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Inhibitory function of CDK12i combined with WEE1i on castration-resistant prostate cancer cells in vitro and in vivo

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Abstract

Objective: The inhibitors of CDK12 and WEE1 (SR-4835 and AZD-1775) have rarely been evaluated in studies on castration-resistant prostate cancer (CRPC) treatment. The research objective of this article is to study the inhibitory effect of SR-4835 and AZD-1775 on CRPC cells and to explore the therapeutic effect of combining the two drugs in the treatment of CRPC cells in vitro and in vivo.

Methods: We performed Western blot, quantitative real-time PCR, Cell Counting Kit-8, colony formation, EdU, and immunofluorescence assays; cell cycle analysis, wound scratch and Transwell assays and nude mice xenograft tumor analysis to identify the mechanism and measure the therapeutic effect of SR-4835, AZD-1775 and the combination in CRPC cells.

Results: Compared with normal prostate cells, the expressions of CDK12 and WEE1 in prostate cancer cells, especially CRPC cells, were significantly increased at protein and mRNA levels. SR-4835 can cause DNA damage in CRPC cells by inhibiting the expression of DNA damage repair genes. AZD-1775 inhibits the G2/M phase checkpoint function. Performing in vivo and in vitro experiments, we found that SR-4835 combined with AZD-1775 significantly enhanced the inhibitory effect on CRPC cell to a greater degree than monotherapy.

Conclusions: In summary, SR-4835 combined with AZD-1775 can eliminate CRPC cells by inducing DNA damage and inhibiting the normal repair machinery. Therefore, we consider this combination therapy to be a promising strategy for CRPC patients.

Keywords: castration-resistant prostate cancer (CRPC); CDK12; clinical translation; drug combination therapy; WEE1

Introduction

Worldwide, According to GLOBOCAN 2020, prostate cancer is the second most common cancer, and the mortality rate is fifth among male patients, with approximately 1,414,259 newly diagnosed cases and it is predicted that the number of new cases will reach 1,700,000 and the number of deaths will reach 499,000 in 2030 [1, 2]. For localized prostate cancer, radical prostatectomy has been the gold standard treatment, while for men with recurrent or metastatic prostate cancer, androgen deprivation therapy (ADT) is most effective but prostate cancer remains incurable [3–5]. Unfortunately, the condition of patients with prostate cancer can rapidly deteriorate as castration-resistant prostate cancer (CRPC) develops after ADT treatment [6, 7]. The median survival time of non-metastatic CRPC (nmCRPC) patients is 25–30 months whereas the median survival time of metastatic CRPC (mCRPC) patients is shortened to 14 months [8–11]. Enzalutamide is the most prescribed FDA-approved treatment for nmCRPC and mCRPC, yet almost all patients treated with enzalutamide develop drug resistance through multiple mechanisms [12]. Therefore, it is extremely urgent to explore a novel treatment plan for patients with CRPC.
In summary, CDK12 is a promising therapeutic target in repair of DNA damage [27]. CDK12 is to regulate the protein expression of DNA damage repair (DDR) genes by phosphorylating the heptad repeat in Ser2 [21–23]. Considering the CDK12 function, the combination of CDK12 inhibition and treatments to induce DNA-damage, such as radiotherapy and chemotherapy, is extremely promising, as confirmed in previous studies [24]. SR-4835 is a CDK12/13 inhibitor [25]. In contrast to THZ531, which inhibits CDK12 via covalent binding, SR-4835 is an ATP-competitive inhibitor of CDK12/13 [25, 26]. In previous studies, SR-4835 efficiently inhibited CDK12 function, and the expression of DDR genes regulated by CDK12 were also inhibited [20]. However, studies of SR-4835 in prostate cancer are lacking.

WEE1, a tyrosine kinase, phosphorylates the Tyr15 residue in CDK1. After DNA is damaged, CDK phosphorylated by WEE1 cannot form a Cyclin B-CDK1 complex, which causes cell cycle arrest in the G2 phase, enabling normal repair of damaged DNA [27–29]. In the study of other tumors, compared with normal tissues, the expression of WEE1 was significantly increased in tumor tissues, and the expression of WEE1 was negatively correlated with prognosis [30–32]. In previous studies, tumor cell proliferation was significantly inhibited when WEE1 action was inhibited [33, 34]. Therefore, WEE1 is worth exploring as a CRPC treatment target.

AZD-1775 is an ATP-competitive WEE1 inhibitor. AZD-1775 effectively promotes the formation of the Cyclin B-CDK1 complex, causing cancer cells to enter mitosis prematurely and inhibiting normal repair of damaged DNA [35]. Previous studies suggested that AZD-1775 effectively inhibited the esophagus carcinoma [34]. Importantly, the research data on small-cell neuroendocrine prostate cancer (NEPC) showed that AZD-1775 can significantly inhibit NEPC [36].

At first, we detected CDK12 and WEE1 levels in CRPC cells. Secondly, in vitro, CRPC cell lines were treated with SR-4835, AZD-1775 and the combination of the two drugs, and the mechanism and the inhibitory effect of drugs were explored. Finally, in vivo, CRPC cells were treated with SR-4835, AZD-1775 and the combination of the two drugs respectively, and the inhibitory effect on tumor was explored. The results of this study lay the theoretical basis for better treatment of patients with CRPC.

**Methods**

**Cell culture**

PC-3 cells (CL-0185), purchased from Procell Biotech (Wuhan, China) and certified by STR, were cultured at 37 °C with 5 % CO2. The PC3 were cultured in Kaighn’s modified Ham’s F-12 medium containing 1 % P/S (streptomycin and penicillin) and 10 % fetal bovine serum (FBS). RWPE-1, LnCap, 22RV1, C42 and DU145 cells (CL-0075) were purchased from Procell Biotech and were certified by STR and, were cultured at 37 °C with 5 % CO2. RWPE-1, LnCap, 22RV1, C42 and DU145 cells were cultured in RPMI 1640 medium containing 1 % P/S (streptomycin and penicillin) and 10 % FBS.

**Chemicals and antibodies**

SR-4835 (HY-130250) and AZD-1775 (HY-10993) were acquired from MedChemExpress (Shanghai, China). A Cell Counting Kit-8 (CCK-8) was purchased from APEXBio (Houston, USA). Paraformaldehyde, crystal violet desalination solution and other chemicals were purchased from Solarbio (Beijing, China). Anti-CDK12 (26816-1-AP), anti-GAPDH (10494-1-AP), anti-BRCA1 (22362-1-AP), anti-ATR (19787-1-AP), anti-phosphorylated-histone H3 (Ser10) (66863-1-Ig), anti-ATM (67586-1-Ig), anti-Chk1 (25887-1-AP), anti-RAD51 (67024-1-Ig) and other secondary antibodies were obtained from Proteintech (Wuhan, China). Anti-gamma H2A. X (S139) (ab81299), anti-CDK1 (Y15) (ab275958), anti-CDK1 (ab133327), anti-CDK12 (ab246887), anti-WEE1 (ab288727) and anti-CTD (Ser2) (ab193468) antibodies were purchased from Abcam (Cambridge, UK).

**Western blot assay**

First, cells were treated with different drugs and concentrations. Then, the original medium was discarded, the cells were rinsed twice, RIPA and PMSF (100:1) were mixed together, and the mixture was added to cells to lyse cells at 4 °C for 60 min. The mixture was centrifuged at 11,000 rpm for 19 min, and the protein supernatant was collected. Proteins were separated by electrophoresis and transferred to nitrocellulose (NC) membranes. Then, the NC membranes were soaked with 5 % BSA for 1 h and P/S (streptomycin and penicillin) and 10 % fetal bovine serum. The membranes were rinsed twice with TBST and then soaked with the original medium. After blocking with a protein-free blocking solution, the membranes were incubated overnight with the corresponding primary antibody. The bands were visualized with an enhanced chemiluminescence (ECL) reagent (ProteinTech, Wuhan, China). Signals were detected using a Tanon (Shanghai, China) chemiluminescence gel imaging system.

**Quantitative real-time PCR (qRT–PCR) and mRNA extraction**

Total mRNA was obtained with TRIzol (Ambion, Shanghai, China). For reverse transcription, a BIOG cDNA Synthesis Kit (BioDai, Changzhou, China) was used. qRT–PCR was performed using a BIOG Elitefast SYBR Kit (BioDai, Changzhou, China). The 2−ΔΔCt calculation method was used to obtain expression data for evaluating semiquantitative differences.
The internal control gene was GAPDH. The following primers were used: ATM-forward: 5′-GCTGCAATCATCAACAAGT-3′; ATM-reverse: 5′-GGTTCTCAGACTATGGGACA-3′; ATR-forward: 5′-GCTGCAACCAGTGAA-3′; ATR-reverse: 5′-CAATTAGCCTGTAACATC-3′; BCRA1-forward: 5′-CCTGTCAGGAGGATCCTCCTA-3′; BCRA1-reverse: 5′-GCTTCTAGTACACATTCCTCG-3′; FANCL-forward: 5′-CACCACCTTACGCCCTTGC-3′; FANCL-reverse: 5′-ATTCCCTCGAAGCTCAGAC-3′; FANCD2-forward: 5′-CCCCAAGTCTGATGCTCCT-3′; FANCD2-reverse: 5′-CCATCATCAGCAGGAAGAAA-3′; RADS1-forward: 5′-GCTGATGATTTGGTGAAGCC-3′; RADS1-reverse: 5′-GGAGAGAGAGAGCTGTAGA-3′; CHK1-forward: 5′-ATATAGAAGCCTGCCCTAGACT-3′; and CHK1-reverse: 5′-TGCTTATGCTGCTCTATCTC-3′.

**Cell counting kit-8 (CCK-8) assay**

After we placed cells in 96-well plates, the cells were mixed with different concentrations of different agents and cultured for 48 h. The CCK-8 reagent (APExBIO) was added to the medium, and the treated cells were incubated for two and a half hours. Experimental data were obtained with a full wavelength scanning multifunctional readout meter (BioTek, Montpelier, USA). The data were analyzed by Gen5 software.

**Colony formation assay**

After the cells were treated with different agents for 2 days, we subcultured the cells in 6-well plates. We used paraformaldehyde (Solarbio, Beijing, China) to fix the cells for 17 min, and then, we utilized a crystal violet desalting solution (Solarbio, Beijing, China) to stain the colonies of Tianjin Medical University. All methods were performed in accordance with the regulations of the Laboratory Animal Management and Use Committee of Tianjin Medical University and ARRIVE guidelines.

**Wound scratch assay**

Cells were placed in six-well plates and cultured with different agents added at different concentrations, to confluence. Then, the cell monolayer was scratched with a 200 μL pipette tip. We took pictures of the cells under a microscope (Olympus, Tokyo, Japan).

**Transwell assays**

Matrigel (Corning, USA) was coated onto the upper chamber of each Transwell insert (Nest, Wuxi, China), and 200 μL of basal medium was added to the upper chamber, while 600 μL complete medium was added to the lower chamber. After the cells were treated with different drugs for 48 h, the cells (5 × 10⁴ cells) were plated in a Transwell chamber. We fixed the cells with parafformaldehyde and stained them with a crystal violet desalination solution for 19 min. Finally, photographs were taken with a microscope.

**Combination index analysis**

Based on the IC₅₀ values of PC-3 and DU145 cell lines for SR-4835 and AZD-1775, we determined a 1:1 ratio (25, 50, 100, 200 and 400 nM). The 5-point dilution concentration response curves were generated. Loewe additivity is a dose-response model that states that additivity occurs in a two-drug combination when the sum of the ratio of the dose to the mean effect for each individual drug equals 1. In this model, the combination index (CI) values estimate the interaction between the two drugs. If CI<1, the drugs have a synergistic effect, and if CI>1, the drugs have an antagonistic effect. CI<1 means that the drugs have an additive effect. CI coefficients were calculated based on the Chou-Talalay median effect model as implemented in CalcuSyn v2.11 [20].

**Tumor xenografts in nude mice**

Approximately 5 × 10⁶ PC3 cells were subcutaneously injected into 16 male BALB/c nude mice. After the mice were housed together for approximately 2 weeks, we randomly assigned the mice into a control group, an SR-4835 group, an AZD-1775 group and a combination group. Mice in the control group received 200 μL of PBS containing 5 % DMSO daily, while mice in the SR-4835 group received 200 μL of SR-4835 (10 mg/kg) daily. Mice in the AZD-1775 group received 200 μL (30 mg/kg) of AZD-1775 daily, and mice in the combination treatment group received 200 μL of a solution containing SR-4835 (10 mg/kg) and AZD-1775 (30 mg/kg) daily. We measured body weight and tumor size of mice every other day. On Day 28, the mice were killed, and the tumors were removed from the mice. The volume of the tumors (calculated as 0.5 × length × width²) and weight were measured. All animal experiments were approved by the Laboratory Animal Management and Use Committee of Tianjin Medical University. All methods were performed in accordance with the regulations of the Laboratory Animal Management and Use Committee of Tianjin Medical University and ARRIVE guidelines.
Statistical analysis

All experimental data were analyzed via GraphPad Prism. All experimental data are presented as the mean ± standard deviation. To compare differences between two groups, the T tests were performed. When comparing four groups, one-way ANOVA was performed. \( p<0.05 \) was considered to be statistically significant (*\( p<0.05 \), **\( p<0.01 \), ***\( p<0.001 \)), and all tests were two tailed.

Results

CDK12 and WEE1 levels are elevated in prostate cancer

To measure the levels of CDK12 and WEE1 in different prostate cancer cells, Western blot analysis was performed (Figure 1A). Compared with normal prostate cell line (RWPE-1), the protein expressions of CDK12 and WEE1 were significantly increased in prostate cancer cell lines (22RV1, C42, PC-3 and DU145), except for LnCap cell line. Similarly, the CDK12 and WEE1 mRNA levels were measured in normal prostate cell line and prostate cancer cell lines (Figure 1B). In summary, the data from this experiment demonstrate that CDK12 and WEE1 are highly expressed in prostate cancer cell lines, including mCRPC cell lines (PC-3 and DU145).

SR-4835 inhibits CRPC cell activity, proliferation, migration and invasion by reducing DDR gene expression

To investigate the inhibitory effect of SR-4835 on CRPC cell activity, we performed CCK-8 experiments with in PC-3 and DU145 cells treated with increasing drug concentrations (Figure 2A). The results suggested that with increasing concentrations of SR-4835, the viability of these CRPC cells decreased. Furthermore, the IC50 values of PC-3 and DU145 were calculated to be 107 and 97 nM, respectively. We verified the effect of SR-4835 on the proliferation of CRPC cells via colony formation experiments (Figure 2B). We found that with increasing concentrations of SR-4835, the size and number of colonies were markedly decreased. Scratch healing was significantly slower in PC3 and DU145 cells treated with increasing concentrations of SR-4835 in the wound scratch assay (Figure 2C). Likewise, the number of PC3 and DU145 cells that crossed the PC membrane was significantly decreased after treatment with increasing concentrations of SR-4835 (Figure 2D). The results of Western blot experiments suggested that the protein expression of DDR genes (ATM, ATR, BRCA1, RAD51 and CHK1) and RNA polymerase II CTD Ser2 decreased significantly, while that of γH2A.X (a DNA damage marker) and cleaved caspase-3 (Asp175) (an apoptosis marker) was elevated with increasing

![Figure 1](image_url)
Figure 2: The effects of SR-4835 on CRPC cells. (A) The viability of cells incubated with 10–1,000 nM SR-4835. (B) The colonies formed by different cells cultured with different concentrations of SR-4835. (C) Changes in the migration ability of CRPC cells after SR-4835 treatment. (D) Changes in the invasion ability of PC3 and DU145 cells treated with SR-4835. (E) Changes in CDK12 level and that of its downstream proteins in different cells cultured with different concentrations of agents. (F) The relative expression of DDR genes in various cells treated with different concentrations of SR-4835.
concentrations of SR-4835 (Figure 2E). These results indicated that SR-4835 increased the injury to and apoptosis rate of CRPC cells by inhibiting DDR gene expression. Similarly, the mRNA levels of DDR genes were significantly reduced (Figure 2F). In summary, SR-4835 inhibited the activity, proliferation, migration and invasion of CRPC cells by inhibiting the repair of damaged DNA.

**AZD-1775 strongly inhibits CRPC cell activity, proliferation, migration and invasion by inducing premature mitosis**

Through CCK-8 assays, we found that the proliferation of CRPC cells was negatively correlated with AZD-1775 concentration (Figure 3A). The IC50 values of AZD-1775 in PC-3 and DU145 cells were 151 and 131 nM, respectively. Similarly, colony formation experiments showed that the proliferative capacity of the CRPC cells was negatively correlated with the concentration of AZD-1775 (Figure 3B). The migratory ability of CRPC cells was significantly affected after treatment with the increasing concentration of AZD-1775 in a wound scratch assay (Figure 3C). To evaluate the influence of AZD-1775 on the invasive ability of CRPC cells, we performed the Transwell experiment. The number of CRPC cells that passed through the chamber was significantly lower after treatment with the increasing concentration of AZD-1775 (Figure 3D). The Western blot results showed that with increasing concentrations of AZD-1775, the levels of WEE1 and pCDK1 (Y15) decreased significantly, but those of γH2A.X and pH3 (Ser10) (a premature mitosis marker) increased continuously (Figure 3E). Through cell cycle analysis, we confirmed that the number of cells arrested in the G2 phase was significantly reduced by AZD-1775 (Figure 3F). A statistical plot of the proportion of EdU staining is presented in Figure S1. In conclusion, these data clarified that combination therapy with SR-4835 and AZD-1775 inhibited the activity, proliferation, migration and invasion of CRPC cells to a greater extent than single-drug therapy.

**SR-4835 efficiently cooperates with AZD-1775 to inhibit CRPC cells**

To verify that SR-4835 combined with AZD-1775 can more effectively inhibit CRPC cells, we performed a combined treatment analysis of the two drugs in two cell lines, represented by the combination index (CI). The CI isobologram method was used to calculate combined drug effects [37]. CI<1, >1 and 1 indicate additive, synergistic and antagonistic effects, respectively. The results showed that the inhibitory effects of SR-4835 and AZD-1775 on CRPC cells were mutually reinforcing (Figure 4A). Subsequently, we found that, compared with other groups, the activity of CRPC cells in the combined treatment group was significantly reduced by CCK-8 experiment (Figure 4B). To explore the effect of combination therapy on the proliferation ability of CRPC cells, we conducted colony formation experiments and EdU experiments. The result is as we expected (Figure 4C and D). A statistical plot of the proportion of EdU staining is presented in Figure S1. Compared with single drug therapy, the combination therapy weakened the migration and proliferation of CRPC cells more significantly (Figure 4E and F).

Performing immunofluorescence assays, we found that the protein expression level of γH2A.X (a DNA damage marker) in PC3 cells treated with the combination of the two drugs was significantly higher than that in CRPC cells treated with either drug alone, although the expression of Ki67 (a cell proliferation marker) was significantly decreased (Figure 4G). Immunofluorescence analysis of DU145 cells is described in Figure S1. In conclusion, these data clarified that combination therapy with SR-4835 and AZD-1775 inhibited the activity, proliferation, migration and invasion of CRPC cells.

**The combination of SR-4835 and AZD-1775 effectively inhibits CRPC cell proliferation in vivo**

We injected PC3 cells subcutaneously into male BALB/C nude mice to verify that SR-4835 cooperates with AZD-1775 to inhibit CRPC cell proliferation in vivo. When the tumor had grown to approximately 100 mm3, we randomly assigned the nude mice to four groups of four mice each. These four groups of nude mice were treated with 0.5 % DMSO, 10 mg/kg SR-4835, 30 mg/kg AZD-1775 and the combination of the two drugs (0.2 mL) by oral gavage daily for 14 days. In this 14-day period, we measured the body weight of the mice every other day, and we showed that the body weights of the nude mice were not significantly influenced by either SR-4835 or AZD-1775 treatment (Figure 5A). Notably, the size of the tumors in the other groups was significantly larger than the size of the tumors in the combination treatment group (Figure 5B). Then, the tumors were removed. We found that the combination treatment group presented with a lower tumor weight and size than the other groups (Figure 5C–E). We plotted a line graph of tumor volume in mice over
Figure 3: The effects of AZD-1775 on CRPC cell proliferation. (A) The viability of cells incubated with AZD-1775 at 10–1,000 nM. (B) Colonies formed by different cells cultured with different concentrations of AZD-1775. (C) The migratory ability of various cells treated with different concentration of AZD-1775 as determined with scratch tests. (D) The invasive ability of various cells treated with different concentration of AZD-1775 was verified by Transwell assay. (E) Changes in WEE1 level and that of its downstream proteins in different cells cultured with different concentrations of agents. (F) Cell cycle changes in cells treated with increasing concentrations of AZD-1775.
Figure 4: Inhibitory effects on CRPC caused by the SR-4835 and AZD-1775 combination treatment. (A) Combination index of the two drugs in PC3 and DU145 cell lines. (B) Cell viability in different treatment groups. (C) Colonies formed by different cells cultured in different combinations of drugs. (D) The proliferation of cells incubated with different combination drugs as determined via EdU assay. (E) Migratory ability of PC3 and DU145 cells treated with different agents in combination as determined with scratch tests. (F) The invasive ability of various cells treated with different agents in combination was verified by Transwell assay with Matrigel. (G) Changes in γH2A.X and Ki67 level in various cells treated with different agents in combination.
14 days. We found that the tumor volume in the combined treatment group was significantly lower than in the other groups (Figure 5F). Together, the combination of SR-4835 and AZD-1775 strongly inhibited CRPC tumor growth in vivo.
Discussion

In 1972, ADT became the standard option for prostate cancer treatment after Hodges and Huggins proposed that prostate cancer cells were androgen dependent [38]. Combining an androgen receptor blocker (ARB) with prior castration is called complete androgen blockade (CAB) [39]. Unfortunately, prostate cancer is prone to progression to CRPC at an average of 24 months after receiving ADT or CAB, and although CAB outperforms ADT in both progression-free survival and overall survival, it comes at the cost of greater toxicity [38, 39]. Over the past decade, several innovative therapies have been developed for CRPC. Treatment options include next generation antihormonal drugs, DNA repair inhibitors, radiopharmaceuticals and immunotherapy (including Enzalutamide, Olaparib, Lutetium-177-PSMA-617 and Pembrolizumab, among others) [40–42]. These novel agents help patients with CRPC by improving PSA response and modest survival benefit. However, CRPC still remains incurable, and an effective treatment method still needs to be explored.

CDK12 and WEE1 play important roles in tumor DDR and cell cycle regulation and are highly expressed in other tumor tissues and cells, as shown in previous reports [20, 34, 43]. In this study, our data showed that compared to those in normal prostate cells and tissues, the expression levels of CDK12 and WEE1 protein and mRNA obviously increased in prostate cancer, especially CRPC. Therefore, this study lays the foundation for subsequent therapeutic research into CDK12 and WEE1.

As a specific CDK12 inhibitor, SR-4835 significantly inhibits the expression of CDK12 and DDR genes. Due to the extremely fast division of CRPC cells and the high risk of DNA damage, SR-4835 prevents CRPC cells from normally repairing damaged DNA, resulting in CRPC apoptosis. This effect has been demonstrated in previous research on breast cancer [20]. In this study, we utilized SR-4835 to treat CRPC cells and achieved excellent results. After treating PC-3 and DU145 cell lines with SR-4835, DDR-related gene expression decreased significantly, and the cells underwent apoptosis.

As a specific WEE1 inhibitor, AZD-1775 effectively inhibits G2/M phase checkpoint arrest regulated by WEE1 [44]. The phosphorylated CDK1 level decreased significantly when WEE1 was inhibited, leading to an increase in Cyclin B-CDK1 complex formation [45]. Because Cyclin B-CDK1 complexes induce cells to prematurely enter mitosis; therefore, after DNA is damaged, cells cannot normally stop at the G2 phase to enable DNA repair. This failure to delay mitosis leads to CRPC cell apoptosis because these cells generally lack p53 expression and rely mainly on G2 phase arrest to enable DNA damage repair [35]. In addition, in previous studies, AZD-1775 showed a very high potential inhibitory effect on tumor cell proliferation [46, 47]. In this study, we used AZD-1775 to treat CRPC cells and found results consistent with the aforementioned reports.

According to the mechanism underlying SR-4835 and AZD-1775 effects, we speculate that these two drugs synergistically inhibit CRPC cell proliferation (Figure 6). Indeed, our experimental data showed that SR-4835 weakened the ability of CRPC cells to repair DNA by inhibiting the transcription of DDR genes, which made the CRPC genome extremely unstable, eventually leading to reduced cell proliferation and eventually to apoptosis. AZD-1775 inhibited CRPC cells by weakening the G2/M phase checkpoint function of WEE1, prompting CRPC cells to prematurely enter mitosis, preventing G2/M phase arrest, which is needed for

![Figure 6: Schematic diagram showing the molecular mechanism by which the SR-4835 and AZD-1775 combination treatment affects CRPC cells.](image)
DNA damage repair. Importantly, CRPC cells generally rely on G2/M phase arrest to repair damaged DNA, not G1 phase arrest, which greatly increases the specificity of drug therapy. In combination, SR-4835 and AZD-1775 enhanced the treatment effects of each other, and the therapeutic effect was much higher than that of single-drug therapy. SR-4835 prevented the normal transcription of DDR genes in CRPC cells and causes DNA damage, while AZD-1775 inhibited the G2/M phase checkpoint, inhibiting DNA repair in the G2 phase. To verify the effects of the SR-4835 and AZD-1775 combination treatment in vivo, we established a xenogeneic CRPC mouse model. The therapeutic effect of the combination was much greater than that of the control group and the single drug treatment group, and no obvious influence on the growth of mice was observed. In a previous clinical trial, adavosertib (AZD-1775) 175 mg was administered orally twice daily on days 1–3 and 8–10 of a 21-day treatment cycle, while in our study, mice were administered a dose of AZD 30 mg/kg [48]. Therefore, the effective therapeutic dose in mice needs to be re-evaluated to optimize plasma and tumor delivery in humans and to achieve a potent antitumor effect in the treatment of CRPC. In future research, we will investigate the mechanism underlying the effects of SR-4835 and AZD-1775 in combination and evaluate the feasibility and efficacy of using this combination as a clinical anti-CRPC therapy.

Conclusions

We found that compared with single drug, SR-4835 combined with AZD-1775 more significantly inhibited CRPC cells by preventing DNA damage repair and promoting cells to enter mitosis prematurely. These data indicate that CDKi combined with WEE1i may be a promising therapeutic approach for CRPC.

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Informed consent: Not applicable.

Ethical approval: All animal experiments were approved by the Laboratory Animal Management and Use Committee of Tianjin Medical University (approval no. 2023006) and all methods were performed in accordance with the regulations of the Laboratory Animal Management and Use Committee of Tianjin Medical University and ARRIVE guidelines.

Author contributions: Zheng Qin: Conceptualization, Writing – original draft, Methodology, Formal analysis. Yueyao Zhang, Dongze Liu, Xiaoxu Chen, Xiao Zhu and Shengxian Xu: Supervision, Validation, Data curation, Resources. Baolong Peng, Shiqiang Dong, Dingkun Hou and Liang Zhu: Visualization, Investigation, Software. Haitao Wang: Funding acquisition, Project administration, Writing – review & editing. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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