Androgen receptor negatively regulates mitotic checkpoint signaling to induce docetaxel resistance in castration-resistant prostate cancer

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ORIGINAL ARTICLE

Androgen receptor negatively regulates mitotic checkpoint signaling to induce docetaxel resistance in castration-resistant prostate cancer

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Abstract

Background: Despite multiple treatment advances for castration-resistant prostate cancer (CRPC), there are currently no curative therapies and patients ultimately to succumb to the disease. Docetaxel (DTX) is the standard first-line chemotherapy for patients with metastatic CRPC; however, drug resistance is inevitable and often develops rapidly, leading to disease progression in nearly all patients. In contrast, when DTX is deployed with androgen deprivation therapy in castration-sensitive disease, more durable responses and improved outcomes are observed, suggesting that aberrant androgen receptor (AR) signaling accelerates DTX resistance in CRPC. In this study, we demonstrate that AR dysregulates the mitotic checkpoint, a critical pathway involved in the anticancer action of DTX.

Methods: Androgen-dependent and independent cell lines were used to evaluate the role of AR in DTX resistance. Impact of drug treatment on cell viability, survival, and cell-cycle distribution were determined by plate-based viability assay, clonogenic assay, and cell-cycle analysis by flow cytometry, respectively. Mitotic checkpoint kinase signal transduction and apoptosis activation was evaluated by Western blotting. Pathway gene expression analysis was evaluated by RT-PCR. A Bliss independence model was used to calculate synergy scores for drug combination studies.

Results: Activation of AR in hormone-sensitive cells induces a rescue phenotype by increasing cell viability and survival and attenuating G2/M arrest in response to DTX. Analysis of mitotic checkpoint signaling shows that AR negatively regulates spindle checkpoint signaling, resulting in premature mitotic progression and evasion of apoptosis. This phenotype is characteristic of mitotic slippage and is also observed in CRPC cell lines where we demonstrate involvement of AR splice variant AR-v7 in dysregulation of checkpoint signaling. Our findings suggest that DTX resistance is mediated through mechanisms that drive premature mitotic exit. Using pharmacologic inhibitors of anaphase-promoting complex/cyclosome and polo-like kinase 1, we show that blocking mitotic exit induces mitotic arrest, apoptosis, and synergistically inhibits cell survival in combination with DTX.

Conclusion: Our results suggest that targeting the mechanisms of dysregulated mitotic checkpoint signaling in AR-reactivated tumors has significant clinical potential to extend treatment benefit with DTX and improve outcomes in patients with lethal prostate cancer.

KEYWORDS
cell-cycle checkpoint, CRPC, mitotic slippage, taxane therapy

1 | INTRODUCTION

Virtually all prostate cancer-related deaths occur due to the development of castration resistance. Despite numerous treatment advances for castration-resistant prostate cancer (CRPC), patients relapse due to drug resistance, and ultimately succumb to the disease. Androgen receptor (AR) signaling is frequently a dominant driver in CRPC and remains a major therapeutic target for these patients. Second generation AR pathway inhibitors such as enzalutamide, apalutamide and abiraterone acetate demonstrate robust initial responses and increased survival benefit; however, resistance can occur rapidly, leading to therapy failure and disease progression. Resistance frequently occurs through alterations in AR that result in reactivation of oncogenic AR signaling (i.e., AR amplification, ligand binding domain mutations, and splice variant expression).[1]

Docetaxel (DTX), a taxane-based chemotherapy, is an important first-line systemic therapy that extends survival for men with advanced prostate cancer.[2–6] Unfortunately, up to 50% of CRPC patients do not respond to DTX, and those patients that initially respond will become refractory within 8 months.[7] Intriguingly, when DTX is deployed with androgen deprivation therapy (ADT) in castration-sensitive prostate cancer (CSPC), survival is improved significantly more than for CRPC.[8,9] The mechanisms that mediate this superior response to DTX in CSPC are currently unclear. Some studies suggest that aberrant AR alterations that are found in CRPC may be involved in resistance to DTX.[2,10]

The anticancer mechanism of action for DTX involves binding and disrupting microtubule dynamics, which activates the mitotic checkpoint, leading to protracted mitotic arrest and apoptosis. Critical to DTX's cell killing is inducing sustained mitotic arrest through activation of the mitotic checkpoint, eventually triggering apoptosis through mitotic catastrophe. The mitotic checkpoint is part of the G2/M checkpoint involving multiple levels of regulation to ensure correct chromosome alignment and segregation to maintain mitotic fidelity during mitosis. Importantly, defects at several nodes of the checkpoint pathway can lead to resistance to microtubule targeting agents such as DTX, frequently through aberrant G2/M transition, weakened spindle assembly checkpoint (SAC), or premature mitotic exit, a process known as mitotic slippage.[11–13] In this study, we sought to determine whether AR was involved in dysfunctional mitotic checkpoint signaling in response to DTX and whether AR dysregulated a specific mechanism that could be targeted to increase DTX sensitivity in CRPC.

2 | METHODS

2.1 | Cell lines and reagents

LNCaP, VCaP, 22Rv1 (American Type Culture Collection) and LNCaP-AR cells (a gift from Drs. Robert Reiter and Charles Sawyers) were maintained in RPMI supplemented with 10% fetal bovine serum (FBS). All experiments were conducted under regular FBS conditions unless specified in figure legend. In androgen deprivation conditions (ADT), cells were grown in phenol red-free RPMI media supplemented with charcoal-stripped serum for the duration of the experiment. Androgen (R1881) was supplemented at a concentration of 1 nM, unless specified in the figure. Cell line authentication was performed using short tandem repeat sequencing (GenePrint 10 kit; Promega). All cells were routinely tested for mycoplasma using a mycoplasma enzyme-based luciferase assay (MycoAlertTM PLUS Mycoplasma Detection Kit; Lonza). Low passage (<15) cultures were used for all experimental testing. Enzalutamide (MDV3100), apcin, barasertib (AZD112), volasertib (BI6727), and DTX were purchased from Selleck Chemicals. proTAME was purchased from R&D Systems. R1881 was purchased from Sigma-Aldrich.

2.2 | Viability assays

Viability was measured using the CellTiter-GLO luminescent assay according to the manufacturer's instructions (Promega). Briefly, cells were seeded into 96-well plates 24 h before drug treatment at a density to permit exponential growth throughout the length of the assay, and viability was measured 72 h after drug treatment. The luminescent signal was detected in 96-well plates using a Victor X1 Luminescence Plate Reader (Perkin Elmer). The IC_{50} values were calculated using Prism v5.02 from GraphPad Software.

2.3 | Clonogenic survival

Cells were seeded into six-well plates at a density to permit exponential growth throughout the length of the assay 24 h before drug treatment. Cells were treated every 72 h over the course of 14 days after which surviving colonies were stained with 0.1% crystal violet and quantified using ImageJ software.

2.4 | Western blot analysis

Immunoblotting was conducted as previously described with minor modifications.[14] Antibodies used in the study include cleaved PARP (#5625), cleaved Caspase-3 (#9664), phospho-AURKB (Thr232) (#2914), phospho-Histone H3(Ser10) (#53348), cyclin B (#12231) purchased from Cell Signaling Technology; AR (AR-N20, Santa Cruz) and AR-v7 (AG10008, Precision); glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (sc47724, Santa Cruz) was used as protein loading control. Proteins were detected by infrared imaging using the Odyssey Imager and Odyssey Imaging software (LI-COR Biosciences).

2.5 | Cell-cycle analysis

Cells were seeded into 100 mm plates and harvested at the indicated time points. Cells were washed in 1X phosphate buffered saline, fixed with 70% ethanol and then stained with propidium iodine according to manufacturer's protocol (Abcam). Approximately 20,000 gated events were collected, and cell-cycle distributions were analyzed by BDFACS Diva software (BD Biosciences). Each flow cytometry analysis was performed three times.

2.6 | Quantitative real-time PCR

Total RNA (1 μg) was reverse-transcribed using High Capacity Complementary DNA (cDNA) Reverse Transcription Kit from Life Technologies. A total of 1 μl of cDNA was PCR amplified in a 20 μl reaction including TaqMan 2X Universal Master Mix and TaqMan gene expression probe/primer set for *TTK*, *BUB1R*, *AURKA*, *AURKB*, *KIF2B*, *KIF2C*, *CDKN1A*, *CDKN1B*, *PLK1*, *CENPF*, *CDC20*, *CDC27*, *ANAPC5*, *ANAPC16*, *UBE2C*, *CCNB1*, and *GAPDH* as an internal control for normalized gene expression. Samples were run in triplicate for a total of three separate experiments.

2.7 | Statistical analysis

Statistical significance was assessed by the Student *t* test (two-tailed distribution, two-sample, unequal variance) and considered statistically significant with *p* value <.01.

3 | RESULTS

3.1 | AR activation in CSPC cells and AR reactivation in CRPC cells induces DTX resistance

To determine the impact of AR activation on DTX response, we used the cell lines LNCaP and VCaP as cell line models of CSPC since both cell lines are dependent on androgen for growth and are responsive

to androgen withdrawal. We define in vitro ADT conditions as described in the Section 2.

We first evaluated the impact of AR activation on DTX sensitivity. Cell viability was measured in response to a dose range of DTX in cells under ADT conditions or cells in androgen-replete conditions where the media is supplemented with androgen (R1881) (Figure 1A). In both LNCaP and VCaP cells, there is more than a 2.5-fold increase in DTX sensitivity under ADT conditions compared to conditions of activated AR. We observe a similar effect on clonogenic survival where we demonstrate that 30% more cells survive cytotoxic levels of DTX when supplemented with androgen (Figure 1B). The predominant mechanism of cell killing by DTX is through apoptosis, and activation of apoptosis is essential for in vitro sensitivity. We then evaluated the effect of AR activation on apoptotic cell death in response to DTX by determining expression of the apoptotic markers, cleaved caspase-3 (cl-Casp3) and cleaved poly (ADP-ribose) polymerase (cl-PARP) (Figure 1C). Strikingly, the high-level apoptotic response to DTX under ADT conditions is significantly reduced in both LNCaP and VCaP cells lines when supplemented with androgen.

To evaluate the role of AR reactivation in DTX sensitivity, we employed the CRPC cell line models LNCaP-AR and 22Rv1, representing two models of hormone-refractory CRPC. 22Rv1 cells express the AR-v7 splice variant which confers constitutive AR activity in the absence of androgen; and LNCaP-AR cells overexpress AR, analogous to AR amplification.[15] Evaluation of viability in response to DTX showed decreased viability in all cell lines tested; however, we observed a fivefold decrease in sensitivity in the CRPC cell lines when comparing IC_{50} values (Figure 2A). Importantly, the doses required to reach the IC_{50} in the CRPC cells are not clinically achievable.[16,17] In addition, evaluation of clonogenic survival demonstrates significantly decreased DTX response in the CRPC cell line models (Figure 2B and Figure S1). Since we showed that AR activation blocked DTX-induced apoptosis in CSPC cells, we asked whether this was also the case in the AR-reactivated CRPC cells. Using a dose range of DTX, we observe that apoptosis is activated even at low doses of DTX in the CSPC cells; however, the CRPC cells showed minimal apoptosis activation at the highest doses of DTX (Figure 2C). Taken together, these results show that CRPC cells demonstrate reduced sensitivity and diminished apoptotic response to DTX, suggesting that oncogenic AR signaling promotes DTX resistance.

3.2 | AR-mediated DTX resistance is characterized by dysregulated checkpoint signaling

To elucidate the role of AR activation on G2/M signaling in response to DTX, we first performed cell-cycle analysis of DNA content from LNCaP cells under ADT or androgen-replete conditions. As expected, we show that DTX induces a robust G2/M arrest in androgen-depleted conditions (Figure 3A). Conversely, adding androgen reduces the DTX-induced G2/M arrest more than 2-fold, suggesting that AR activation dysregulates G2/M
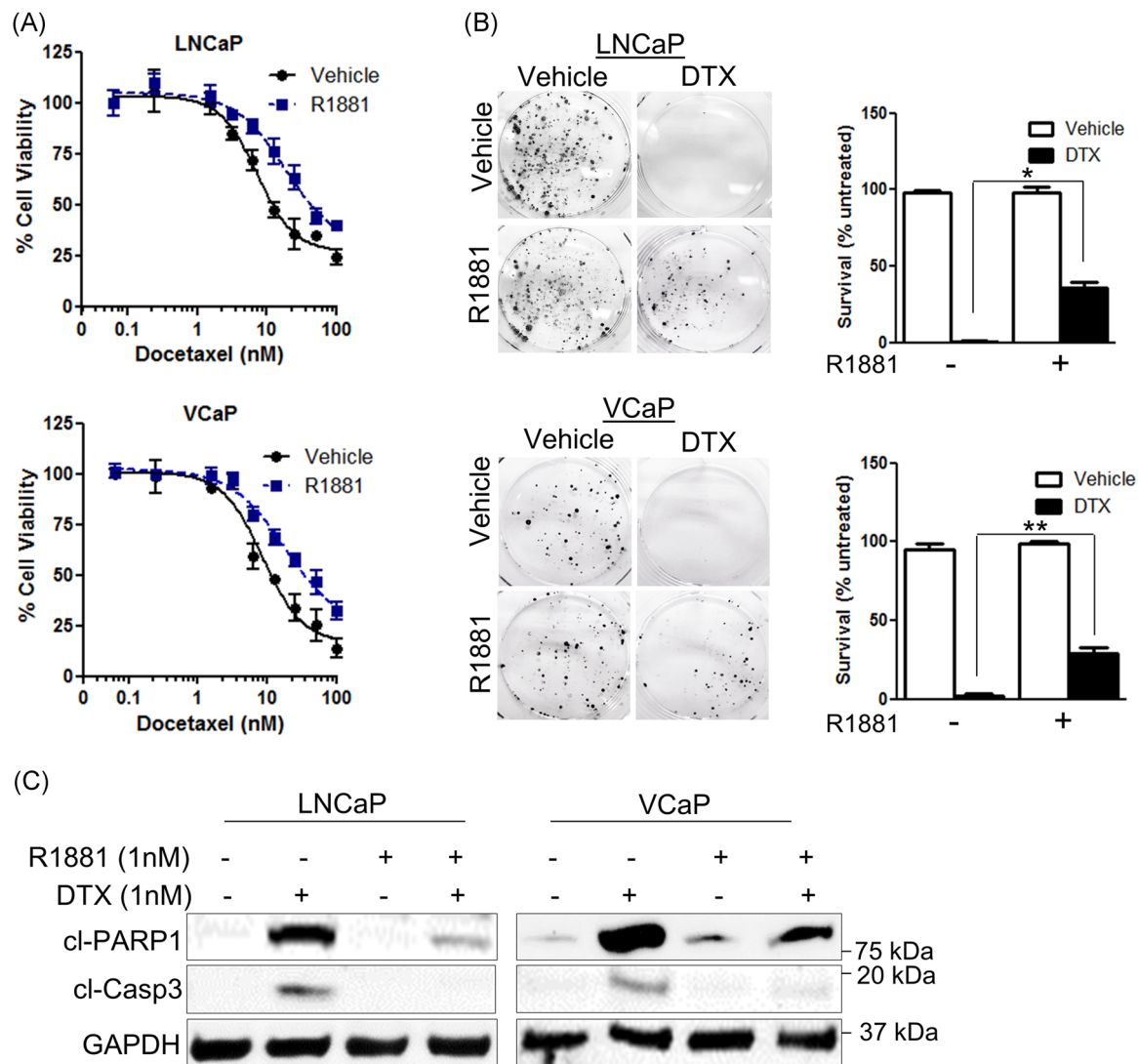
FIGURE 1 Androgen receptor activation induces DTX resistance. (A) Viability of castration-sensitive prostate cancer (CSPC) cell lines LNCaP and VCaP grown in media supplemented with charcoal-stripped serum (CSS) treated with 1 nM DTX with or without androgen (1 nM R1881). (B) Clonogenic survival of CSPC cells treated with DTX with or without androgen. (C) Apoptosis evaluated by Western blot. LNCaP and VCaP cells were treated with DTX with or without androgen for 18 h and lysates were probed with the indicated antibodies. *$p < .01$. DTX, docetaxel [Color figure can be viewed at wileyonlinelibrary.com]

checkpoint signaling and drives mitotic entry. To characterize the checkpoint response in CRPC cells, we performed cell-cycle analysis in 22Rv1 and LNCaP-AR cell lines, which revealed modest G2/M arrest in LNCaP-AR cells and no G2/M arrest in 22Rv1 cells upon treatment with DTX (Figure 3B). We compared the CRPC cells to LNCaP or VCaP cells under the same treatment conditions (regular, androgen-replete media), and observe that the CSPC cells induce G2/M arrest nearly twofold more than the CRPC cells in response to DTX. To further analyze AR-mediated checkpoint dysregulation, we evaluated the key signaling that regulates mitotic arrest; the SAC. As shown in LNCaP and VCaP cells under ADT conditions, DTX activates the SAC; indicated by phosphorylation of Aurora B kinase (AURKB; Thr232) and histone H3 (pH3; Ser10) (Figure 3C). In addition, we show that DTX

induces mitotic arrest (indicated by accumulation of cyclin B), consistent with the increase in G2/M population in response to DTX under ADT conditions. In contrast, when androgen is added SAC activity is diminished, and cells escape mitotic arrest.

To determine whether increased DTX resistance in the CRPC cells involves dysregulated SAC signaling, we evaluated SAC activation and mitotic arrest activity in 22Rv1 and LNCaP-AR cells compared to the CSPC cells (Figure 3D). When treated with DTX, the LNCaP-AR and 22Rv1 cells demonstrate very low AURKB activation, while cyclin B expression is unchanged in both CRPC cell lines in response to DTX indicating failed mitotic arrest in response to DTX, consistent with the G2/M cell-cycle data. In contrast, the CSPC cells, treated under the same conditions as the CRPC cells, show activated SAC and mitotic arrest.
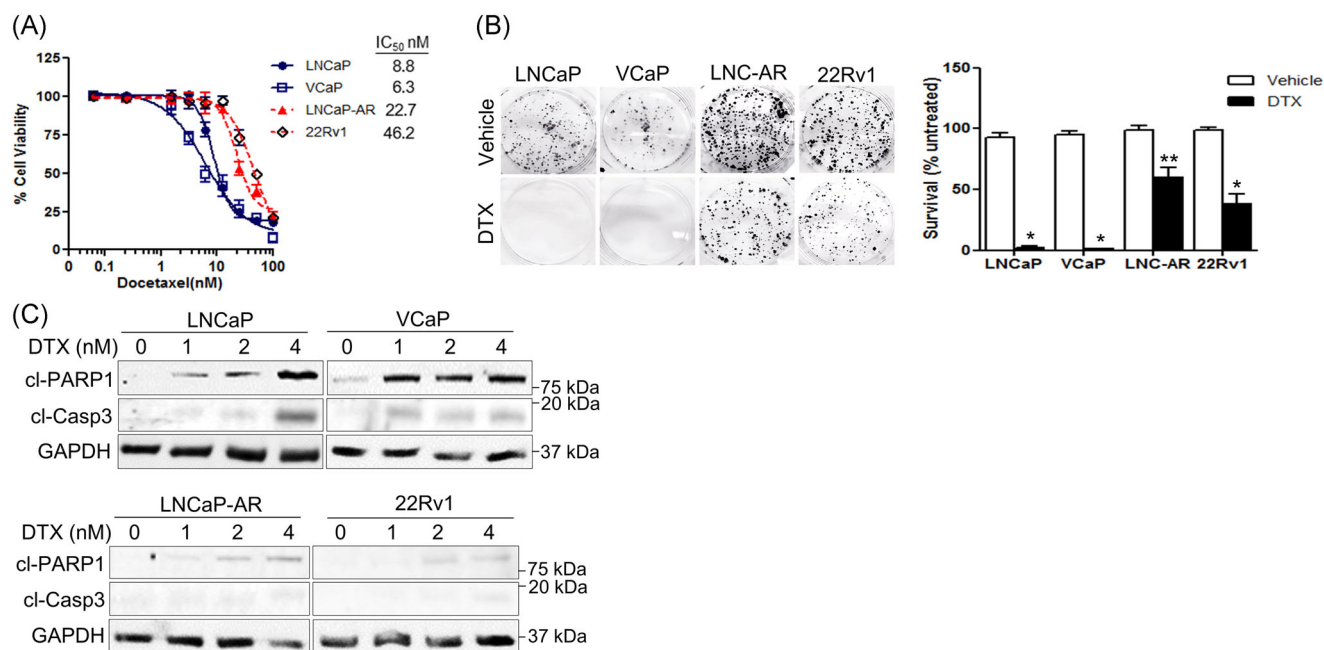
FIGURE 2 Divergent sensitivity to docetaxel in castration-resistant prostate cancer cells. (A) Viability in CSPC (LNCaP, VCaP) and CRPC (LNCaP-AR, 22Rv1) cell lines treated with DTX. The IC_{50} values were calculated in GraphPad. (B) Clonogenic survival of CSPC and CRPC cells treated with DTX. (C) Apoptosis evaluated by Western blot. Cells were treated with the indicated doses of DTX for 18 h and lysates were probed with the indicated antibodies. *$p < .01$ and *$p < .05$. CRPC, castration-resistant prostate cancer; CSPC, castration-sensitive prostate cancer; DTX, docetaxel [Color figure can be viewed at wileyonlinelibrary.com]

We next tested whether AR signaling was involved in failure to activate the checkpoint and induce mitotic arrest in the CRPC cells. We treated LNCaP-AR cells with DTX alone or with addition of the AR antagonist, enzalutamide (Figure 3E). Here we demonstrate significant mitotic checkpoint activation when enzalutamide is added, and this is associated with a robust apoptosis response. Collectively, these results indicate that AR dysregulates mitotic checkpoint signaling in response to DTX and suggest that AR activation induces mitotic slippage as a mechanism of DTX resistance.

3.3 | AR overrides the mitotic checkpoint through negative regulation of AURKB signaling

To further assess the role of AR signaling in disrupting mitotic checkpoint signaling, we evaluated expression of genes involved in critical phases of the mitotic checkpoint; G2/M transition, SAC, anaphase, and mitotic exit in CSPC and CRPC cell lines (Figure S2). When we analyzed gene expression in response to enzalutamide we found several AR-responsive checkpoint effectors, consistent with previous reports.[18–20] Strikingly, when examining the expression of genes in the different phases of checkpoint signaling, we observed that expression of several genes involved in SAC signaling are altered in response to AR inhibition (Figure 4A). Aurora B kinase (AURKB) is essential for SAC activation and preventing premature anaphase. AURKB is recruited to the site of kinetochore-microtubule (k-MT) attachment in response to microtubule stabilization where it activates

the k-MT signaling network. KIF2B and MCAK encode two kinesin-13 family kinases that make up the k-MT signaling network by sensing microtubule tension and stability.[21] To determine if these genes are responsive to AR activation, we measured mRNA transcript levels in LNCaP cells in response to increasing doses of androgen (Figure 4B). Here we observe decreasing expression of AURKB, KIF2B, and MCAK in response to increasing doses of androgen; suggesting AR negatively regulates SAC signaling. This data is consistent with the signal transduction data above, where AR activation attenuates DTX-induced SAC activation, suggesting that negative regulation of AURKB signaling is critical for overriding the mitotic checkpoint in response to DTX.

We next assessed whether these genes are AR-responsive in the CRPC cells. As shown, treatment with enzalutamide increases AURKB, KIF2B, and MCAK gene expression in both LNCaP-AR and 22Rv1 cell lines; however, response is reduced compared with LNCaP cells, likely reflecting reduced drug sensitivity in these cells (Figure 4C). Since full-length AR has been shown to mediate regulation of mitotic checkpoint processes, both in previous studies and the data reported here, we sought to determine whether expression of AR splice variants (ARVs) also influence the mitotic checkpoint. Importantly, ARVs have been shown to activate a distinct transcriptional program enriched for mitotic regulatory genes.[20] To determine whether checkpoint pathway effectors were influenced by expression of AR-v7 expressed in 22Rv1 cells, we evaluated gene expression in response to knockdown of AR-v7. Consistent with AR-mediated negative regulation observed in LNCaP cells, knockdown of
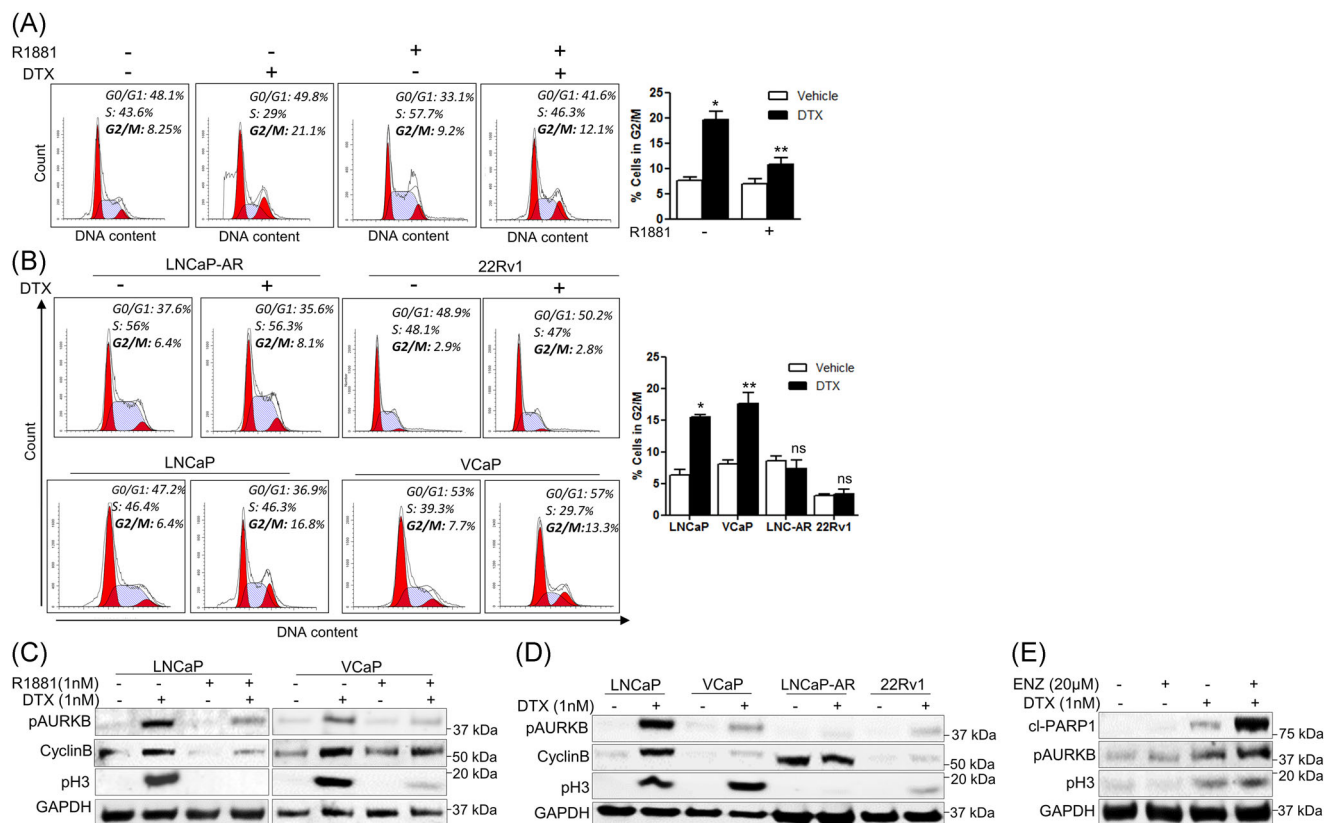
FIGURE 3 AR-mediated DTX resistance is characterized by attenuated mitotic arrest. (A) DNA content of LNCaP cells in CSS media treated with 1 nM DTX with or without androgen for 18 h, stained with propidium iodide (PI) and quantified by flow cytometry. Left, representative flow cytometry plots of DNA content. Right, bar chart of the percentage of change in the G2/M cell population after DTX treatment in cells deprived or supplemented with androgen. (B) DNA content of the indicated cell lines treated with 1 nM DTX for 18 h, stained with PI and quantified by flow cytometry. Left, representative flow cytometry plots of DNA content after DTX treatment for each pair of cell lines. Right, bar chart of the percentage of change in the G2/M cell population after DTX treatment. (C) Western blot analysis for markers of mitotic arrest in LNCaP and VCaP cells in CSS media treated with 1 nM DTX for 18 h with or without androgen. (D) Western blot analysis for markers of mitotic arrest in the CSPC and CRPC cell lines treated with DTX for 18 h. (E) Western blot analysis for markers of mitotic arrest and apoptosis in LNCaP-AR cells treated with DTX alone or in combination with 20 µM enzalutamide (ENZ) for 18 h. *$p < .01$. AR, androgen receptor; CRPC, castration-resistant prostate cancer; CSPC, castration-sensitive prostate cancer; CSS, charcoal-stripped serum; DTX, docetaxel [Color figure can be viewed at wileyonlinelibrary.com]

AR-v7 (siCE3) in the 22Rv1 cells results in increased *KIF2B* and *MCAK* expression, while *AURKB* expression was unchanged in AR-v7 silenced cells (Figure 4D). We next evaluated the expression of known AR-v7 target genes that are involved in mitotic checkpoint signaling including *AURKA*, *UBE2C*, and *CDC20*. As expected, expression was significantly decreased in response to AR-v7 knockdown (Figure 4E). Intriguingly, these genes are involved in control of anaphase progression and mitotic exit, suggesting AR-v7 may promote premature anaphase activation which has been implicated in resistance to DTX.

Since SAC inhibition leads to premature mitotic progression and AURKB signaling is critical for sustained SAC activation, we hypothesized that AR-mediated negative regulation of AURKB is a potential mechanism of DTX resistance. We evaluated the effect of blocking AURKB signaling on DTX sensitivity through pharmacologic inhibition of AURKB. In LNCaP cells, we measured viability in response to DTX with or without addition of AURKB inhibitor barasertib (AZD1152) and show that blocking AURKB signaling induces

resistance to DTX (Figure 4F). Investigation of checkpoint signaling and apoptosis showed that DTX in combination with AURKB inhibitor blocks checkpoint activation and rescues cells from apoptosis (Figure 4G). Collectively, these findings show that AR and AR-v7 negatively regulate mitotic checkpoint signaling and may promote premature anaphase progression, suggesting that mitotic exit can be targeted as a potential therapeutic strategy to overcome DTX resistance in CRPC.

3.4 | Blocking mitotic exit sensitizes CRPC cells to DTX

We demonstrate that aberrant AR activation overrides SAC signaling in response to DTX-induced mitotic arrest; a mechanism that has been shown to limit efficacy of microtubule-targeting agents.[22] Premature exit from mitotic arrest occurs through activation of
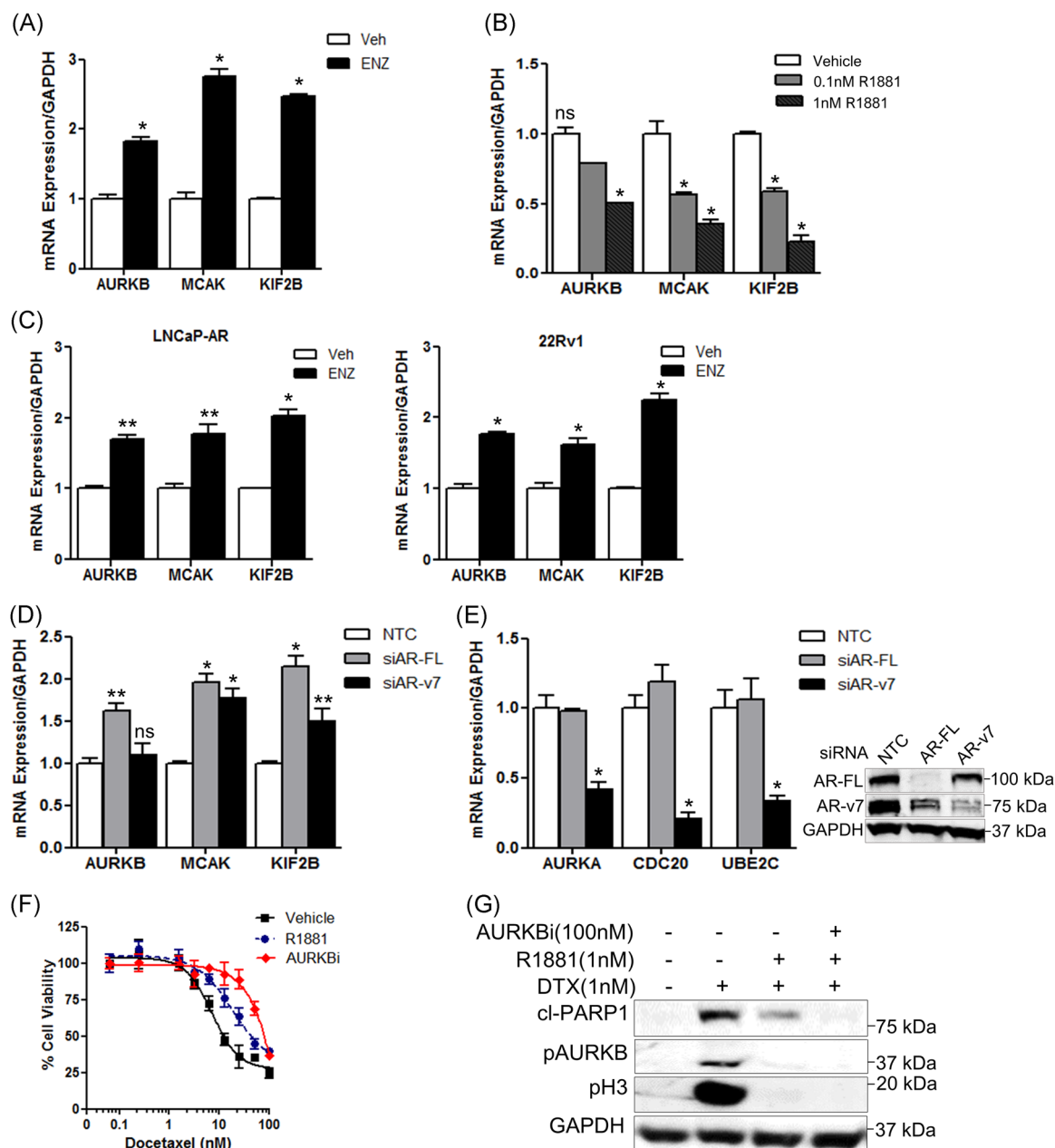
FIGURE 4 AR overrides the mitotic checkpoint through negative regulation of AURKB signaling. (A) mRNA expression of the indicated genes in LNCaP cells treated with 20 μM enzalutamide (ENZ). (B) mRNA expression in LNCaP cells in CSS media treated with the indicated doses of androgen. (C) mRNA expression of the indicated genes in LNCaP-AR and 22Rv1 cells treated with 20 μM ENZ. (D,E) mRNA expression in 22Rv1 cells transfected with the indicated siRNA. Right, Western blot analysis showing siRNA knockdown. (F) Viability of LNCaP cells in CSS media treated with DTX alone, with addition of androgen, or in combination with AURKB inhibitor (AZD1152) for 72 h. (G) Western blot analysis for markers of mitotic arrest and apoptosis in LNCaP cells in CSS media treated DTX alone, with addition of androgen, or in combination with AURKB inhibitor (AZD1152) for 18 h. *$p < .01$ and *$p < .05$. CSS, charcoal-stripped serum; DTX, docetaxel; mRNA, messenger RNA; siRNA, small interfering RNA [Color figure can be viewed at wileyonlinelibrary.com]

anaphase-promoting complex/cyclosome (APC/C), a ubiquitin ligase that targets specific mitotic substrates, such as cyclin B and securin, for degradation.[23] We, therefore, hypothesized that blocking APC/C activity would force mitotic arrest and potentiate the apoptotic effect of DTX. We tested two small molecule inhibitors of APC/C, apcin and proTAME, that inhibit APC/C activity through different mechanisms. Apcin competitively inhibits ubiquitination of APC/C co-activator

CDC20 substrates, preventing substrate recognition and binding.[24] Where as proTAME disrupts the interaction between APC and CDC20 resulting in CDC20 degradation and stabilization of cyclin B.[25]

We first evaluated the single-agent efficacy of these compounds in the CRPC cell lines LNCaP-AR and 22Rv1 (Figure S3). Apcin demonstrated minimal activity as a single agent in both cell

lines tested. Interestingly, LNCaP-AR cells were more sensitive to proTAME as single-agent; however, the drug had only moderate activity in both cell lines. To evaluate the effect of APC/C inhibition in cell lines that display mitotic slippage in response to DTX, CRPC cell lines LNCaP-AR and 22Rv1 were treated with three increasing doses of apcin or proTAME in combination with DTX. Evaluation of cell viability showed that only high doses of apcin (50 and 75 μM) increased DTX sensitivity (Figure 5A). To determine if any of the dose combinations synergistically decreased cell viability, we calculated the combination indexes (CI) from the drug dose combinations using the Bliss model of synergy where CI values <0.5 indicates synergism, 0.5–1.0 is additive; and >1.0 no synergism.[26] As shown in the CI heatmap for LNCaP-AR, most of the doses of apcin tested did not synergize with DTX. In 22Rv1 cells, the majority of low doses of apcin showed minimal effect on cell viability in combination with DTX, however, several high doses of apcin were effective and appeared to be moderately synergistic. Combination with proTAME proved to be more effective in sensitizing the CRPC cells to DTX, demonstrating a more than threefold increase in DTX sensitivity at the lowest dose of proTAME in both cell lines tested (Figure 5B).

Furthermore, the CI values showed significant synergy in a range of doses for both LNCaP-AR and 22Rv1. Since 5 μM of proTAME synergistically reduced cell viability with several low doses of DTX, we evaluated clonogenic survival of the drug combinations in CRPC cells (Figure 5C). Here, both cell lines show a significant reduction in clonogenic survival when treated with 5 μM of proTAME in combination with 1 nM DTX. Although targeting mitotic exit through inhibition of APC/C has significant clinical potential, neither apcin nor proTAME have been tested clinically. Based on our findings, we hypothesized that therapies that activated the spindle checkpoint in cells undergoing mitotic stress would be effective at overcoming resistance to anti-mitotic agents such as DTX. Polo-like kinase 1 (PLK1) regulates several cell-cycle processes including k-MT stabilization and activation of APC/C.[27,28] Furthermore, PLK1 is a druggable target with several inhibitors currently in clinical trials.[29] We evaluated volasertib, an ATP-competitive inhibitor of PLK1, currently tested in phase III clinical trials in AML. We first tested volasertib single-agent efficacy in both CRPC cell lines (Figure S4). Interestingly, both cell lines showed sensitivity at high doses of volasertib with LNCaP-AR cells exhibited more than fivefold greater sensitivity
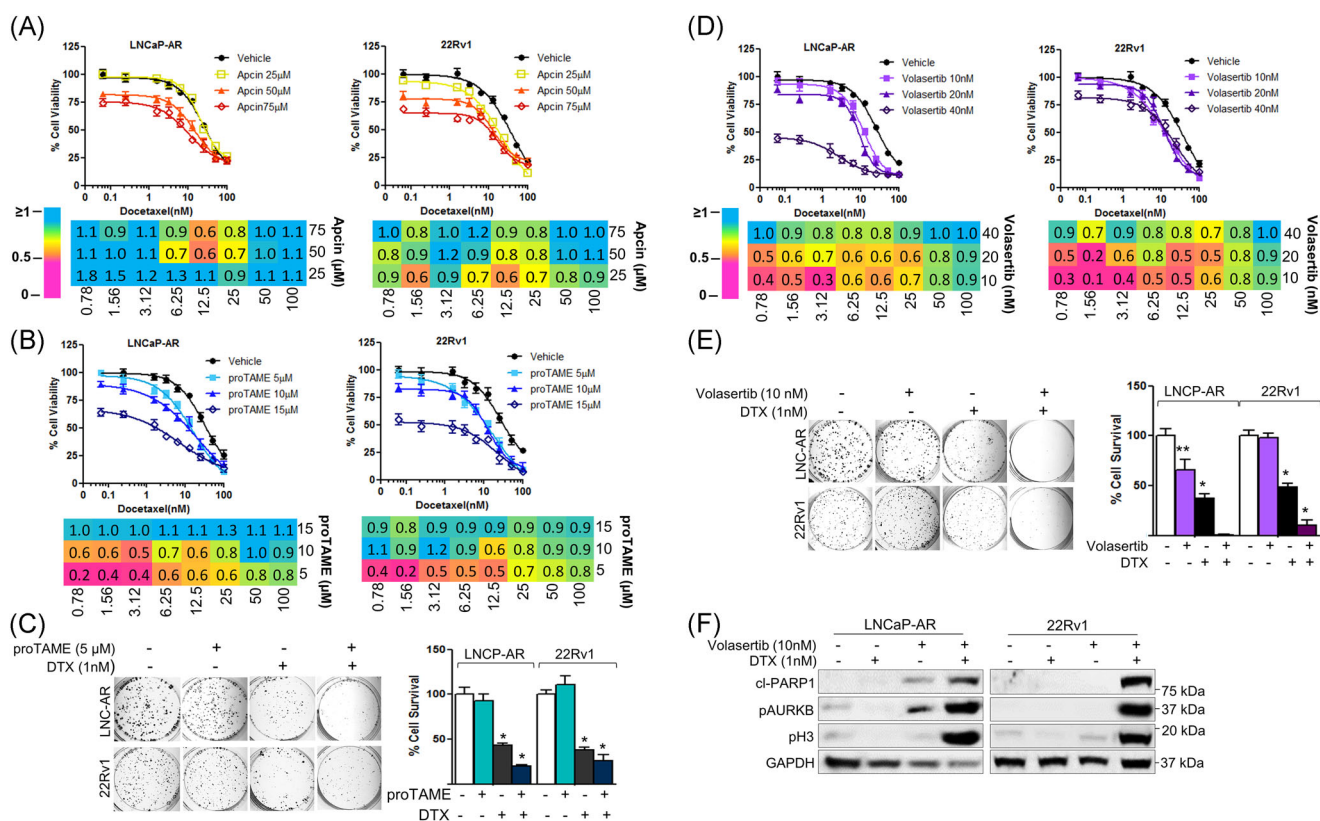

FIGURE 5 Blocking mitotic progression sensitizes CRPC cells to docetaxel. (A, B, D) Viability of LNCaP-AR and 22Rv1 cells treated with DTX alone or in combination with the indicated doses of apcin (A) or proTAME (B) or volasertib (D) for 72 h. Heatmaps show combination index (CI) for all dose combinations. Synergy score was calculated using the Bliss independence Model. CI < 0.5 indicates synergism, 0.5–1.0 is additive; and >1.0 no synergism. (C), Clonogenic survival of CRPC cells treated with DTX, proTAME, or the combination. (E) Clonogenic survival of CRPC cells treated with DTX, volasertib, or the combination. (F) Western blot analysis for markers of mitotic checkpoint activation and apoptosis in LNCaP-AR and 22Rv1 cells treated with DTX alone or in combination with volasertib for 18 h. *p < .01. CRPC, castration-resistant prostate cancer; DTX, docetaxel [Color figure can be viewed at wileyonlinelibrary.com]

compared to 22Rv1 cells. To evaluate the effect of PLK1 inhibition in combination with DTX, we tested three doses of volasertib that included IC_{25} and IC_{50} of both cell lines. Our results demonstrated that combination of volasertib and DTX caused stronger inhibition of cell viability than what was conferred by each drug alone in both CRPC cell lines tested (Figure 5D). Furthermore, calculation of the CI values showed strong synergy with DTX at low doses of volasertib (10 nM, 20 nM) in both cell lines. We then evaluated clonogenic survival using the lowest dose of volasertib (10 nM) in combination with DTX and demonstrate that the drug combination significantly decreases clonogenic survival in both LNCaP-AR and 22Rv1 cells (Figure 5E). To support our hypothesis that blocking mitotic exit would sensitize cells to DTX by forcing AURKB activation and mitotic arrest, we evaluated mitotic checkpoint signaling with the drug combination in LNCaP-AR and 22Rv1 cells. Indeed, addition of volasertib resulted in strong AURKB activation and mitotic arrest; and robustly induced apoptosis compared to DTX alone (Figure 5F). Taken together, these results suggest that DTX resistance in CRPC cells is mediated through mechanisms that override spindle checkpoint activation, suggesting that blocking mitotic exit is an attractive therapeutic target to overcome DTX resistance CRPC.

4 | DISCUSSION

Although DTX has been shown to provide a survival benefit in patients with metastatic CRPC, drug resistance remains a major clinical challenge. Knowledge of the mechanisms governing DTX resistance in CRPC is critical to develop novel therapeutic strategies to improve outcomes in these patients. Several proposed mechanisms of DTX resistance include aberrations in drug efflux pump activity, alterations in microtubule structure and function, activation of cell survival pathways, or defects in apoptosis signaling.[30] However, these mechanisms are ubiquitous among anti-mitotic drugs and do not address the potential role of biological drivers underlying resistance to taxane-based therapies.

The development of CRPC from castration-sensitive disease frequently involves acquisition of AR alterations including activating mutations, copy-number gains, amplification, and expression of ARVs that can maintain adequate AR activity to fuel tumor growth. In this study, we investigated the role of AR activation in DTX resistance to identify AR-dysregulated pathways that can be therapeutically targeted to overcome drug resistance. We showed that activating AR in CSPC cells decreased DTX sensitivity through mechanisms involving the G2/M checkpoint. Cell-cycle analysis revealed that AR activation attenuated the G2/M arrest in response to DTX, suggesting aberrant AR signaling is involved in dysregulating mitotic checkpoint processes. Further evaluation of checkpoint signaling showed that AR negatively regulates AURKB signaling, effectively overriding the checkpoint and inducing a phenotype of mitotic slippage. Importantly, we observed decreased checkpoint activation and mitotic arrest in response to DTX in AR-reactivated CRPC cells, supporting our hypothesis that aberrant AR signaling was involved in DTX resistance.

We mechanistically examined the interaction of AR on mitotic checkpoint signaling and identified a novel mechanism where AR disrupts SAC signaling in response to DTX by negatively regulating AURKB activation. AURKB is part of the SAC and functions in destabilizing microtubules to achieve correct bi-orientation of chromosomes during mitosis. Since the mechanism of action of DTX is through microtubule binding and subsequent SAC activation, it is likely that DTX resistance in AR reactivated tumors is driven by mitotic checkpoint override, resulting in premature mitotic exit and evasion of apoptosis. To therapeutically target mitotic exit, we tested two APC/C inhibitors, apcin and proTAME, and showed that only proTAME was synergistic with DTX in the CRPC cells. The contrasting response observed with two APC/C inhibitors likely reflects the different mechanisms of action of the two drugs, suggesting that blocking the interaction between APC/C and CDC20 with proTAME is more effective due to increased dependence on CDC20 for APC/C activation in the CRPC cells. However, since APC/C inhibitors have not been tested clinically, we searched for a target whose inhibition would block mitotic exit and is clinically actionable. PLK1 is a multi-functional kinase that has been shown to promote anaphase progression by increasing CDC20 binding and APC/C activation. Importantly, it is frequently overexpressed in many cancers including prostate and was recently shown to induce resistance to T-DM1, an anti-mitotic therapy in breast cancer, through mitotic slippage.[31] Combination of volasertib and DTX in the CRPC lines showed strong synergism with low, clinically achievable doses of DTX, demonstrating the potential to overcome DTX and eliminate dose-related toxicities.

Increased knowledge of the role of aberrant AR activation on DTX response has revealed a potential link between DTX mechanism of action and AR signaling. Several studies have shown that the microtubule-targeting activity of DTX disrupts AR translocation into the nucleus, effectively blocking AR signaling and may mediate the majority of DTX's efficacy.[32,33] In this context, the increased prevalence of mutated or splice variant of forms of AR found in CRPC may be a critical determinant of DTX response. This was demonstrated in a recent prospective study where detection of AR-v7 was associated with inferior PSA decrease in response to taxane treatment.[34] Another potential outcome from the inhibitory effect of taxanes on AR transactivation is that it provides a selective environment to overcome taxane-induced disruption of microtubule dynamics. Indeed, several recent studies have reported enrichment of dysregulated G2/M checkpoint and mitotic phase signaling in CRPC, suggesting that underlying molecular alterations that confer DTX resistance are acquired in CRPC.[20,35,36] Our findings show that signaling controlling microtubule dynamics and mitotic progression is dysregulated under AR-driven conditions and suggest that targeting this aberrant signaling is a potential therapeutic strategy to overcome DTX resistance in CRPC (Figure 6).
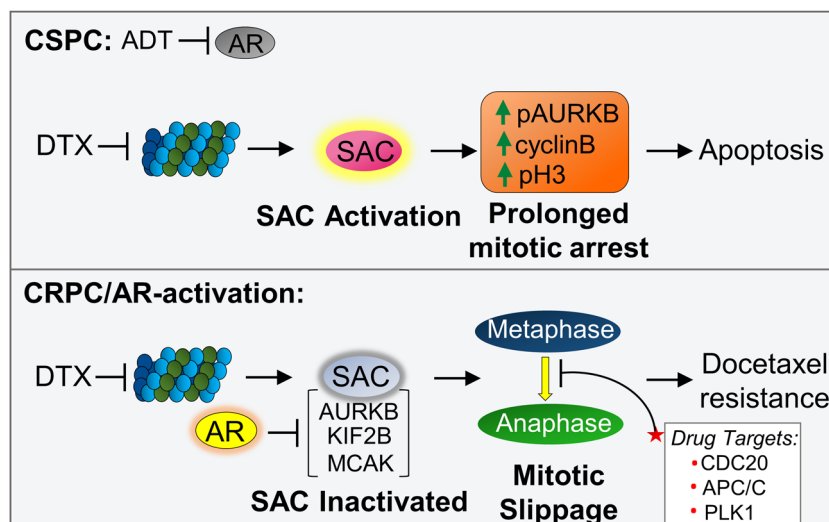
FIGURE 6 Schematic representation of the proposed model of AR-dysregulated mitotic checkpoint signaling and docetaxel resistance. Upper panel shows model of DTX cytotoxicity when AR is inhibited in CSPC. DTX binds microtubules and induces SAC activation, leading to prolonged mitotic arrest and apoptosis. Lower panel depicts DTX resistance when AR is activated or aberrantly reactivated, as is frequently observed in CRPC. Activated AR inhibits SAC activation, resulting in failed mitotic arrest, followed by premature mitotic progression (mitotic slippage) and DTX resistance. Targeting anaphase activators such as CDC20, APC/C or PLK1 is a potential therapeutic strategy to overcome DTX resistance in CRPC. APC/C, anaphase-promoting complex/cyclosome; AR, androgen receptor; CRPC, castration-resistant prostate cancer; CSPC, castration-sensitive prostate cancer; DTX, docetaxel; PLK1, polo-like kinase 1 [Color figure can be viewed at wileyonlinelibrary.com]

5 | CONCLUSIONS

In summary, we uncover a DTX resistance mechanism that is driven by AR signaling indicating a potential mechanism underlying reduced DTX benefit in CRPC compared to castration-sensitive disease. Our studies identify an AR-driven mechanism of DTX resistance that interrupts the anticancer action of DTX. Since DTX is an important therapeutic option for CRPC patients and the majority of CRPC cases are AR driven, our findings demonstrate a clinically relevant therapeutic strategy to target aberrant mitotic checkpoint signaling in combination with DTX. Further, identifying the specific checkpoint effectors aberrantly regulated by AR allows direct targeting of this signaling, as opposed to AR inhibition, thus circumventing inevitable AR antagonist resistance. DTX remains a major anticancer therapy for CRPC, thus identifying strategies to extend benefit and overcome drug resistance is of critical importance for improving CRPC outcomes.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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