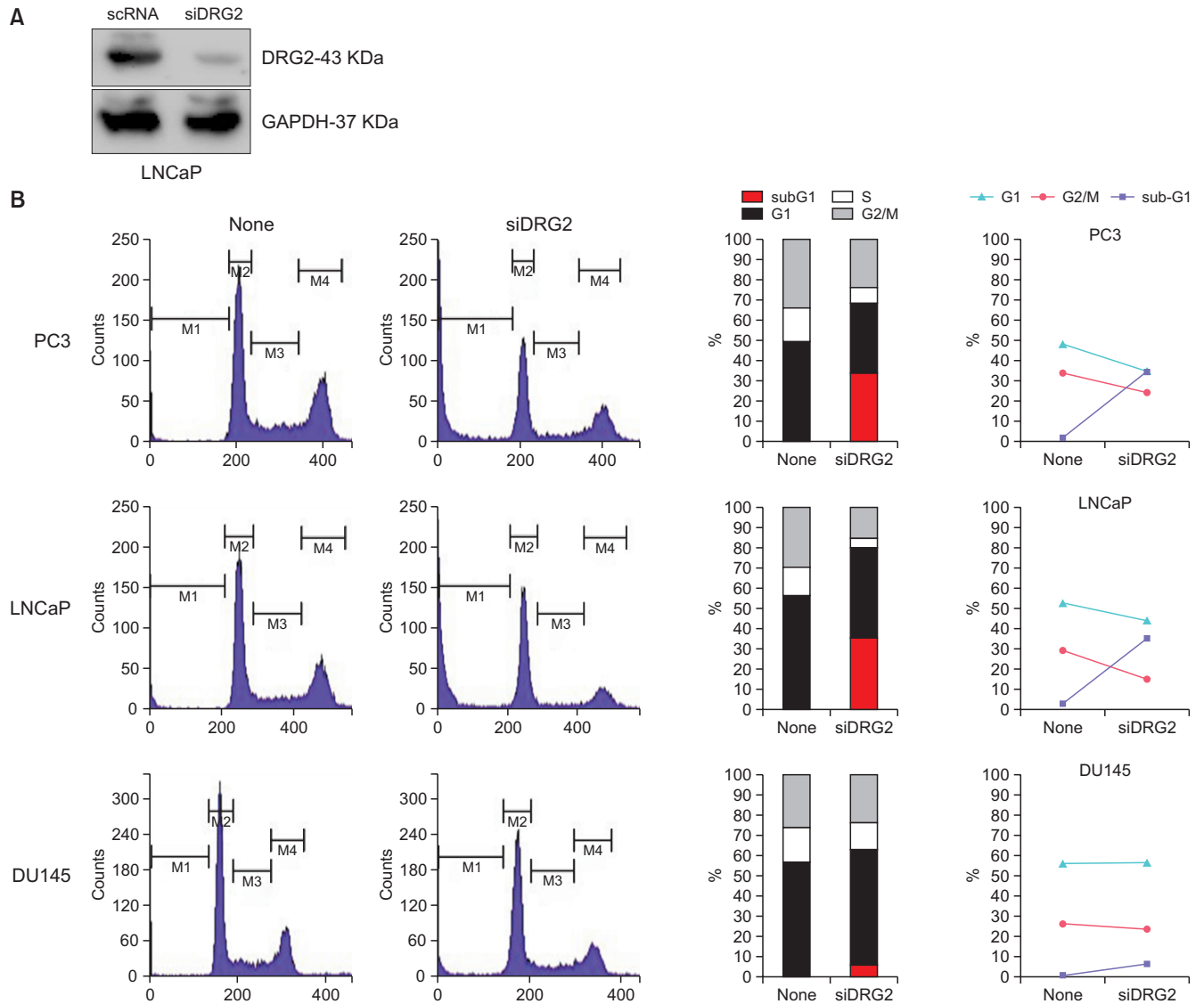
## DISCUSSION

Because DRG2 affects microtubule dynamics through the cyclin B1-cdk1 complex, several studies have examined the relation between microtubules and chemotherapy with taxane, an anti-microtubule agent. These studies have shown that an increase in DRG2 enhances G2/M arrest [17]. DRG2 expression is reduced with docetaxel administration, and docetaxel-induced apoptosis is decreased with DRG2 overexpression; these results indicate a correlation between DRG2 and docetaxel-induced apoptosis [16,19]. However, the association between DRG2 and docetaxel-induced apoptosis could not be explained simply by microtubule dynamics because the same results were observed with doxorubicin, etoposide, and thapsigargin, which are not related to microtubules [19]. Rather, these studies showed that these chemotherapeutic drugs regulate the caspase 3-PARP pathway according to DRG2 expression and that DRG2 acts as a DNA repair protein for genotoxic stress.

Against genotoxic stress, cells undergo cell death by three different pathways: apoptosis and stress-induced premature senescence (SIPS) related to the G1 checkpoint and multinucleated giant cells (MNGCs) related to the G2 check point [11,22]. Upon genotoxic stress such as docetaxel exposure, the p53-dependent pathway induces apoptosis or SIPS at the G1 checkpoint in cells with damaged DNA, resulting in cell death or repair [23]. On the contrary, the p53-independent pathway results in the formation of MNGCs through G2/M arrest at the G2 check point, thereby leading to cell death

through the mitotic catastrophe pathway [24-26]. However, MNGCs surviving the mitotic catastrophe can contribute to cancer relapse by entering a state of dormancy and giving rise to progeny with stem cell-like properties [27,28]. Accordingly, stress-induced growth arrest in cancer cells—reflecting either SIPS (predominantly in p53 wild-type cells) or the creation of MNGCs (predominantly p53-deficient cells)—can provide a “survival” mechanism, ultimately resulting in the emergence of cancer-repopulating progeny [12,29]. However, the G2 checkpoint is important for DNA damage in cancer cells because loss of G1 checkpoint control is a common feature of cancer cells (e.g., p53 mutation). Thus, most cancer cells show cell cycle arrest at the G2 checkpoint with most DNA-damaging chemotherapy [30]. For this reason, reducing MNGCs and increasing apoptosis could be a way to reduce recurrence and improve treatment efficacy in cancer.

In the present study, the level of DRG2 expression differed in each prostate cancer cell line, being highest in LNCaP cells and lowest in DU145 cells. These results contradict those of a previously reported study on melanoma [20]. That study reported that VEGF-A expression was enhanced by DRG2 expression, thus promoting tumor growth and metastasis. As such, the study reported that DRG2 is positively correlated with the malignancy of melanoma. In contrast, among prostate cancer cell lines, DRG2 was expressed the most in the LNCaP cell line, a hormone-sensitive prostate cancer cell line, but the least in the DU145 cell line, which is a CRPC cell line. Currently, the reason for this is unclear. Considering the high frequency of DNA repair gene muta-



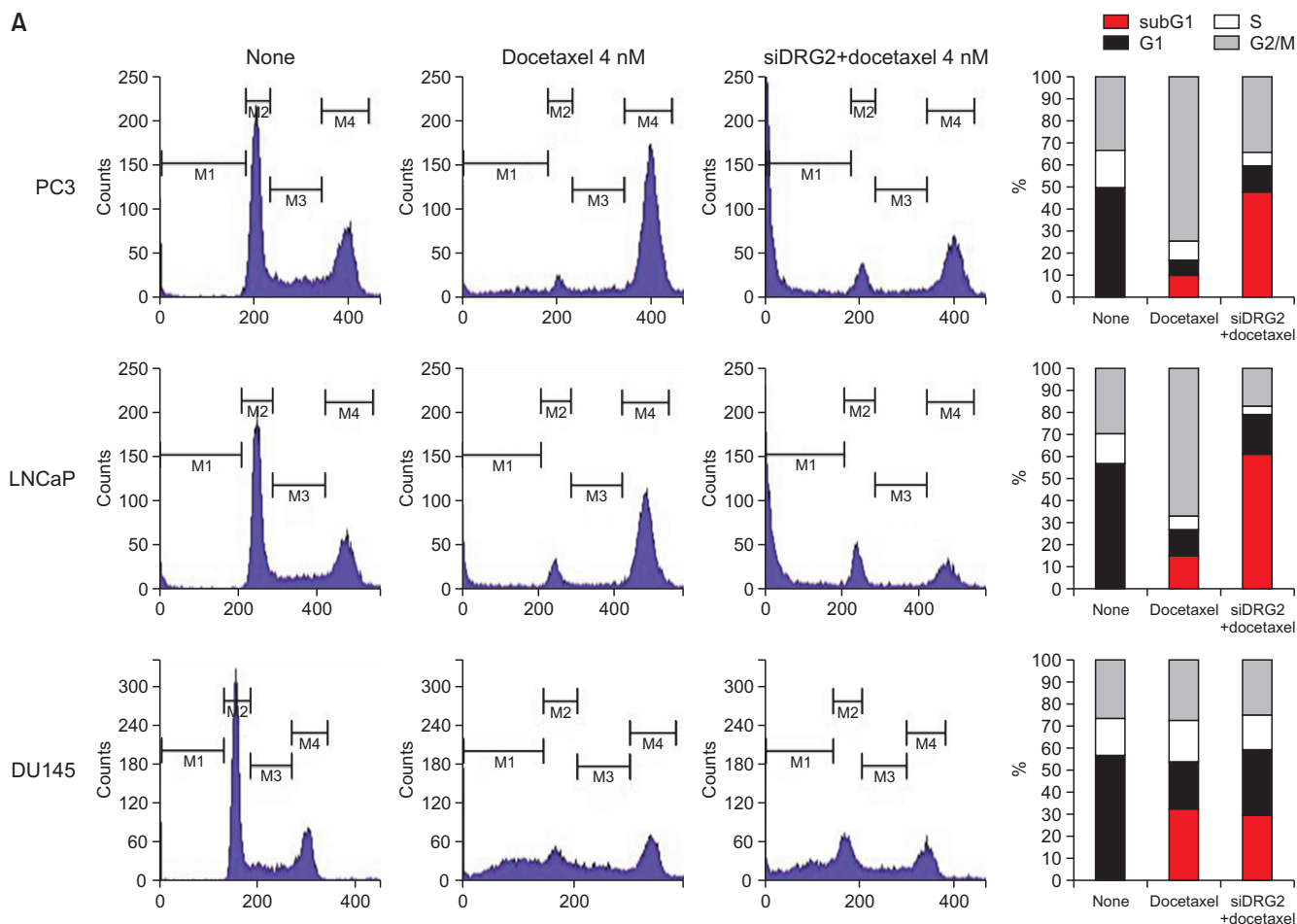
**Fig. 5.** Cell cycle changes in prostate cancer cells after developmentally regulated GTP-binding protein 2 (DRG2) knockdown. (A) Knockdown efficiency of siDRG2 in LNCaP cells. (B) PC3 and LNCaP cells with DRG2 knockdown showed a marked increase in the sub-G1 phase, but DU145 cells did not.

tions in CRPC, the possibility of DRG2 mutation may also be considered.

As with previous studies, this study showed a typical G2 checkpoint arrest in response to docetaxel treatment in which prostate cancer cells showed a decreased G1 and increased G2/M upon 48 hours of exposure to low-dose docetaxel. Due to this arrest, apoptosis hardly occurred in PC3 and DU145 cells exposed to 4 nM docetaxel, which is much lower than their  $IC_{50}$ . However, the response to docetaxel exposure was somewhat different between these two CRPCs. In the case of the PC3 cell line, the increase in G2/M was prominent, whereas this was not observed in the DU145 cell line. PC3 cells with DRG2 knockdown showed a response similar to that of DU145 cells, with no increase of G2/M in response

to docetaxel exposure. These results suggest that DRG2 may regulate the G2/M arrest that occurs upon docetaxel treatment. Several previous studies support these findings. These studies have also reported that DRG2 knockdown causes more cell death with low-dose paclitaxel [18], and that overexpression of DRG2 increases G2/M arrest and suppresses apoptosis [15,19].

When docetaxel is administered to DRG2-knockdown prostate cancer cells, the sub-G1 phase increases without an increase in the G2/M. This response can be confirmed as an increase in apoptosis observed by the increase in Annexin V (+) cells. These results indicate that reduction of DRG2 tends to promote apoptosis rather than the mitotic catastrophe, a typical response of cancer cells to low-dose docetaxel



**Fig. 6.** Effect of developmentally regulated GTP-binding protein 2 (DRG2) knockdown by DRG2 siRNA (siDRG2) on docetaxel-induced apoptosis in prostate cancer cell lines. (A) Flow cytometry showing the difference in the cell cycle after treatment with 4 nM docetaxel before and after DRG2 knockdown. (B) DRG2 knockdown reversed the increase in the G2/M phase caused by docetaxel treatment. (C) Flow cytometry analysis of prostate cancer cells treated with 4 nM docetaxel. Cells were stained with Annexin V fluorescein and propidium iodide. Annexin V (+) indicates apoptosis. (D) Difference in the proportion of Annexin V (+) cells after treatment of prostate cancer cells with 4 nM docetaxel before and after DRG2 knockdown. N, none; D, 4 nM docetaxel; S, siDRG2.

treatment. However, DRG2 knockdown is more likely to be resistant to high-dose docetaxel treatment because mitotic catastrophe, an important cell death mechanism for large amounts of genotoxic stress, is disturbed [18]. Further research is needed to investigate the effects of DRG2 on apoptosis and mitotic catastrophe while increasing the dose or exposure time of docetaxel.

Although LNCaP showed high DRG2 expression in this study, apoptosis occurred frequently. Because an increase in G2/M also occurs with cell death, mitotic catastrophe and apoptosis are considered to occur simultaneously. However, we inferred that the cell death in LNCaP cells was more common than in PC3 cells with low-dose docetaxel treatment despite maintaining high DRG2 expression because LNCaP cells have wild-type p53. In addition, more than 75% of this cell death was early apoptosis (Annexin V (+)/PI (-)).

The present study has several limitations. First, the

reason for the differing DRG2 expression in each cell line was not confirmed. As the malignancy of prostate cancer increases, the prevalence of mutations in the DNA repair gene also increases [7]. Therefore, it is necessary to confirm whether DRG2 gene mutation affects subsequent DRG2 expression. Second, changes in DRG2 over time after docetaxel treatment were not confirmed. MNGCs produced after treatment with docetaxel gradually lead to cell death through the mitotic catastrophe [10,11]. In this process, it is necessary to simultaneously confirm the change in DRG2 expression and to check the changes in cell death during the incubation period when the drug was not administered. Finally, the relationship with other DNA repair proteins was not confirmed. Of these proteins, p53 seems to have little relation with DRG2. However, the role of DRG2 is similar to the effect of DNA repair proteins that promote G2/M arrest; thus, it is necessary to check its relationship with other



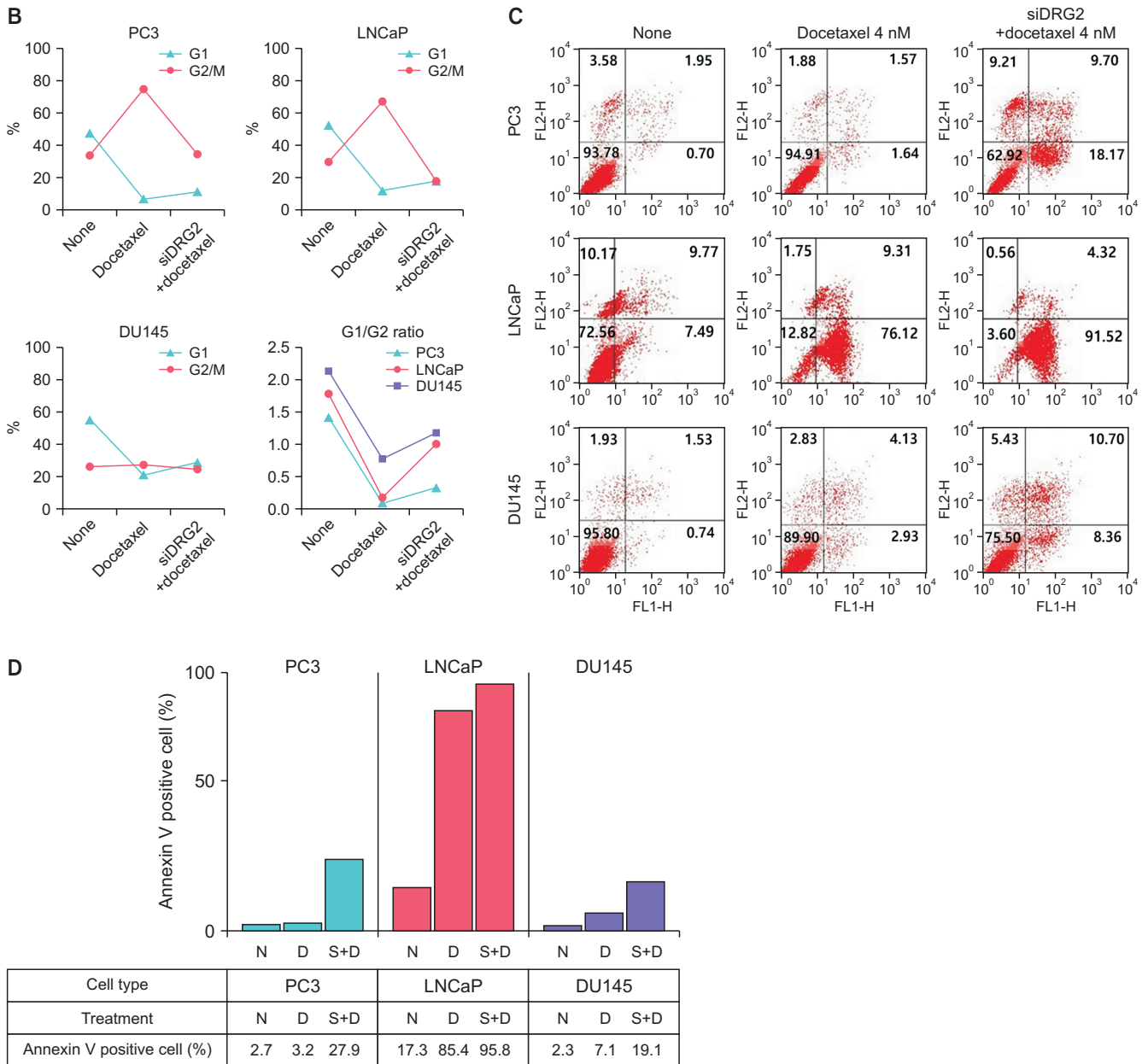


Fig. 6. Continued.

DNA repair proteins. In particular, it is necessary to clarify whether there is a relationship between DRG2 and p21 because DRG2 controls the cell cycle through cyclin B1-Cdk1, similar to p21, which is an anti-apoptotic p53 protein [17].

### CONCLUSIONS

In prostate cancer cells, DRG2 regulates G2/M arrest after docetaxel treatment. DRG2 expression differs in each prostate cancer cell line, resulting in different responses to docetaxel treatment. In prostate cancer cells with DRG2 knockdown, apoptosis increases without G2/M arrest in re-

sponse to docetaxel treatment. These results show that inhibition of DRG2 expression can be useful to enhance docetaxel-induced apoptosis despite low-dose treatment in CRPC.

### CONFLICTS OF INTEREST

The authors have nothing to disclose.

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## AUTHORS' CONTRIBUTIONS

Research conception and design: Sungchan Park. Data acquisition: Won Hyeok Lee, Song Hee Kim, Young Min Kim, Abdumadjidov Alisher Abdulkhayevich, Sang Hun Lee, and Kyung Hyun Moon. Statistical analysis: Won Hyeok Lee and Song Hee Kim. Data analysis and interpretation: Seong Cheol Kim, Won Hyeok Lee, and Sungchan Park. Drafting of the manuscript: Seong Cheol Kim. Critical revision of the manuscript: Seong Cheol Kim. Obtaining funding: Sungchan Park. Administrative, technical, or material support: Jeong Woo Park. Supervision: Jeong Woo Park and Sungchan Park. Approval of the final manuscript: Seong Cheol Kim.

## REFERENCES

1. Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N Engl J Med* 2004;351:1502-12.
2. Petrylak DP, Tangen CM, Hussain MH, Lara PN Jr, Jones JA, Taplin ME, et al. Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. *N Engl J Med* 2004;351:1513-20.
3. Lowrance WT, Murad MH, Oh WK, Jarrard DF, Resnick MJ, Cookson MS. Castration-resistant prostate cancer: AUA guideline amendment 2018. *J Urol* 2018;200:1264-72.
4. Kentepozidis N, Soultati A, Giassas S, Vardakis N, Kalykaki A, Kotsakis A, et al. Paclitaxel in combination with carboplatin as salvage treatment in patients with castration-resistant prostate cancer: a Hellenic oncology research group multicenter phase II study. *Cancer Chemother Pharmacol* 2012;70:161-8.
5. Ross RW, Beer TM, Jacobus S, Bubley GJ, Taplin ME, Ryan CW, et al. A phase 2 study of carboplatin plus docetaxel in men with metastatic hormone-refractory prostate cancer who are refractory to docetaxel. *Cancer* 2008;112:521-6.
6. Nuhn P, De Bono JS, Fizazi K, Freedland SJ, Grilli M, Kantoff PW, et al. Update on systemic prostate cancer therapies: management of metastatic castration-resistant prostate cancer in the era of precision oncology. *Eur Urol* 2019;75:88-99.
7. Antonarakis ES. Germline DNA repair mutations and response to hormonal therapy in advanced prostate cancer. *Eur Urol* 2017;72:43-4.
8. Kroemer G, El-Deiry WS, Golstein P, Peter ME, Vaux D, Vandenberg P, et al. Classification of cell death: recommendations of the Nomenclature Committee on Cell Death. *Cell Death Differ* 2005;12 Suppl 2:1463-7.
9. Ricci MS, Zong WX. Chemotherapeutic approaches for targeting cell death pathways. *Oncologist* 2006;11:342-57.
10. Morse DL, Gray H, Payne CM, Gillies RJ. Docetaxel induces cell death through mitotic catastrophe in human breast cancer cells. *Mol Cancer Ther* 2005;4:1495-504.
11. Fabbri F, Amadori D, Carloni S, Brigliadori G, Tesei A, Ulivi P, et al. Mitotic catastrophe and apoptosis induced by docetaxel in hormone-refractory prostate cancer cells. *J Cell Physiol* 2008;217:494-501.
12. Erenpreisa J, Kalejs M, Cragg MS. Mitotic catastrophe and endomitosis in tumour cells: an evolutionary key to a molecular solution. *Cell Biol Int* 2005;29:1012-8.
13. Schenker T, Lach C, Kessler B, Calderara S, Trueb B. A novel GTP-binding protein which is selectively repressed in SV40 transformed fibroblasts. *J Biol Chem* 1994;269:25447-53.
14. Li B, Trueb B. DRG represents a family of two closely related GTP-binding proteins. *Biochim Biophys Acta* 2000;1491:196-204.
15. Song H, Kim SI, Ko MS, Kim HJ, Heo JC, Lee HJ, et al. Overexpression of DRG2 increases G2/M phase cells and decreases sensitivity to nocodazole-induced apoptosis. *J Biochem* 2004;135:331-5.
16. Ko MS, Lee UH, Kim SI, Kim HJ, Park JJ, Cha SJ, et al. Overexpression of DRG2 suppresses the growth of Jurkat T cells but does not induce apoptosis. *Arch Biochem Biophys* 2004;422:137-44.
17. Jang SH, Kim AR, Park NH, Park JW, Han IS. DRG2 regulates G2/M progression via the cyclin B1-Cdk1 complex. *Mol Cells* 2016;39:699-704.
18. Dang T, Jang SH, Back SH, Park JW, Han IS. DRG2 deficiency causes impaired microtubule dynamics in HeLa cells. *Mol Cells* 2018;41:1045-51.
19. Chen J, Shen BY, Deng XX, Zhan Q, Peng CH. SKP1-CULLIN1-F-box (SCF)-mediated DRG2 degradation facilitated chemotherapeutic drugs induced apoptosis in hepatocellular carcinoma cells. *Biochem Biophys Res Commun* 2012;420:651-5.
20. Yoon NA, Jung SJ, Choi SH, Ryu JH, Mani M, Lee UH, et al. DRG2 supports the growth of primary tumors and metastases of melanoma by enhancing VEGF-A expression. *FEBS J* 2020;287:2070-86.
21. Hong MJ, Yoo SS, Choi JE, Kang HG, Do SK, Lee JH, et al. Functional intronic variant of SLC5A10 affects DRG2 expression and survival outcomes of early-stage non-small-cell lung cancer. *Cancer Sci* 2018;109:3902-9.
22. Mirzayans R, Andrais B, Kumar P, Murray D. Significance of wild-type p53 signaling in suppressing apoptosis in response to chemical genotoxic agents: impact on chemotherapy outcome. *Int J Mol Sci* 2017;18:928.
23. Roninson IB. Tumor cell senescence in cancer treatment. *Can-*

- cer Res 2003;63:2705-15.
24. Bunz F, Dutriaux A, Lengauer C, Waldman T, Zhou S, Brown JP, et al. Requirement for p53 and p21 to sustain G2 arrest after DNA damage. *Science* 1998;282:1497-501.
  25. Giono LE, Manfredi JJ. The p53 tumor suppressor participates in multiple cell cycle checkpoints. *J Cell Physiol* 2006;209:13-20.
  26. Portugal J, Mansilla S, Bataller M. Mechanisms of drug-induced mitotic catastrophe in cancer cells. *Curr Pharm Des* 2010;16:69-78.
  27. Weihua Z, Lin Q, Ramoth AJ, Fan D, Fidler IJ. Formation of solid tumors by a single multinucleated cancer cell. *Cancer* 2011;117:4092-9.
  28. Mirzayans R, Andrais B, Scott A, Wang YW, Kumar P, Murray D. Multinucleated giant cancer cells produced in response to ionizing radiation retain viability and replicate their genome. *Int J Mol Sci* 2017;18:360.
  29. Erenpreisa J, Cragg MS. Three steps to the immortality of cancer cells: senescence, polyploidy and self-renewal. *Cancer Cell Int* 2013;13:92.
  30. Massagué J. G1 cell-cycle control and cancer. *Nature* 2004;432:298-306.