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Galaxy Publication

Rictor-Dependent mTORC2 Signaling Drives Docetaxel Resistance in CRPC: Implications for Therapeutic Reversal

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ABSTRACT

The mammalian target of rapamycin (mTOR) is a major regulator of cell growth, proliferation, and the cell cycle. The primary component of mTORC2 is markedly upregulated in prostate cancer cells that have developed resistance to docetaxel. However, its specific molecular effects in prostate cells have not been fully defined. A docetaxel-resistant prostate cancer cell line (PC-3/DTX) was established to examine the function of mTORC2 in drug resistance. Lentiviral vectors were used to silence Rictor expression, and cell viability was evaluated with the Cell Counting Kit-8 (CCK-8). Flow cytometry was conducted to assess cell cycle distribution, while changes in related signaling pathways were analyzed through immunohistochemistry (IHC) and Western blot assays. PC-3/DTX cells with sh-RNA targeting Rictor showed the lowest IC50 for docetaxel. Reduced Rictor expression led to a greater proportion of cells arrested in the G0/G1 phase. When docetaxel treatment was repeated, IC50 values were lower in the AZD8055-treated group than in the Rapamycin group. Additionally, the AZD8055 group demonstrated a higher percentage of G0/G1-arrested PC-3/DTX cells compared with Rapamycin. IHC analysis of prostate cancer tissue from a CRPC patient showed elevated Rictor expression, while Raptor levels remained unchanged.This study examined the contribution of mTORC2 signaling to acquired docetaxel resistance in PC-3 cells to explore potential therapeutic strategies. mTORC2 activity is crucial for maintaining docetaxel resistance in PC-3 cells. The dual mTORC1/2 inhibitor AZD8055 more effectively disrupted mTORC2 kinase activity than the mTORC1-specific inhibitor Rapamycin, resulting in reduced resistance to docetaxel. Targeting this pathway may offer a promising approach to overcoming docetaxel resistance in patients with metastatic castration-resistant prostate cancer.

Keywords: Prostate cancer cells, Docetaxel, Drug resistance, MTORC2, Reversal

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Introduction

Globally, female breast cancer, lung cancer, prostate cancer, and nonmelanoma skin cancer remain the most frequently diagnosed cancers and are responsible for the highest mortality rates [1]. Among men in the United States, prostate cancer has shown the greatest annual increase in incidence [2]. It is one of the most common male malignancies worldwide, ranking second in global cancer incidence among men, and has become the fastest-growing cancer in the male population of China. Although prostate cancer occurs less frequently in China than in Western countries, its proportion of global prostate cancer–related deaths is significantly higher [2, 3]. The disease is also rising rapidly in Asia, where it was historically considered uncommon.

Androgen deprivation therapy (ADT) is the primary treatment for advanced prostate cancer. While most patients respond initially, many eventually progress to castration-resistant prostate cancer (CRPC) within several years. Limited therapeutic options have contributed to the continued increase in prostate cancer incidence in both China and the United States [4]. Metastatic CRPC (mCRPC) poses a significant clinical challenge and is the leading cause of prostate cancer–associated mortality [5]. Docetaxel has long been the standard first-line therapy for

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mCRPC, offering a survival advantage. However, nearly half of patients show minimal response, and even those who initially respond typically experience disease progression within one year [6, 7]. Alternative treatments—such as cabazitaxel and next-generation androgen receptor (AR) inhibitors like abiraterone and enzalutamide—provide benefit but are not curative. Moreover, because AR expression is highly heterogeneous in late-stage disease, AR-directed therapies may be ineffective in tumors with low AR expression [8]. AR amplification is detected in fewer than 30% of mCRPC cases [9], emphasizing the need for new therapeutic strategies, particularly for patients with weak or absent AR signaling.

The mammalian target of rapamycin (mTOR) is a key regulator of cell growth, proliferation, and cell cycle progression [10]. mTOR pathway hyperactivation is strongly associated with prostate cancer initiation, progression, and treatment resistance, making it a critical target for therapeutic intervention [11]. The PTEN/PI3K/AKT pathway upstream of mTOR plays a central role in driving prostate cancer development [12]. Loss or mutation of PTEN is the major cause of abnormal mTOR activation in prostate tumors [13]. PTEN loss occurs in approximately 40% of primary prostate cancers and up to 70% of metastatic cases, and this dysregulation is linked to tumor growth and poor patient outcomes [14, 15]. Notably, PTEN loss is present in about 80% of mCRPC tumors [16], underscoring its strong association with aggressive, metastatic disease. Preclinical studies have shown that mice lacking PTEN eventually progress to CRPC [8], and multiple findings confirm robust activation of the downstream Akt/mTOR axis in advanced and castration-resistant prostate cancer [15]. These observations highlight the importance of the mTOR pathway as a potential therapeutic target to better understand CRPC mechanisms and enhance the effectiveness of chemotherapy.

mTOR functions through two major complexes: mTORC1 and mTORC2. Raptor and Rictor serve as essential components of mTORC1 and mTORC2, respectively. Activation of mTORC1 promotes phosphorylation of downstream effectors such as p70S6 kinase (pS6K1) and 4E-binding protein 1 (4E-BP1), which regulate protein synthesis and cell proliferation [17]. In contrast, mTORC2 directly phosphorylates Akt, regulating cytoskeletal structure, cell survival, and cell movement.

Docetaxel works by stabilizing microtubules, preventing their depolymerization and inducing G2/M arrest, ultimately leading to apoptosis and mitotic catastrophe [18]. Nonetheless, several mechanisms have been proposed to explain docetaxel resistance in patients, including activation of compensatory survival pathways independent of AR signaling [19]. Preclinical studies have demonstrated that docetaxel increases phosphorylation of Akt at serine 473—an mTORC2-specific target—in prostate cancer cells [20]. Our previous findings also revealed heightened mTORC2 signaling activity in the docetaxel-resistant cell line PC-3/DTX, which shows elevated expression of Rictor, the defining subunit of mTORC2 [21].

These observations suggest that mTORC2 may play a crucial role in regulating acquired resistance to docetaxel in CRPC. The current study investigates the influence of mTORC2 signaling on docetaxel resistance in PC-3 cells and examines protein expression in tissue samples from patients with metastatic castration-resistant disease. Additionally, the study evaluates whether dual mTORC1/2 inhibitors can reverse chemoresistance, providing potential therapeutic opportunities for mCRPC patients who fail docetaxel therapy.

Cell line culture

The PC-3 prostate cancer cell line (CRL-1435) was obtained from ATCC (Manassas, VA, USA). Cells were maintained in F-12 medium (Gibco, UK) containing 10% fetal bovine serum (Gibco, Australia) along with 100 U/mL each of penicillin and streptomycin (Gibco, Germany). The docetaxel-resistant derivative, PC-3/DTX, was generated following the protocol described in our earlier work.21 Both PC-3 and PC-3/DTX cells were cultured under identical environmental conditions at 37°C in an atmosphere of 5% CO_2.

Chemicals and antibodies

Docetaxel was purchased from Sanofi. Rapamycin (S1039) and AZD8055 (S1555) were obtained from Selleck Chemicals. For Western blotting, antibodies against Rictor (2114), Raptor (2280T), and GAPDH (5174T) were acquired from Cell Signaling Technology. IHC antibodies targeting Rictor (70374) and Raptor (40768) were purchased from Abcam. The CCK-8 assay kit (C0037) and the apoptosis/cell-cycle detection kit (C1052) came from Beyotime (Suzhou, China).

Cell viability assay

Cell viability was quantified using the CCK-8 method. After seeding 2.0×10^3 cells per well into 96-well plates (three wells per condition), cells were allowed to attach for 24 h at 37°C. CCK-8 reagent (10 µL) was then added, and absorbance at 450 nm was measured after 1 h using a microplate reader (ELX808, Bioteck). Growth curves were generated by repeating the assay daily over a 5-day period. Resistance indices were determined by comparing IC_{50} values between experimental groups.

Western blotting

Rictor and Raptor expression levels were assessed via Western blot. Protein concentration was quantified using the Bio-Rad assay (Beyotime). A total of 10 µg protein per sample was separated on 10% SDS–PAGE gels. Electrophoresis was followed by transfer to 0.45-µm PVDF membranes (EMD Millipore). Membranes were blocked in 5% milk and incubated overnight at 4°C with primary antibodies (1:1000). After washing, membranes were incubated with HRP-linked goat anti-rabbit IgG (Beyotime; 1:1000). Protein bands were visualized using ECL detection reagents.

shRNA and lentiviral transduction

Rictor knockdown constructs and the control sh-NC plasmid were obtained from Obio Technology (Shanghai, China). Three shRNAs targeting Rictor (sh-1, sh-2, sh-3) were cloned into the pLKD-CMV-EGFP-2A-Puro backbone. Viral packaging and infection were completed according to the manufacturer's guidelines. Transduction efficiency was evaluated 72 h later with fluorescence microscopy (Nikon ECLIPSE TS100). Stable clones expressing sh-Rictor were selected using puromycin (initially 6 µg/mL, then reduced to 2 µg/mL), and maintained for subsequent experiments.

Immunohistochemistry

Paraffin-embedded samples from primary prostate cancer and tissues collected before development of CRPC were processed for IHC analysis. Rictor and Raptor were detected using Abcam antibodies at dilutions of 1:500 and 1:100, respectively. After deparaffinization, rehydration, and quenching of endogenous peroxidase, sections were incubated with primary antibodies overnight at 4°C. Afterward, the ABC kit (Vector Laboratories) was applied, and staining was visualized with DAB and counterstained with hematoxylin. Positively stained cells were counted in three randomly selected high-power fields. Negative controls omitted the primary antibody.

Cell-cycle analysis

Cell-cycle profiles were determined using the Beyotime C1052 kit. Cells were collected, washed, and fixed in 70% ethanol overnight. After washing and resuspension in binding buffer (2×10^5 cells/mL), cells were stained with PI and RNase A for 30 min at room temperature. Samples were analyzed using a BD FACSCalibur flow cytometer, and DNA content was quantified with ModFit software.

Statistical evaluation

SPSS version 19 was used to perform all analyses. Quantitative results are reported as mean ± SD. Experiments were performed at least three times. Comparisons between groups were carried out using one-way ANOVA followed by Fisher's test. Statistical significance was set at $p < 0.05$.

Results and Discussion

Reduction of rictor expression limits growth of PC-3/DTX cells

In our earlier findings,21 PC-3/DTX cells showed markedly elevated Rictor levels compared with the parental PC-3 line, suggesting a possible link between Rictor and docetaxel resistance. Based on this observation, we examined whether Rictor contributes to cell growth in resistant cells. Three distinct shRNA constructs were introduced into PC-3/DTX cells to silence Rictor. Western blot analysis confirmed successful knockdown **(Figures 1a and 1b)**. CCK-8 assays showed that suppression of Rictor significantly reduced the proliferation of PC-3/DTX cells 72 h after transfection **(Figures 1c and 1d).** Cells treated with the negative control sh-NC did not show any measurable change in growth relative to untreated resistant cells.

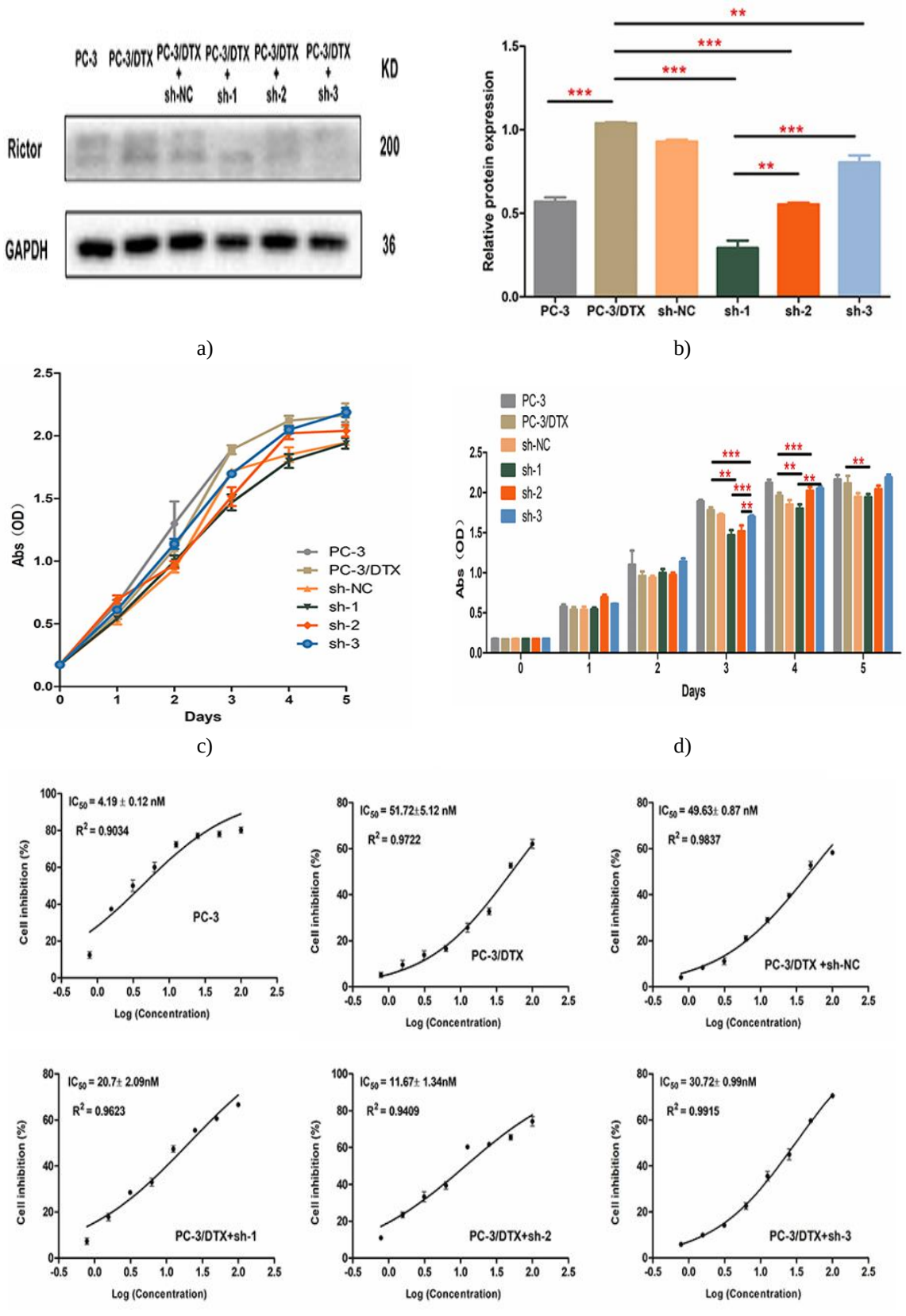


Figure 1. Rictor suppression via shRNA reduces the proliferation of PC-3/DTX cells.
(a) Western blot showing Rictor expression in cells transfected with different shRNA constructs or the control vector, with GAPDH used as a loading reference.
(b) Densitometric analysis of Rictor protein levels.

(c) Growth curves for the six experimental groups. Knockdown of Rictor led to reduced cell growth as measured by the CCK-8 assay. Data are presented as mean ± SD (n = 3).
(d) Statistical comparison of proliferation rates among the groups.
(e) IC_{50} values determined using CCK-8 following treatment with increasing concentrations of docetaxel in PC-3/DTX cells transfected with shRNAs or the mock control. Results represent mean ± SD of three independent experiments. **P < 0.01, ***P < 0.001 versus control.
Abbreviations: CCK-8, Cell Counting Kit-8; PC-3/DTX, docetaxel-resistant PC-3 cells; IC_{50}, 50% inhibitory concentration.

Cell proliferation results

Figures 1c and 1d show the growth trends of all six cell groups. No significant changes were observed during the first 48 hours. After this point, PC-3/DTX cells transfected with Rictor-targeting shRNAs exhibited the slowest proliferation compared with both parental PC-3 cells and untreated PC-3/DTX cells. Among the three shRNA constructs, sh-1 and sh-2 produced a more pronounced reduction in cell growth than sh-3. These observations indicate that silencing Rictor effectively suppresses the proliferation of PC-3/DTX cells.

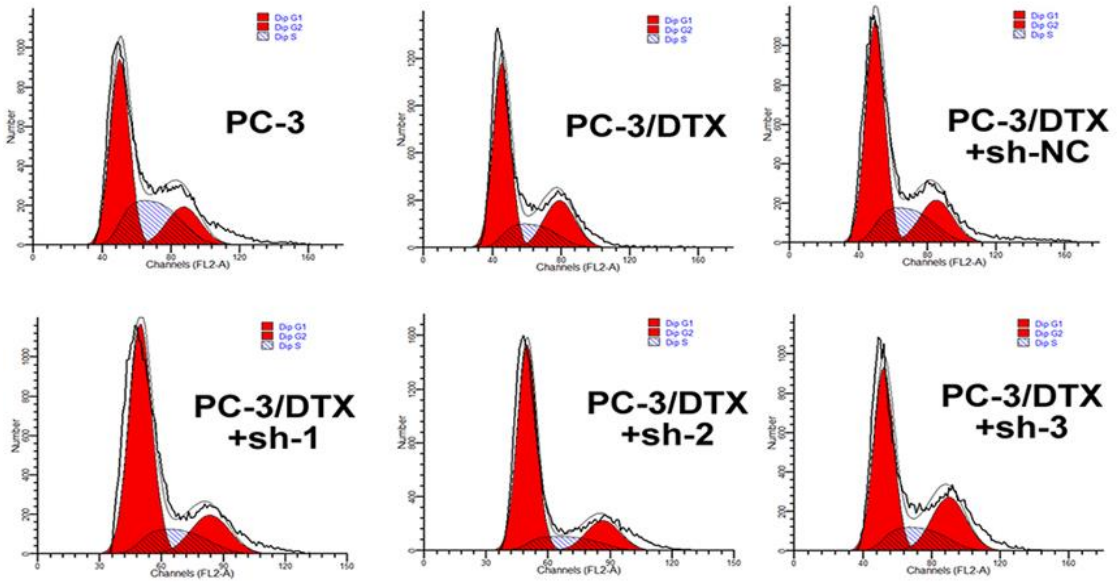
To further evaluate how Rictor influences docetaxel response, a CCK-8–based drug-sensitivity assay was performed **(Figure 1e)**. The IC_{50} values for docetaxel in PC-3, PC-3/DTX, PC-3/DTX + sh-NC, PC-3/DTX + sh-1, PC-3/DTX + sh-2, and PC-3/DTX + sh-3 were 4.19±0.12 nM, 51.72±5.12 nM, 49.63±0.87 nM, 20.7±2.09 nM, 11.67±1.34 nM, and 30.72±0.99 nM, respectively. All Rictor-silenced groups displayed markedly lower IC_{50} values than the sh-NC and untreated resistant cells. Notably, sh-1 and sh-2 produced the strongest reversal of resistance. These findings suggest that Rictor contributes to the development of docetaxel resistance, and its inhibition enhances the responsiveness of PC-3/DTX cells to docetaxel.

Cell-cycle effects of rictor knockdown

To explore how Rictor downregulation suppresses cell growth, flow cytometry was used to assess alterations in cell-cycle distribution. The percentages of PC-3, PC-3/DTX, sh-NC, sh-1, sh-2, and sh-3 groups in G0/G1 phase were 50.29±1.72%, 58.08±1.41%, 58.93±0.41%, 65.13±0.32%, 67.65±0.88%, and 58.82±0.66%, respectively. Corresponding S-phase proportions were 32.91±4.98%, 19.62±2.4%, 21.38±0.74%, 16.37±0.91%, 14.59±1.43%, and 18.34±1.25%.

As shown in **Figure 2**, sh-1 and sh-2 caused a clear reduction in the number of cells progressing through S phase, accompanied by a significant accumulation in G0/G1. The G2/M-phase proportions were 16.8±3.27%, 22.3±1.09%, 19.68±0.68%, 18.51±0.61%, 17.76±0.55%, and 22.85±0.91% for the respective groups.

Overall, stronger Rictor knockdown (sh-1 and sh-2) produced a more pronounced G0/G1 arrest than sh-3, with fewer cells entering G2/M. These results demonstrate that Rictor depletion hinders PC-3/DTX cell proliferation largely by blocking the cell cycle at the G0/G1 checkpoint.



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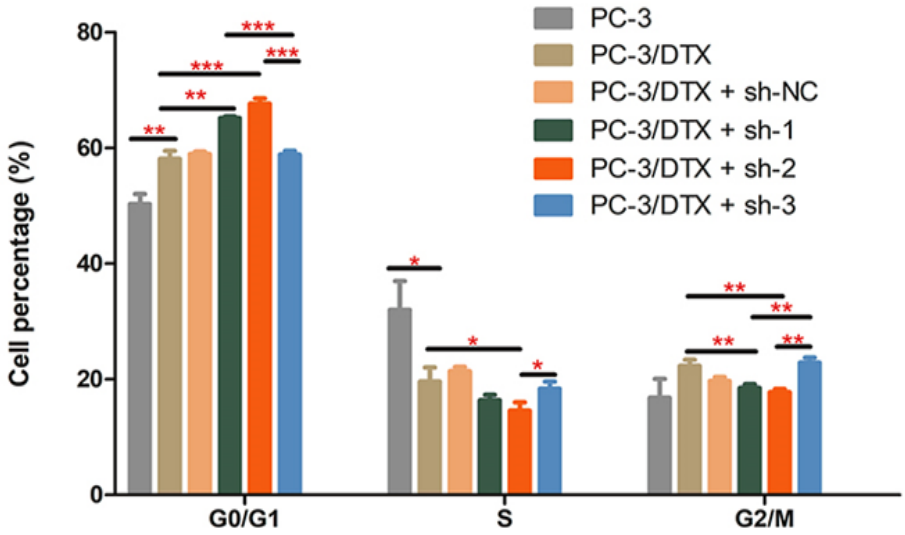

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Figure 2. Rictor knockdown induces cell-cycle arrest

(a) Flow cytometry analysis demonstrated that sh-Rictor transfected PC-3/DTX cells were predominantly arrested in the G0/G1 phase.

(b) Quantitative analysis of cell-cycle distribution. Data represent mean ± SD from three independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 versus control.

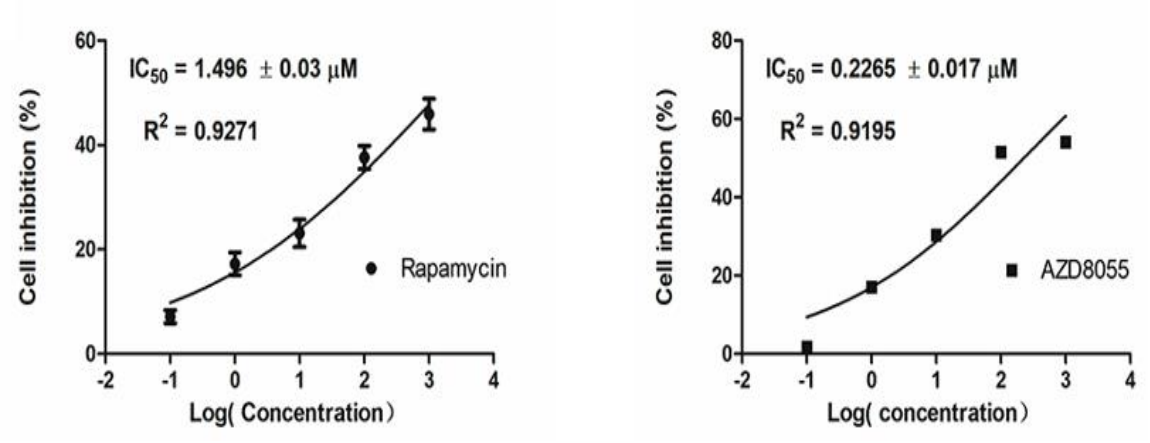
Effects of rapamycin and AZD8055 on docetaxel resistance and cell proliferation

Given the central role of mTOR signaling in regulating cell proliferation and survival, we examined the effects of the mTORC1 inhibitor Rapamycin and the dual mTORC1/2 inhibitor AZD8055 on docetaxel-resistant PC-3 cells. The IC_{50} values of PC-3/DTX cells were determined using the CCK-8 assay. Rapamycin exhibited an IC_{50} of 1.496 ± 0.030 μM, while AZD8055 showed a lower IC_{50} of 0.2265 ± 0.017 μM **(Figure 3a).**

PC-3/DTX cells were then exposed to each inhibitor at their respective IC_{50} concentrations for 48 hours, followed by a return to standard culture conditions. Both treatments reduced Raptor protein levels, but AZD8055 caused a more pronounced decrease in Rictor expression compared to Rapamycin and untreated controls **(Figure 3b).**

Next, we assessed docetaxel sensitivity after pre-treatment with the inhibitors. Cells co-cultured with various concentrations of docetaxel for 24 hours showed that AZD8055 pre-treatment led to a stronger inhibition of cell

proliferation than Rapamycin. These results suggest that dual inhibition of mTORC1/2 is more effective than mTORC1 inhibition alone in reversing docetaxel resistance in PC-3/DTX cells.



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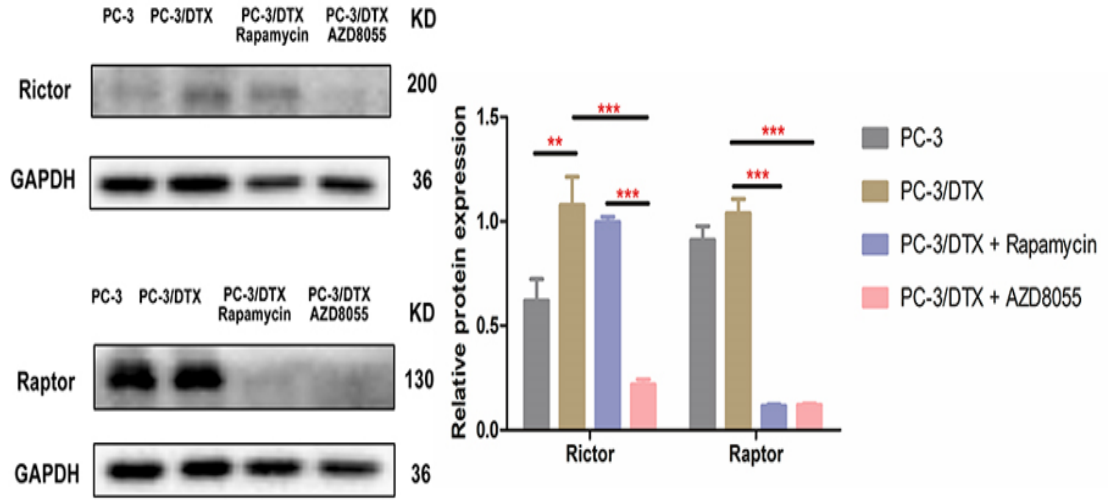

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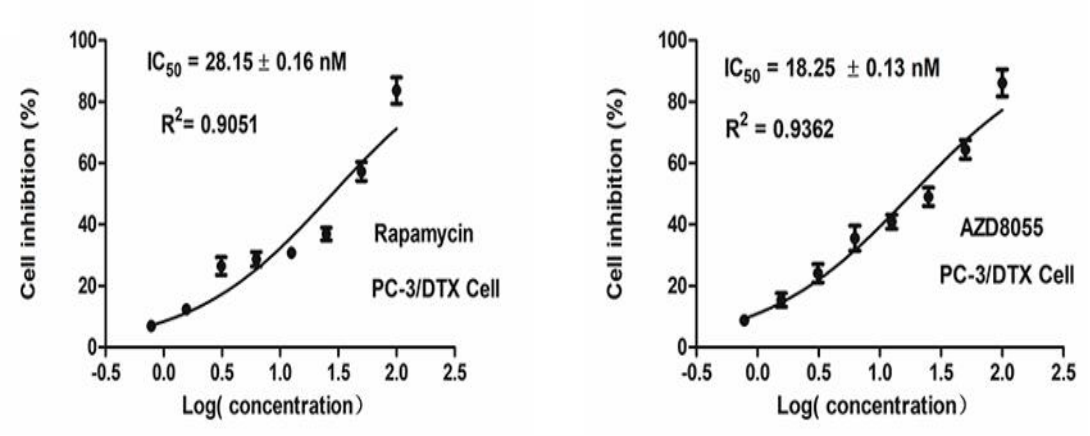


c)

Figure 3. Effects of rapamycin and AZD8055 on docetaxel-resistant PC-3 cell proliferation

(a) IC_{50} values were determined using the CCK-8 assay after treatment of PC-3/DTX cells with increasing concentrations of Rapamycin or AZD8055.

(b) Western blot analysis showing Rictor and Raptor protein levels in PC-3/DTX cells treated with each inhibitor at their IC_{50} for 48 hours.

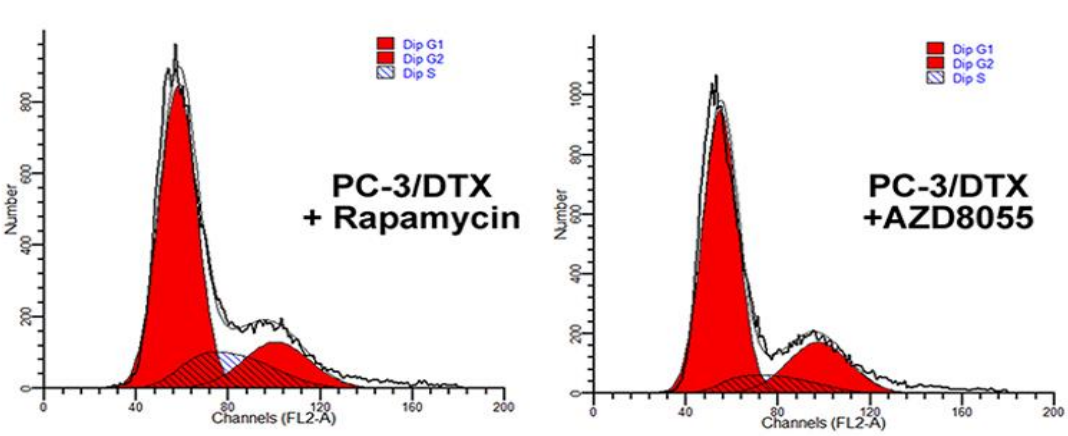
(c) After 48 hours of treatment with Rapamycin or AZD8055, cells were exposed to escalating doses of

docetaxel, and IC_{50} values were measured by CCK-8. Data represent mean ± SD from three independent experiments. **P < 0.01, ***P < 0.001 versus control.

Docetaxel sensitivity after mTOR inhibition

The IC_{50} of docetaxel in PC-3/DTX cells pre-treated with Rapamycin and AZD8055 was 28.15 ± 0.16 nM and 18.25 ± 0.13 nM, respectively. Both inhibitors enhanced docetaxel sensitivity compared with untreated cells, with AZD8055 showing a stronger effect **(Figure 3c)**.

Flow cytometry analysis revealed corresponding cell-cycle changes **(Figure 4)**. PC-3/DTX cells treated with Rapamycin or AZD8055 showed G0/G1 phase percentages of 65.72 ± 0.84% and 67.92 ± 0.70%, respectively, while the S-phase fractions decreased to 16.56 ± 1.17% and 9.93 ± 2.32%. Compared to untreated PC-3 and PC-3/DTX cells, both treatments significantly increased G0/G1 arrest, demonstrating effective blockage of cell-cycle progression into S phase. These results indicate that dual mTORC1/2 inhibition by AZD8055 more efficiently enhances docetaxel sensitivity and promotes G1-phase cell-cycle arrest than Rapamycin alone.



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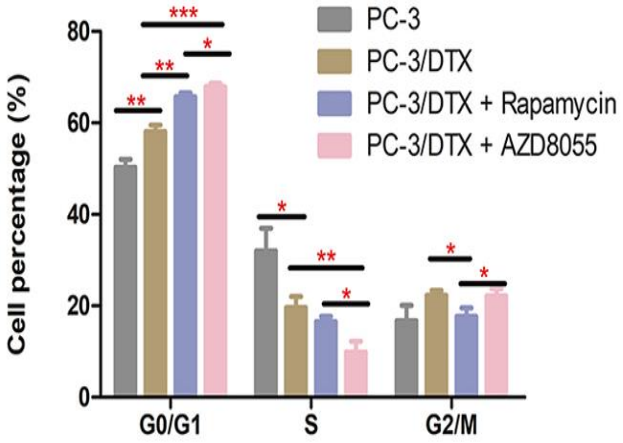


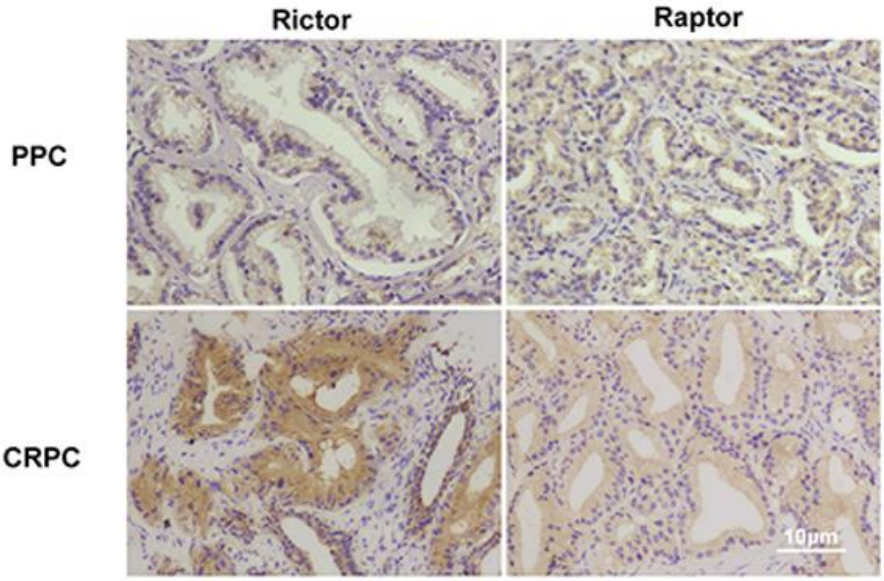
b)

Figure 4. effects of rapamycin and AZD8055 on cell-cycle progression in docetaxel-resistant cells
(a) Flow cytometry analysis demonstrated that treatment with Rapamycin or AZD8055 induced G0/G1 phase arrest in PC-3/DTX cells.
(b) Quantitative assessment of cell-cycle distribution. Data represent mean ± SD from three independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 versus control.

Elevated rictor expression correlates with docetaxel resistance in prostate cancer tissue

To explore the clinical relevance of mTORC2 in docetaxel resistance, we analyzed primary prostate cancer (PPC) specimens and pre-CRPC tissue samples using immunohistochemistry. Tumor regions displayed varying levels of Rictor and Raptor expression. Notably, CRPC tissues exhibited higher Rictor expression compared with PPC tissues, while Raptor levels remained largely unchanged **(Figure 5)**. These findings suggest that Rictor

upregulation may be associated with acquired docetaxel resistance. This supports the rationale for targeting mTORC2 signaling in preclinical CRPC models to investigate its role in drug-resistant phenotypes.



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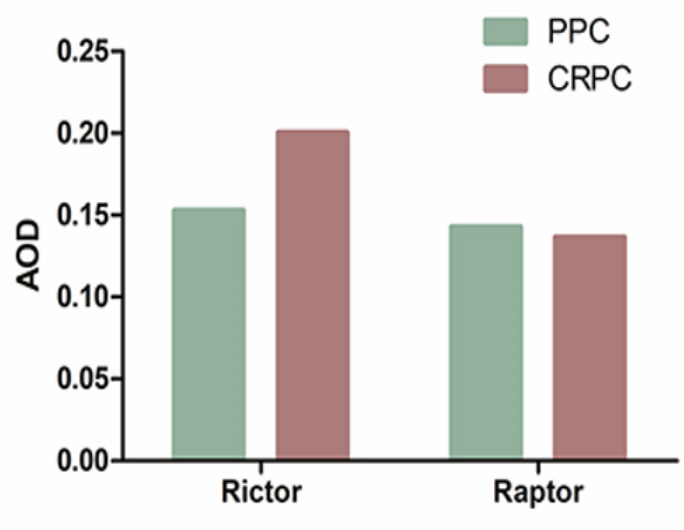

b)

Figure 5. Elevated rictor expression in docetaxel-resistant prostate cancer tissue
(a) Immunohistochemical staining showing levels of Rictor and Raptor in PPC and CRPC tissues (×200).
(b) Quantification of Rictor and Raptor expression in PPC and CRPC tissues using average optical density (AOD).
Abbreviations: PPC, primary prostate cancer; CRPC, castration-resistant prostate cancer; AOD, average optical density.

mTOR is a pivotal regulator of cell growth, metabolism, and tumor proliferation [22]. It exists in two structurally and functionally distinct complexes: mTORC1 and mTORC2. mTORC2, consisting of the scaffold protein Rictor and mSIN1, plays a key role in cell metabolism [23]. Notably, mTORC2 is critical for prostate cancer progression in the absence of PTEN, whereas normal prostate epithelial cells do not require it [12]. P-Akt, a downstream target of mTORC2, is elevated in prostate cancer cells that acquire resistance to docetaxel after prolonged exposure [20]. These observations suggest that mTORC2 specifically promotes tumor development without inducing hyperplasia in normal tissue and may contribute to docetaxel resistance in CRPC, a mechanism not fully investigated previously.

In earlier work, docetaxel-resistant CRPC models were established by chronically treating sensitive mouse cells with the drug, resulting in activation of mTORC2 downstream pathways. The present study aimed to evaluate the role of mTORC2 in acquired docetaxel resistance and assess dual mTORC1/2 inhibitors as a potential therapeutic approach.

To examine whether mTOR complexes mediate docetaxel resistance, PC-3/DTX cells were transfected with shRNA targeting Rictor to stably knock down mTORC2 [24]. Cells with Rictor knockdown displayed significantly slower growth compared to control PC-3 or PC-3/DTX cells. The decreased IC50 for docetaxel in these cells indicates that Rictor contributes to drug resistance and that its inhibition enhances docetaxel sensitivity. Rictor knockdown also induced G0/G1 cell-cycle arrest and apoptosis, which may restore docetaxel responsiveness in vitro and partially reverse chemoresistance.

Next, we investigated the therapeutic potential of mTOR inhibitors. PC-3/DTX cells were treated with Rapamycin (mTORC1 inhibitor) and AZD8055 (dual mTORC1/2 inhibitor) at their IC50 concentrations, followed by docetaxel exposure. Both treatments reduced the IC50 for docetaxel, with AZD8055 showing a more pronounced effect, confirming the involvement of mTOR in drug resistance. Flow cytometry revealed that both inhibitors caused G0/G1 phase arrest, with AZD8055 inducing a significantly higher percentage of G0/G1-phase cells compared to Rapamycin, highlighting the enhanced efficacy of dual mTORC1/2 inhibition.

Immunohistochemistry of CRPC patient tissues showed elevated Rictor expression without changes in Raptor levels, supporting in vitro findings that mTORC2 is associated with docetaxel resistance. These results provide a rationale for targeting mTORC2 in high-risk or metastatic prostate cancer, particularly in patients with PTEN loss and tumors with low or heterogeneous AR expression [25]. The study underscores the potential of dual mTORC1/2 inhibitors as an alternative strategy to overcome docetaxel resistance in mCRPC.

Conclusion

The study demonstrates that mTORC2 is essential for maintaining docetaxel resistance in PC-3 cells. Dual mTORC1/2 inhibitors are more effective than Rapamycin in overcoming resistance by inhibiting mTORC2 activity. Targeting mTORC2 could serve as a promising therapeutic strategy for mCRPC patients with docetaxel-resistant tumors.

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Conflict of Interest: None

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Ethics Statement: This study followed the principles of the Helsinki Declaration. All procedures carried out on human tissues were approved by the Experimental Human Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine. The informed consent was exempted with the approval of the ethics committee for the reason as follows:

1. The biological specimens used in this study were discarded specimens obtained in previous clinical diagnosis and treatment;
2. The risk to subjects in this study is not greater than the minimum risk;
3. Waiving informed consent will not adversely affect the rights or health of the subject;
4. The subjects were orally informed before the study and did not reject the use of discarded samples;
5. The confidentiality of subjects' privacy and identifying information is guaranteed.

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