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Collateral resistance to taxanes in enzalutamide-resistant prostate cancer through aberrant androgen receptor and its variants

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Currently, the optimal sequential use of androgen receptor (AR) axis-targeted agents and taxane chemotherapies remains undetermined. We aimed to elucidate the resistance status between taxanes and enzalutamide, and the functional role of the AR axis. Enzalutamide-resistant 22Rv1 cells showed collateral resistance to taxanes, including docetaxel and cabazitaxel. However, taxane-resistant cells showed no collateral resistance to enzalutamide; taxane-resistant cells expressed comparable protein levels of full-length AR and AR variants. Knockdown of both full-length AR and AR variants rendered cells sensitive to taxanes, whereas knockdown of AR variants sensitized cells to enzalutamide, but not to taxanes. In contrast, overexpression of full-length AR rendered cells resistant to taxanes. Consistently, the prostate-specific antigen response and progression-free survival in docetaxel chemotherapy were worse in cases with prior use of ARAT agents compared with cases without. Collateral resistance to taxanes was evident after obtaining enzalutamide resistance, and aberrant AR signaling might be involved in taxane resistance.

KEYWORDS
androgen receptor, cabazitaxel, docetaxel, enzalutamide, prostate cancer

1 | INTRODUCTION

Currently, androgen-deprivation therapy (ADT) with or without upfront docetaxel or abiraterone is the standard treatment for metastatic prostate cancer.[1-3] Although most prostate cancers are dependent on androgens for tumor growth and respond prominently to ADT, they eventually overcome low circulating levels of androgens and progress in a castration-resistant manner, despite consecutive ADT. Taxanes including docetaxel and cabazitaxel are chemotherapeutic agents that confer a survival benefit to patients with castration-resistant prostate cancer (CRPC). In addition to taxanes, the radioisotope radium-223 and novel androgen receptor (AR) axis-targeted (ARAT) agents including the CYP17 inhibitor abiraterone and antiandrogen enzalutamide have shown tremendous benefits, including survival in patients with CRPC, in clinical trials.[4-11] Thus, multiple agents are currently available for the treatment of CRPC, mainly in a sequential manner. However, there is scarce evidence on their sequential use; therefore, optimal sequential use of ARAT agents and taxane chemotherapies remains undetermined.[12]

To date, various cross- or collateral resistance among those agents has been reported. Several studies have consistently shown robust cross-resistance between enzalutamide and abiraterone in both preclinical and clinical studies.[13] Similarly, collateral partial resistance to cabazitaxel in docetaxel-resistant cells was also reported in

a preclinical study.[14] In addition, van Soest et al[15] have reported collateral resistance to docetaxel and cabazitaxel in enzalutamide-resistant cells in an in vitro experiment, but not to cabazitaxel in an in vivo experiment.[16] Conversely, Al Nakouzi et al[17] have reported no collateral resistance to cabazitaxel in enzalutamide-resistant tumors, and no collateral resistance in cabazitaxel-resistant cells to abiraterone or enzalutamide. However, there are no reports on cellular resistance to ARAT agents in docetaxel-resistant cells.

Thus, there are controversial or unreported findings on the resistance status between taxanes and ARAT agents. In addition, aberrant AR signaling by ligand-dependent activation of full-length AR and ligand-independent activation of AR variants lacking a ligand-binding domain and exerting constitutive activation without the ligand has been suggested as playing a critical role in cross- or collateral resistance among agents for CRPC.[18] Therefore, in this study, we aimed to elucidate the resistance status between taxanes and enzalutamide, as well as the functional role of the AR axis in the resistance status between taxanes and enzalutamide using CRPC 22Rv1 cells, which express both full-length AR and AR variants.

2 | MATERIALS AND METHODS

2.1 | Cell culture

Castration-resistant prostate cancer 22Rv1 cells were obtained from ATCC (Manassas, VA, USA), and cultured in RPMI-1640 media, which were purchased from Thermo Fisher Scientific (Waltham, MA, USA) and contained 10% FBS. Enzalutamide- and docetaxel-resistant derivatives of 22Rv1 cells, specifically 22Rv1/MDV and 22Rv1/DTX cells, respectively, were established and maintained as described previously.[19,20] Cabazitaxel-resistant derivatives of 22Rv1 cells, specifically 22Rv1/CBZ cells, were established by long-term culture under the appropriate media with gradually increasing concentrations of cabazitaxel and maintained under media containing 10 ng/mL cabazitaxel. The cell lines were maintained in a 5% CO_2 atmosphere at 37°C.

2.2 | Antibodies and reagents

Antibodies against AR (N-20, sc-816) and β-actin (A3854) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and Sigma (St. Louis, MO, USA), respectively. Enzalutamide was purchased from Selleck Chemicals (Houston, TX, USA).

2.3 | Knockdown analysis using siRNAs

The following double-stranded RNA oligonucleotides were commercially generated by Thermo Fisher Scientific: 5′-CAUAGUGACACCCAGAAGCUUCAUC-3′ (sense) and 5′-CCGUAACCAUUAUAGACGCUAUCCA-3′ (antisense) for AR #1; 5′-UAGAGAGCAAGGCUGCAAAGGAGUC-3′ (sense) and 5′-GACUCCUUUGCAGCCUUGCUCUCUA-3′ (antisense) for AR #2; and 5′-GUAGUUGUGAGUAUCAUGATT-3′ (sense) and 5′-UCAUGAUACUCACAACUACTT-3′ (antisense) for AR V7. 22Rv1 cells were transfected with siRNA (40 nmol/L for AR #1 and AR #2; 5 nmol/L for AR V7) using Lipofectamine 2000 (Thermo Fisher Scientific) according to the manufacturer's protocol.

2.4 | Plasmid construction and stable transfectants

The AR-GFP plasmid was kindly provided by Dr. Toshihiko Yanase (Fukuoka University, Fukuoka, Japan).[21] AcGFP plasmid expressing GFP was purchased from Clontech (Mountain View, CA, USA). Stable transfectants of AcGFP plasmid (22Rv1-AcGFP) and AR-GFP plasmid (22Rv1-AR-GFP) to 22Rv1 cells were established as described previously.[22]

2.5 | RNA isolation, reverse transcription, and quantitative real-time PCR

The RNA isolation and reverse transcription were carried out as described previously.[19,20,22-24] Quantitative real-time PCR was undertaken using TaqMan Gene Expression Assays (Thermo Fisher Scientific) for full-length *AR* (Hs00171172_m1), *AR V7* (order-made), and *GAPDH* (Hs02758991_g1) and TaqMan Gene Expression Master Mix (Thermo Fisher Scientific) on a CFX Connect Real-Time System (Bio-Rad, Hercules, CA, USA). The transcript levels of full-length *AR* and *AR V7* were corrected by the corresponding *GAPDH* transcript levels. All values represent the results of at least three independent experiments.

2.6 | Western blot analysis

Whole-cell extracts were prepared as described previously.[19,20,22-24] The bound antibodies were visualized using an ECL kit (GE Healthcare Bio-Sciences, Piscataway, NJ, USA), and images were obtained using an image analyzer (Ez-Capture MG; ATTO, Tokyo, Japan).

2.7 | Cytotoxicity analysis

Cytotoxicity analyses were carried out as described previously.[19,20,22-24] Briefly, 22Rv1 cells (2.5×10^3) transfected with the indicated siRNA, or stably transfected with the indicated plasmids, were seeded in 96-well plates. On the following day, various concentrations of the indicated agents were applied. After 48 hours (docetaxel and cabazitaxel) or 72 hours (enzalutamide), the surviving cells were stained using the alamarBlue assay (TREK Diagnostic Systems, Cleveland, OH, USA) at 37°C for 180 minutes. The absorbance of each well was measured using the ARVO MX plate reader (PerkinElmer, Waltham, MA, USA). The results are representative of at least three independent experiments.

2.8 | Cell proliferation assay

The cell proliferation assay was carried out as described previously.[19,20] Briefly, 22Rv1 cells (2.5×10^4) were seeded into 12-well plates, then transfected with the indicated siRNA and incubated. After 72 hours, cells were harvested with trypsin and counted daily

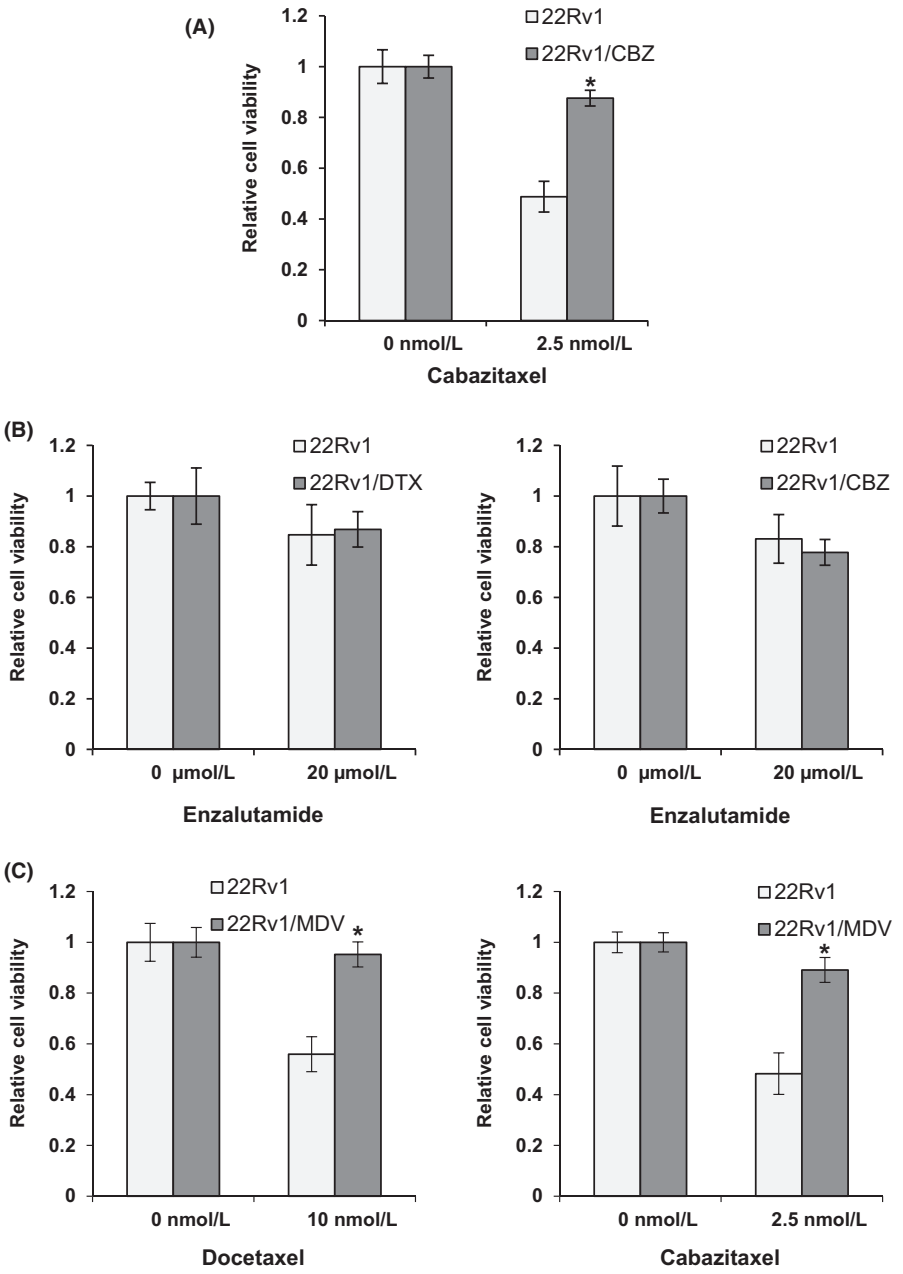

FIGURE 1 Resistance status between taxanes and enzalutamide. A-C, Indicated cells were seeded in 96-well plates. On the following day, the indicated concentration of anticancer agents was applied. After 48 hour for docetaxel and cabazitaxel, or 72 hour for enzalutamide, the cell survival rates were analyzed by cytotoxicity analyses. Cell survival in the absence of drugs was defined as 1. Boxes, mean; bars, ±SD. *$P < .05$ (compared with 22Rv1 cells)

using a cell counter (Beckman Coulter, Fullerton, CA, USA). The results were normalized to cell counts when transfected with control siRNA, and are representative of three experiments.

2.9 | Patients

Japanese patients who had undergone docetaxel chemotherapy for metastatic CRPC at Kyushu University Hospital (Fukuoka, Japan) between 2008 and 2017 were included. This study was undertaken in accordance with the principles described in the Declaration of Helsinki and the Ethical Guidelines for Epidemiological Research enacted by the Japanese Government, and approved by an institutional review board. All patients were histopathologically diagnosed with adenocarcinoma of the prostate. Clinical staging was determined in accordance with the unified TNM criteria based on the results of a digital rectal examination, transrectal ultrasound, computed tomography, MRI, and bone scan.[25] Extent of disease on bone scan score was determined according to a previous report.[26] Prostate-specific antigen (PSA) doubling time was calculated as described previously.[27] Progression was defined according to consecutive PSA increments resulting in 25% increases and 2 ng/mL over the nadir despite consecutive ADT, or progression of soft-tissue lesions or the appearance of two lesions on a bone scan.[28]

2.10 | Statistical analysis

All statistical analyses were undertaken using JMP13 software (SAS Institute, Cary, NC, USA). All data in experiments using cell lines

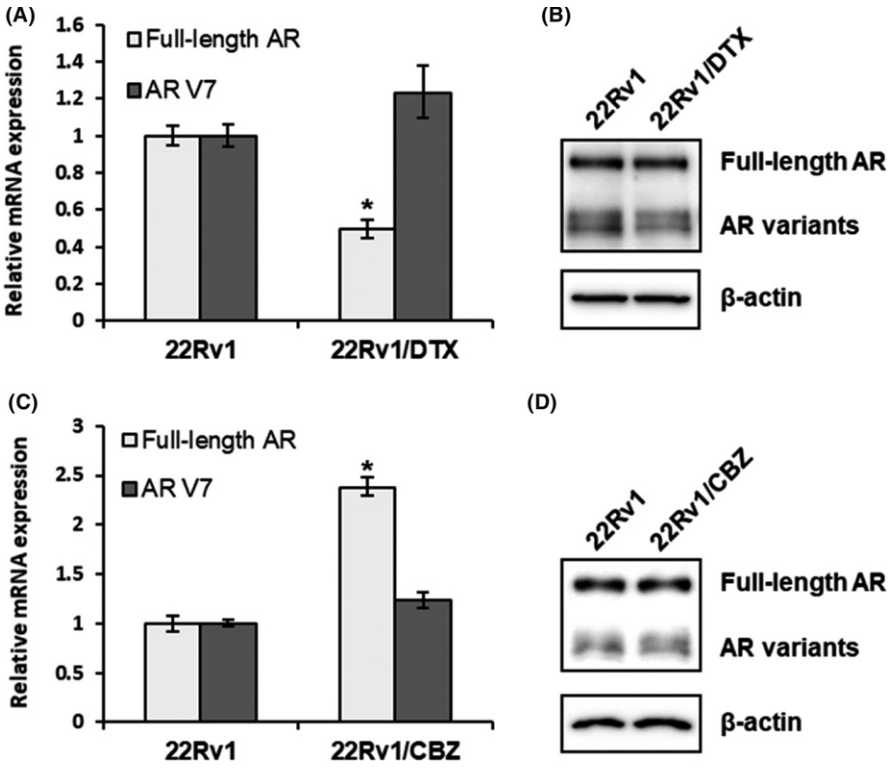

FIGURE 2 Expression level of full-length androgen receptor (AR) and AR variants in taxane-resistant cells. A,C, After extraction of total RNA from 22Rv1, 22Rv1/DTX, and 22Rv1/CBZ cells and synthesis of cDNA, quantitative real-time PCR was carried out for full-length *AR*, *AR V7*, and *GAPDH*. Each target transcript level was corrected relative to the corresponding *GAPDH* transcript level. The level of each target transcript in 22Rv1 cells was defined as 1. Boxes, mean; bars, ±SD. **P* < .05 (compared with 22Rv1 cells). B,D, Whole-cell extracts from 22Rv1, 22Rv1/DTX, and 22Rv1/CBZ cells were subjected to SDS-PAGE, followed by western blotting for the indicated proteins

were assessed using Student's *t* test. The Kruskal-Wallis test and Wilcoxon's exact test were used to analyze PSA response and survival between groups. The survival curve was determined by the Kaplan-Meier method. All *P*-values are two-sided. Levels of statistical significance were set at $P < .05$.

3 | RESULTS

3.1 | Resistance status between taxanes and enzalutamide, and expression level of full-length AR and AR variants in taxane-resistant cells

First, the resistance status between taxanes and enzalutamide was examined using enzalutamide-resistant and docetaxel-resistant 22Rv1 cells,[19,20] as well as cabazitaxel-resistant 22Rv1 cells established in this study (Figure 1A). As shown in Figure 1B, taxane-resistant cells showed no collateral resistance to enzalutamide. However, enzalutamide-resistant cells showed significant resistance to taxanes, including docetaxel and cabazitaxel (Figure 1C).

To explore the mechanism of no collateral resistance in taxane-resistant cells to enzalutamide, the expression of full-length AR and AR variants was investigated in parental and taxane-resistant cells. Although full-length *AR* expression at the mRNA level was downregulated in docetaxel-resistant cells, *AR V7* mRNA was comparable between parental and resistant cells (Figure 2A). However, at the protein level, the expression level of both full-length AR and AR variants were comparable between parental and docetaxel-resistant cells (Figure 2B). Conversely, although full-length *AR* expression at the mRNA level was upregulated in cabazitaxel-resistant cells, *AR V7* mRNA was comparable (Figure 2C). However, at the protein level, the expression level of both full-length AR and AR variants were comparable between parental and cabazitaxel-resistant cells (Figure 2D). In contrast, enzalutamide-resistant 22Rv1 cells expressed increased mRNA and protein levels of both full-length AR and AR variants, as shown previously.[19]

3.2 | Effect of AR signaling on cell proliferation and cellular resistance to taxanes and enzalutamide

To explore the role of full-length AR and AR variants in resistance to taxanes and enzalutamide, we examined cellular sensitivity to taxanes and enzalutamide when AR expression was downregulated using two kinds of AR-specific siRNAs (AR siRNA #1 and #2). As shown in Figure 3A, AR siRNA #1 targeting exon 4 successfully suppressed full-length AR, but not AR variants. Androgen receptor siRNA #2 targeting exon 2 suppressed both full-length AR and AR variants. Using AR siRNA #2, cell proliferation was significantly suppressed, but AR siRNA #1 did not show prominent suppression of

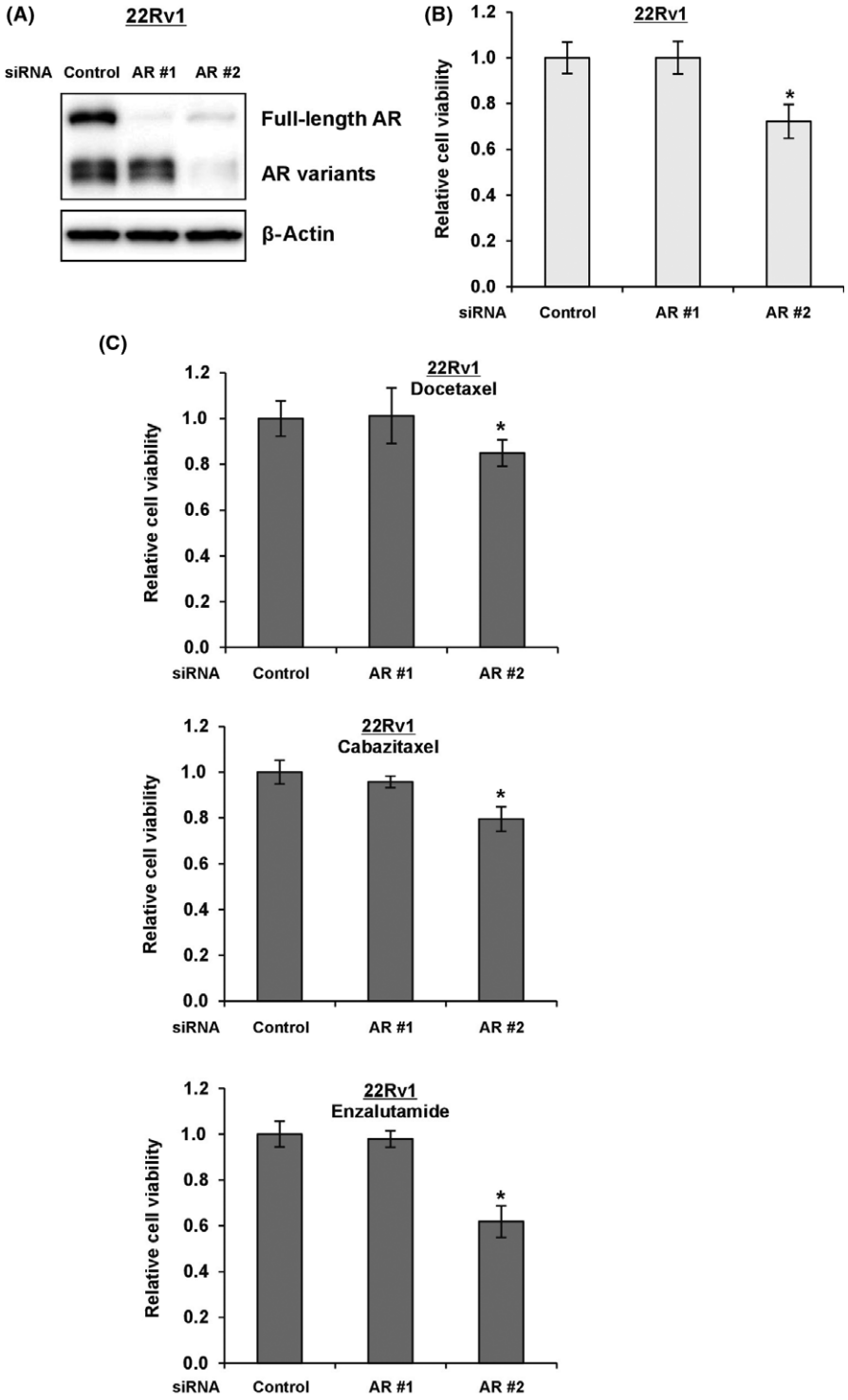

FIGURE 3 Effect of androgen receptor (AR) knockdown on cell proliferation and cellular resistance to taxanes and enzalutamide. A, 22Rv1 cells were transfected with 40 nmol/L of the indicated siRNA and incubated for 72 hours. Whole-cell extracts were subjected to SDS-PAGE, followed by western blotting for the indicated proteins. B, 22Rv1 cells were transfected with 40 nmol/L of the indicated siRNA. After 72 hours, cell numbers were counted. Cell counts when transfected with control siRNA were defined as 1. Boxes, mean; bars, ±SD. *$P < .05$ (compared with control siRNA). C, 22Rv1 cells were transfected with 40 nmol/L of the indicated siRNA and seeded in 96-well plates. On the following day, 10 nmol/L of docetaxel, 2.5 nmol/L of cabazitaxel, and 20 μmol/L of enzalutamide were applied. After 48 hours for docetaxel and cabazitaxel, or 72 hours for enzalutamide, the cell survival rates were analyzed by cytotoxicity analyses. Cell survival when transfected with control siRNA was defined as 1. Boxes, mean; bars, ±SD. *$P < .05$ (compared with control siRNA)

cell proliferation (Figure 3B). This might be due to the compensation of suppressed full-length AR by AR variants. Similarly, cellular sensitivities to taxanes (docetaxel and cabazitaxel) as well as enzalutamide were augmented by AR siRNA #2, although AR siRNA #1 failed to show a significant difference (Figure 3C). These results suggested that AR variants with or without full-length AR in 22Rv1 cells play critical roles in cell proliferation as well as in cellular sensitivity to taxanes and enzalutamide.

22Rv1 cells are reported to express AR variants such as AR V7.[19,21] Thus, we used siRNAs specific to AR V7.[29] As shown in Figure 4A, the expression of AR variants was suppressed by AR V7-specific siRNA. However, cell proliferation was not significantly suppressed by AR V7-specific siRNA, which could be due to the compensation of suppressed AR variants by full-length AR (Figure 4B). Similarly, cellular sensitivities to taxanes, including docetaxel and cabazitaxel, were comparable when AR V7-specific siRNA was

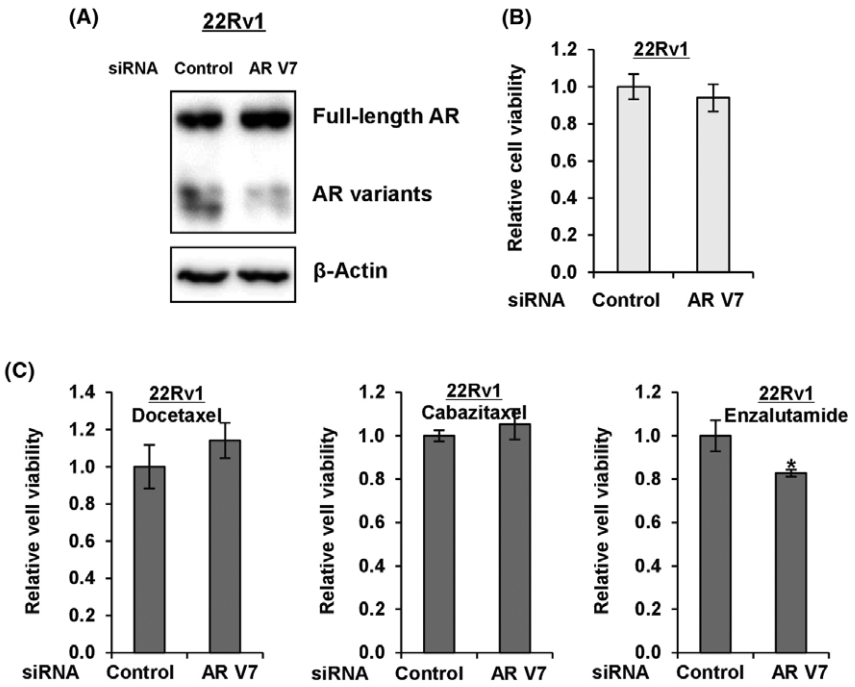

FIGURE 4 Effect of androgen receptor (AR) variant knockdown on cell proliferation and cellular resistance to taxanes and enzalutamide. A, 22Rv1 cells were transfected with 5 nmol/L each of the indicated siRNA and incubated for 72 hours. Whole-cell extracts were subjected to SDS-PAGE, followed by western blotting analyses for the indicated proteins. B, 22Rv1 cells were transfected with 5 nmol/L each of the indicated siRNA. After 72 hours, cell numbers were counted. Cell counts when transfected with control siRNA were defined as 1. Boxes, mean; bars, ±SD. C, 22Rv1 cells were transfected with 5 nmol/L each of the indicated siRNA and seeded in 96-well plates. On the following day, 10 nmol/L docetaxel, 2.5 nmol/L cabazitaxel, or 20 μmol/L enzalutamide was applied. After 48 hours for docetaxel and cabazitaxel, or 72 hours for enzalutamide, the cell survival rates were analyzed by cytotoxicity analyses. Cell survival when transfected with control siRNA was defined as 1. Boxes, mean; bars, ±SD. *$P < .05$ (compared with control siRNA)

used, although cellular sensitivity to enzalutamide was augmented by AR V7-specific siRNA (Figure 4C).

To confirmed the results shown in Figures 3 and 4, we used overexpression of AR in 22Rv1 cells, and knockdown of AR in enzalutamide-resistant 22Rv1 cells. As expected, stable transfectants with AR-GFP expressed abundant AR-GFP, compared with mock transfectants (Figure 5A). As a result, 22Rv1-AR-GFP cells were resistant to taxanes including docetaxel and cabazitaxel (Figure 5B). Similar to 22Rv1 cells, AR siRNA #1 successfully suppressed full-length AR, but not AR variants, whereas AR siRNA #2 suppressed both full-length AR and AR variants in enzalutamide-resistant cells (Figure 5C). Enzalutamide-resistant 22Rv1 cells transfected with AR siRNA #2, but not AR siRNA #1, were sensitive to taxanes including docetaxel and cabazitaxel (Figure 5D).

3.3 | Clinical outcome in docetaxel chemotherapy in cases with or without prior use of ARAT agents

Finally, to explore the clinical implications of these findings, we investigated whether the oncological outcome with docetaxel chemotherapy was affected by prior therapy with ARAT agents, including enzalutamide and abiraterone. Sixty-seven cases treated with docetaxel chemotherapy were identified. Among them, 10 cases were previously treated with ARAT agents before docetaxel chemotherapy (5, 1, and 4 cases treated with enzalutamide, abiraterone, and both enzalutamide and abiraterone before docetaxel, respectively); the remaining 57 cases were not. Patients' characteristics are presented in Table 1, which shows similar clinicopathological characteristics between cases with and without prior use of ARAT agents. Prostate-specific antigen response, defined as >50% PSA decline, was recognized in 2 (20.0%) and 16 cases (28.1%) with or without prior use of ARAT agents, respectively. As shown in Figure 6A, PSA declines were significantly lower in cases with prior use of ARAT agents compared with cases without prior use of ARAT agents ($P = .047$). Similarly, progression-free survival was significantly shorter among cases with prior use of ARAT agents compared with cases without prior use of ARAT agents ($P = .019$) (Figure 6B).

4 | DISCUSSION

This study showed collateral resistance to taxanes, including docetaxel and cabazitaxel, in enzalutamide-resistant cells derived from 22Rv1 cells expressing AR variants in addition to full-length AR. Previously, van Soest et al[15] reported collateral resistance in enzalutamide-resistant PC346C cells to taxanes, including docetaxel and

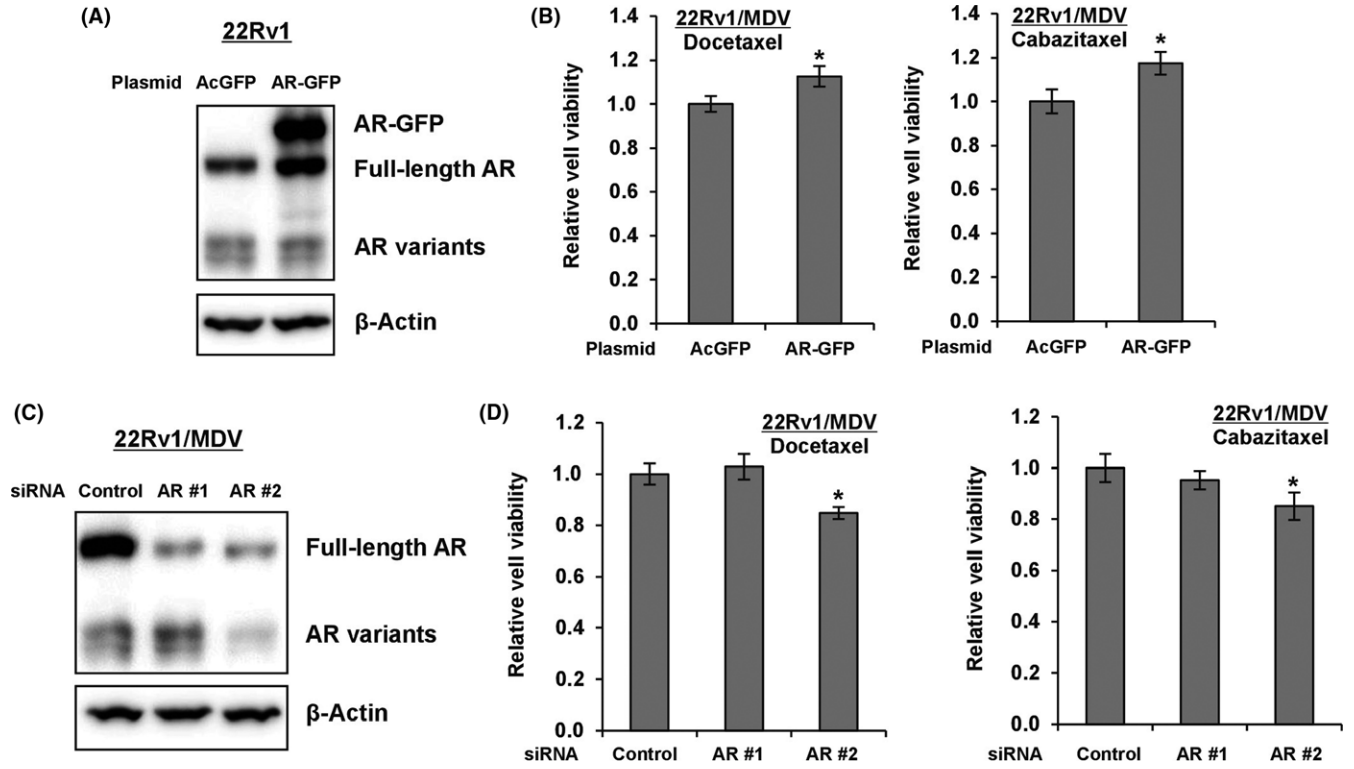

FIGURE 5 Effects of androgen receptor (AR) overexpression in 22Rv1 cells and of AR knockdown in enzalutamide-resistant 22Rv1 cells on cellular resistance to taxanes. A, Whole-cell extracts from stable transfectants of 22Rv1 cells with AcGFP and AR-GFP plasmids were subjected to SDS-PAGE, followed by western blotting for the indicated proteins. B, Stable transfectants of 22Rv1 cells with AcGFP and AR-GFP plasmids were seeded in 96-well plates. On the following day, 10 nmol/L docetaxel or 2.5 nmol/L cabazitaxel was applied. After 48 hours, the cell survival rates were analyzed by cytotoxicity analyses. Cell survival when transfected with AcGFP plasmid was defined as 1. Boxes, mean; bars, ±SD. *$P < .05$ (compared with AcGFP plasmid). C, 22Rv1/MDV cells were transfected with 40 nmol/L of the indicated siRNA and seeded in 96-well plates. On the following day, 10 nmol/L docetaxel or 2.5 nmol/L cabazitaxel was applied. After 48 hours, the cell survival rates were analyzed by cytotoxicity analyses. Cell survival when transfected with control siRNA was defined as 1. Boxes, mean; bars, ±SD. *$P < .05$ (compared with control siRNA)

cabazitaxel. In that study, there was no collateral resistance to cabazitaxel in an in vivo experiment, in which PC346C cells were shown to express full-length AR, but it remains unknown whether AR variants were expressed.[16] In addition, Al Nakouzi et al[17] showed no collateral resistance to cabazitaxel in LNCaP-derived enzalutamide-resistant cells established in an in vivo system. Thus, there were controversial results, and collateral resistance to cabazitaxel in enzalutamide-resistant cells could be dependent on the cell-line type or cellular context. However, there was no collateral resistance to enzalutamide in taxane-resistant cells, which is exactly compatible with the study by Al Nakouzi et al,[17] which showed no collateral resistance to enzalutamide in cabazitaxel-resistant 22Rv1 cells.

Androgen receptor variants are a critical factor affecting cellular sensitivity to ARAT agents, including enzalutamide.[18] Consistently, this study clearly showed that knockdown of AR variants, but not knockdown of full-length AR, successfully enhanced cellular sensitivity to enzalutamide. In addition, this study showed for the first time that suppression of both full-length AR and AR variants exerted augmented cellular sensitivity to taxanes, whereas the suppression of either full-length AR or AR variants showed no effect on cellular resistance to taxanes. Similarly, we have previously shown that knockdown of full-length AR rendered LNCaP cells that expressed only full-length mutated AR sensitive to docetaxel.[30] In addition, Komura et al has shown that androgen deprivation in androgen-dependent LAPC4 cells expressing WT AR increased docetaxel toxicity.[31,32] Conversely, it was reported that expression of AR variants including AR V7 and AR v567es rendered LNCaP cells resistant to taxanes.[33] Consistently, AR signaling was augmented in enzalutamide-resistant cells in which full-length AR and AR variant expression was increased.[19,34] In contrast, Al Nakouzi et al[17] showed that AR knockdown did not influence cellular sensitivity to cabazitaxel in LNCaP-derived castration- or enzalutamide-resistant cells; AR variants in LNCaP-derived cells might bypass taxane toxicity because the AR-specific siRNA targeting ligand-binding domain was used. Thus, these results suggest that AR signaling plays an important role in promoting taxane resistance, and comprehensive shut-down of AR signaling augments taxane toxicity. Partial persistence of active AR signaling by either full-length AR or AR variants support prostate cancer cells to escape from cytotoxicity by taxanes through various mechanisms.[35] However, there is a limitation that only cells derived from a single cell line were utilized in this study.

TABLE 1 Characteristics of patients with castration-resistant prostate cancer according to prior use of androgen receptor axis-targeted (ARAT) agents

Variable	Prior use of ARAT agents		*P*-value
	Presence (n = 10)	Absence (n = 57)	
Median age at docetaxel, years (IQR)	74 (72-77)	72 (65-76)	.13
Performance status, n (%)			
0	9 (90.0)	52 (91.2)	
≥1	1 (10.0)	5 (8.8)	.90
Pain, n (%)			
Absence	6 (60.0)	25 (43.9)	
Presence	4 (40.0)	32 (56.1)	.35
Median hemoglobin at docetaxel, g/dL (IQR)	12.2 (11.7-12.5)	12.0 (11.2-12.9)	.96
Median ALP at docetaxel, IU/L (IQR)	187 (154-639)	355 (243-1126)	.076
Median PSA at docetaxel, ng/mL (IQR)	33.7 (11.3-193.7)	60.4 (21.1-155.4)	.46
Median PSA doubling time at docetaxel, months (IQR)	2.0 (1.3-4.5)	1.6 (1.0-2.9)	.26
Biopsy Gleason score, n (%)			
≤8	4 (40.0)	16 (31.2)	
>8	6 (60.0)	35 (68.6)	.60
NA	0	6	
Metastatic sites at docetaxel, n (%)			
Bone	8 (80.0)	52 (91.2)	
Lung	2 (20.0)	5 (8.8)	
Liver	0 (0.0)	4 (7.0)	.39
EOD score, n (%)			
1	3 (37.5)	15 (29.4)	
2	2 (25.0)	14 (27.5)	
3	3 (37.5)	14 (27.5)	
4	0 (0.0)	8 (15.7)	.65
NA	0	1	

ALP, alkaline phosphatase; EOD, extent of disease on bone scan; IQR, interquartile range; NA, not available; PSA, prostate-specific antigen.

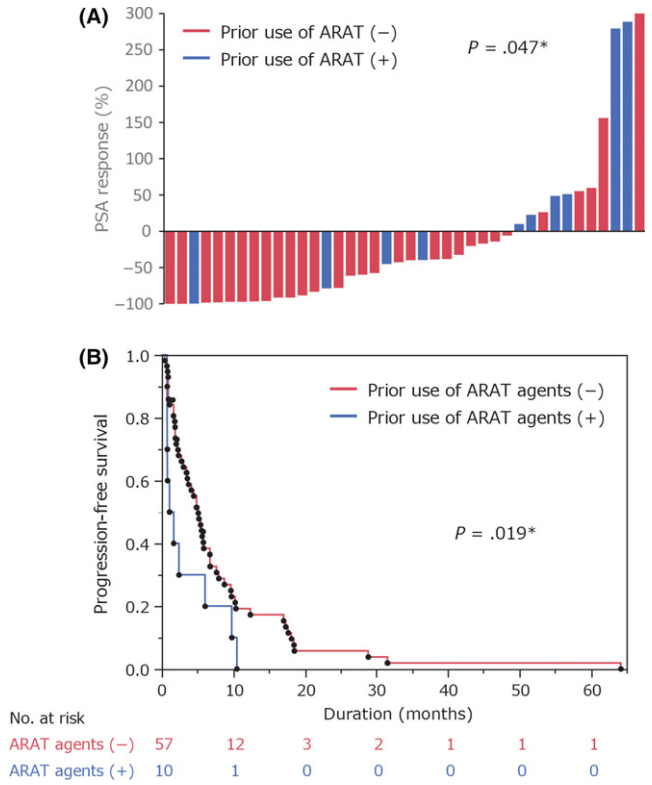

FIGURE 6 Clinical outcome in docetaxel chemotherapy in cases with or without prior use of androgen receptor axis-targeted (ARAT) agents. A,B, Best prostate-specific antigen (PSA) response (A) and progression-free survival (B) in patients with metastatic castration-resistant prostate cancer treated with docetaxel chemotherapy according to prior use of ARAT agents

Several studies have suggested that taxanes exert a cytotoxic effect through impairment of AR signaling because taxane treatment decreases cellular AR activity by inhibiting total AR as well as AR variants.[36-38] However, taxanes at low concentrations have recently shown no impact on AR localization, indicating taxanes do not act through AR.[39] In addition, Al Nakouzi et al[17] have clearly shown that AR suppression is not a cause but a result of cell death by taxanes. Thus, although there is controversy, the results showing that the suppression of AR signaling augments taxane toxicity support the hypothesis that AR inhibition by taxanes is not a cause of cytotoxicity by taxanes but a result of taxane cytotoxicity.

Aberrant AR signaling was suggested to promote taxane resistance. However, it has been shown that AR V7 expression in circulating tumor cells was not a predictive factor in taxane chemotherapy,[40,41] contrary to that in ARAT agents.[42] This suggests that AR V7 causing incomplete suppression of AR signaling in ADT is not involved in the efficacy of taxane chemotherapy. However, both studies in taxane chemotherapy were regrettably carried out after treatment with enzalutamide and/or abiraterone in most patients.[40,41] In this setting, however, aberrant AR signaling promoting resistance to ARAT agents already exists, even in cases with no AR V7 expression, making AR V7 an unreliable parameter of aberrant AR signaling. Expression of AR V7 promoting cellular resistance to ARAT agents as well as taxanes was shown to increase after treatment with ARAT agents, but not after taxane chemotherapy,[43] suggesting that taxane chemotherapy is less effective after treatment using ARAT agents, but not vice versa.

Consistently, the clinical data suggested less sensitivity to docetaxel after ARAT agents, although this study might be biased by long recruitment periods and lead time. Several retrospective studies have shown an inferior response to taxane chemotherapies in cases previously treated with ARAT agents compared with cases without pre-ARAT agents, which is similar to this study's results.[44-46] Conversely, worse responses to ARAT agents after taxane

chemotherapies compared with those in prechemotherapy settings were reported.[8-11] However, those studies were distorted by lead-time bias to an initiation of later-line therapy or other potential biases. In addition, we should take into account that taxane chemotherapy usually requires corticosteroid coadministration, which is reported to be associated with a worse response to ARAT agents.[47,48] Interestingly, enzalutamide therapy after abiraterone with prednisone showed comparable anticancer effects between men with and without prior chemotherapy,[49] which supports the observation that the worse response to ARAT agents after chemotherapy might result from corticosteroid pretreatment. Recently, upfront use of docetaxel or abiraterone has been shown to prolong overall survival in men with metastatic hormone-naïve prostate cancer.[2,3,50,51] In these settings, the efficacy of subsequent therapeutics, such as ARAT agents after upfront docetaxel or taxane chemotherapies after upfront abiraterone, could determine whether there is a clinical resistance between taxanes and ARAT agents. Consistent with the finding in this study, the excellent efficacy of ARAT agents for CRPC after upfront docetaxel chemotherapy for hormone-naïve prostate cancer has recently been reported;[52,53] data on the clinical outcomes of taxane chemotherapies after upfront abiraterone are not yet available.

The findings in this study, that comprehensive AR blockade is required to exert augmented sensitivity to taxanes, raise several implications in the pharmacotherapy for prostate cancer. In castration-sensitive prostate cancer, which depends on almost normal AR signaling by full-length AR, ADT can effectively inhibit AR signaling initially. In fact, upfront docetaxel chemotherapy with ADT has been shown to bring an excellent survival benefit in the CHAARTED and STAMPEDE trials.[50,51] However, in CRPC sensitive to ARAT agents, combination therapy with ARAT agents might augment the antitumor activity of taxanes. Actually, phase 1b studies examining combination therapy using docetaxel and ARAT agents such as enzalutamide and abiraterone have recently shown excellent PSA responses.[54,55] In CRPC refractory to ARAT agents, a novel therapeutic method that blocks ligand-independent AR signaling, in combination with taxanes, would be required to improve the therapeutic outcome. In a preclinical study, a novel antiandrogen agent, EPI, which binds to the N-terminal domain of AR and inhibits AR variants in addition to full-length AR, showed prominent antitumor activity in combination with docetaxel for 22Rv1-xenograft tumors.[56]

Taken together, this study has shown that collateral resistance to taxanes was established after obtaining enzalutamide resistance. Thus, it has been suggested that prior use of taxanes before ARAT agents for hormone-naïve prostate cancer and CRPC could be more advantageous, although it should be examined in clinical trials in the future. Augmented aberrant AR signaling might also be involved in taxane resistance, notably suggesting that comprehensive inhibition of AR signaling might augment cellular sensitivity to taxanes. These findings support upfront use of taxanes for castration-sensitive prostate cancer before aberrant AR signaling emerges, as well as combination therapy of taxanes with ARAT agents or novel agents inhibiting aberrant AR signaling for CRPC.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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