

Original Paper

AZD5153 Inhibits Prostate Cancer Cell
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Key Words

Prostate cancer • BRD4 • AZD5153 • AKT • Chemosensitization

Abstract

Backgrounds/Aims: Bromodomain-containing protein 4 (BRD4) overexpression participates in prostate cancer progression by enhancing the transcriptional activity and expression of several key oncogenes. AZD5153 is a novel BRD4 inhibitor. **Methods:** Prostate cancer cells were treated with AZD5153. Cell survival was tested by MTT assay and clonogenicity assay. Cell proliferation was tested by [³H] DNA incorporation assay. Cell apoptosis was tested by caspase-3/-9 activity assay, Histone DNA ELISA assay, Annexin V FACS assay and TUNEL staining assay. Cell cycle progression was tested by propidium iodide (PI) FACS assay. Signaling was tested by Western blotting assay. The nude mice PC-3 xenograft model was applied to test AZD5153's activity *in vivo*. **Results:** AZD5153 inhibited proliferation and survival of established and primary prostate cancer cells. AZD5153 induced apoptosis activation and cell cycle arrest in prostate cancer cells. AZD5153 was non-cytotoxic to the prostate epithelial cells. AZD5153 downregulated BRD4 targets (cyclin D1, Myc, Bcl-2, FOSL1 and CDK4) in PC-3 and primary prostate cancer cells. Further studies show that AKT could be the primary resistance factor of AZD5153. Pharmacological inhibition or genetic depletion of AKT induced BRD4 downregulation, sensitizing AZD5153-induced cytotoxicity in PC-3 cells. *In vivo*, AZD5153 oral administration inhibited PC-3 xenograft tumor growth in nude mice. Its anti-tumor activity was further enhanced with co-treatment of the AKT specific inhibitor MK-2206. **Conclusion:** Together, our results indicate a promising therapeutic value of the novel BRD4 inhibitor AZD5153 against prostate cancer cells.

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Introduction

Molecularly-targeted therapy is important for prostate cancer treatment [1-4]. Bromodomain and extraterminal (BET) family proteins have four members, including bromodomain-containing protein (BRD) 2, BRD3, BRD4 and testis-specific isoform BRDT [5, 6]. BET proteins are transcriptional co-activators. They regulate a number of key cellular

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behaviors, including cell cycle progression, cell proliferation, apoptosis-resistance, migration and invasion [5, 6]. BET proteins are often overexpressed in human cancers, correlated with cancer initiation, carcinogenesis and progression [5, 6]. BET inhibitors, including JQ1 and CPI203, have demonstrated promising anti-cancer efficiency in preclinical cancer studies and early clinical trials [5, 6].

BRD4 is the most abundant BET family protein [7-10]. It binds to acetylated-histones, acting as a key epigenetic regulator [7-9, 11]. BRD4 helps to maintain the normal chromatin structure in the daughter cells [7-9]. BRD4 recruits P-TEFb (the positive transcription elongation factor b) and the RNA polymerase II, both are essential for transcription elongation [9]. BRD4 targets are mostly key oncogenes, including Bcl-2 [12, 13], Myc [11, 14, 15] and cyclin D1 [16, 17]. Recent studies have proposed BRD4 as a potential therapeutic target of prostate cancer [18-20]. Recent studies have developed a novel, potent and specific BRD4 inhibitor, AZD5153 [21-23]. Unlike other BRD4 inhibitors, AZD5153 is a bivalent BRD4 inhibitor targeting two bromodomains of BRD4 [22]. It displaces BRD4 from chromatin at relatively lower concentration [22]. It's activity against human prostate cancer cells is tested here.

Materials and Methods

Chemicals and reagents

AZD5153 was purchased from Medkoo Bioscience (Beijing, China). The antibodies were all provided by the Cell Signaling Tech (Beverly, MA). The AKT inhibitor MK-2206 was purchased from Sigma (Shanghai, China). The AKT inhibitor AKTi-1/2 [24, 25], the BRD4 inhibitors (JQ1 and CPI203) were obtained from Selleck (Shanghai, China).

Cell lines

Human prostate cancer cell lines, PC-3 and LNCaP, were purchased the iBS cell bank of Fudan University (Shanghai, China). Cells were cultured as monolayer in RPMI-1640 with 10% FBS (fetal bovine serum). RWPE1, the non-transformed prostate epithelial cell line, was provided by Dr. Shuo [26]. RWPE1 cells were cultured in Defined Keratinocyte-SFM medium supplemented with described growth factors [26]. The reagents for cell culture were all obtained from Hyclone (Suzhou, China).

Primary culture of human cells

One patient administrated at the first-affiliated hospital of Soochow University was enrolled in this study. The written-informed patient, male, 52-year old, didn't receive prior chemical, hormonal, or radiation therapy before surgery (see our previous study [27]). The prostate cancer tissues and surrounding epithelial tissues were separated carefully. Tissues were minced, digested, and pipetted to disperse clumps [27]. Cells cultured on collagen-coated tissue-culture plates (BD Biosciences, Suzhou, China) in the described medium [26]. Primary human cells at passage 3-10 were utilized for further experiments, with approval by the ethics committee of Nantong University.

MTT assay

As described [27], methyl thiazolyl tetrazolium (MTT) assay was performed to test cell survival. MTT was dissolved in DMSO. MTT optical density (OD) at 570 nm was recorded.

Clonogenicity assay

Prostate cancer cells (5×10^5 per well) with the indicated treatment were trypsinized and re-suspended in agarose-containing medium. Cells were then plated on the top of six-well plates. AZD5153-containing medium was renewed every two days for a total of 10 days. Afterwards, the colonies were counted.

Western blotting assay

The lysis buffer (Biyuntian, Suzhou, China) was added to cultured cells [28, 29]. Total cellular lysates were resolved by SDS-PAGE gels, and then transferred onto the PVDF membrane. The blot was incubated with PBST with 10% non-fat dry milk and desired primary/secondary antibodies. The enhanced chemiluminescence (ECL) method was applied to detect the immuno-reactive bands. Band intensity was quantified by the ImageJ software (NIH).

Caspase-3/-9 activity assay

Twenty μ g of cytosolic extracts were mixed with the caspase assay buffer [26] and the caspase-3 substrate Ac-DEVD-AFC (15 μ g/mL) or the caspase-9 substrate Ac-LEHD-AFC (15 μ g/mL) (Calbiochem, Darmstadt, Germany). After incubation, the amount of released AFC was tested by the spectrofluorometer (Thermo-Labsystems, Helsinki, Finland) with excitation of 380 nm and emission wavelength of 460 nm.

Annexin V assay

Briefly, cells with the applied treatment were harvested, washed, and incubated with Annexin V and propidium iodide (PI). Afterwards, cells were analyzed by fluorescent-activated cell sorting (FACS) on a FACSCalibur machine (BD Biosciences). Percentage of Annexin V positive cells was recorded.

Histone DNA apoptosis ELISA assay

The Histone-DNA ELISA assay detects apoptotic cell death by quantifying cytoplasmic histone-associated DNA fragments [26]. The assay was performed with the instruction from the manufacturer (Roche, Shanghai, China). Histone-DNA ELISA OD at 450 nm was recorded.

TUNEL assay

TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) In Situ Cell Death Detection Kit (Roche, Shanghai, China) was utilized to quantify cell apoptosis. The percentage of apoptotic cells was determined by the TUNEL percentage (TUNEL/Hoechst \times 100%). At least 100 cells per preparation in five random views were counted.

Cell cycle analysis

After the treatment, cells were labeled with PI (10 μ M), analyzed on a FACSCalibur machine (BD Biosciences). Cell cycle percentages were recorded.

Quantitative reverse transcriptase real-time polymerase chain reaction (qRT-PCR) assay

Total cellular RNA was extracted by the TRIzol reagents [2]. The First-Strand Synthesis Kit (SABiosciences, Frederick, MD) was utilized for cDNA synthesis. qRT-PCR was performed in Quant studio 3 (Applied Biosystems, Foster City, CA) via the SYBR GREEN kit (TaKaRa, Japan). The 2^{- $\Delta\Delta$ Ct} method was applied to quantify *BRD4 mRNA* using *GAPDH* as the internal control. The mRNA primers for *BRD4*: 5'-ACCTCCAACCTAACAAGCC-3' and 5'-TTTCCATAGTGCTTGAGCACC-3' [30], the mRNA primers for *GAPDH*: 5'-GCACCGTCAAGGCTGAGAAC-3' and 5'-TGGTGAAGACGCCAGTGGA-3' [30] were synthesized by Genechem (Shanghai, China).

AKT1 shRNA

AKT1 shRNA lentiviral particles (Santa Cruz Biotech, sc-29195-V) were added to PC-3 cells for 12 hours. Afterwards, puromycin (10 μ g/mL, Sigma) was added to select stable cells. AKT1 knockdown in stable cells was verified by Western blotting assay. Control cells were transfected with lentiviral scramble control shRNA (Genechem).

CRISPR/Cas9 knockout of AKT1

The lentiCRISPR plasmid with the AKT1 CRISPR/Cas9 KO Plasmid, provided by Genepharma (Shanghai, China), was transfected to PC-3 cells. Afterwards, puromycin (10 μ g/mL, Sigma) was added to select stable cells. AKT1 knockout in the stable cells was verified by Western blotting assay.

Xenograft assay

Male nude mice (6-8 weeks old, 17-19 g weight) were provided by the experimental animal center of Soochow University (Suzhou, China). Mice were maintained in accordance with Institutional Animal Care Use Committee guidelines. PC-3 cells (3×10^6 cells per mouse, dissolved in Matrigel, 1:1 ratio, in 0.1 mL total volume) were injected subcutaneously (s.c.) on the right flanks. Four weeks post tumor implantation, the volume of each tumor was close to 100 mm^3 . Mice were then randomly assigned into four groups. Tumor volumes, mice body weights and tumor weights were determined as described [27].

Statistical analysis

Results were expressed as the mean \pm standard deviation (SD). Ordinary one-way ANOVA test was employed for comparison between groups. Multiple comparisons were performed using Tukey's honestly significant difference procedure. To determine significance between two treatment groups, a two-tailed unpaired t test was applied. A P value < 0.05 was considered statistically different.

Results

AZD5153 inhibits prostate cancer cell survival and proliferation

First, PC-3 pancreatic cancer cells [31] were treated with applied concentration of AZD5153 (1-1000 nM). MTT assay results in Fig. 1A demonstrate that AZD5153 dose-dependently inhibited PC-3 cell survival, causing significant MTT OD reduction (Fig. 1A). The anti-survival effect by AZD5153 was also time-dependent (Fig. 1A). At least 48 hours

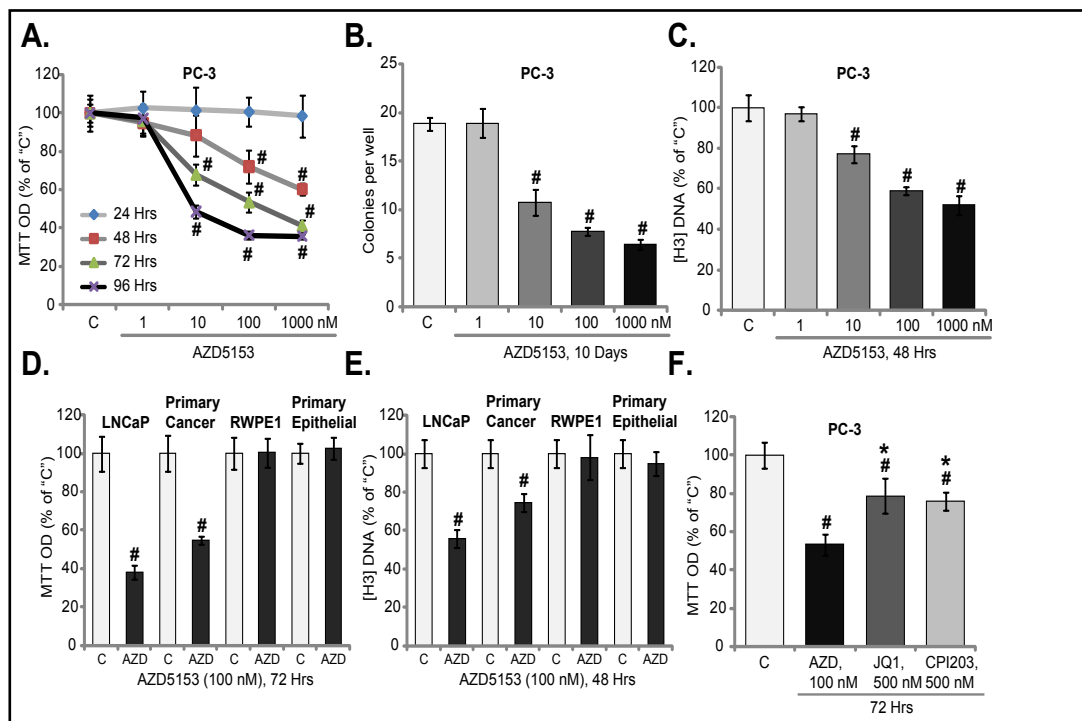


Fig. 1. AZD5153 inhibits prostate cancer cell survival and proliferation. The established (LC-3 and LNCaP lines) or the primary human (patient-derived) prostate cancer cells, the prostate epithelial RWPE1 cells or the primary human (patient-derived) prostate epithelial cells were cultured in complete medium, and cells were treated with applied concentration of AZD5153 (A-F), JQ1 (500 nM) (F) or CPI203 (500 nM) (F) for indicated time, cell survival was tested MTT assay (A, D and F) and clonogenicity assay (B); Cell proliferation was tested by the $[H^3]$ DNA incorporation assay (C and E). "C" stands for cells without treatment. For each assay, n=5. Data are mean \pm SD. # P < 0.05 vs. "C" cells. * P < 0.05 vs. AZD5153 treatment. Experiments in this Fig. were repeated four times, and similar results were observed.

were required for AZD5153 to achieve significant activity (Fig. 1A). AZD5153's IC-50 (the concentration using 50% inhibition of cell survival) was close to 100 nM at 72 hours and around 10 nM at 96 hours (Fig. 1A). Clonogenicity assay was performed to test cell survival as well. Results in Fig. 1B show that the number of viable PC-3 cell colonies was significantly decreased after AZD5153 (10-1000 nM) treatment. To test cell proliferation, [³H] DNA incorporation assay [32] was performed, results show that AZD5153 dose-dependently inhibited the amount of incorporated [³H] DNA in PC-3 cells (Fig. 1C), suggesting that AZD5153 inhibited PC-3 cell proliferation.

Next, we tested the potential effect of AZD5153 on other prostate cancer cells. In established (LNCaP cell line) and primary (patient-derived) human prostate cancer cells, treatment with 100 nM of AZD5153 significantly decreased cell viability (MTT OD, Fig. 1D) and [³H] DNA incorporation (Fig. 1E). RWPE1, the non-transformed prostate epithelial cells [26] and the primary cultured prostate epithelial cells were treated with AZD5153 as well. By performing MTT assay and [³H] DNA incorporation assay, we show that AZD5153 failed to affect survival (Fig. 1D) and proliferation (Fig. 1E) of epithelial cells. Significantly, 100 nM of

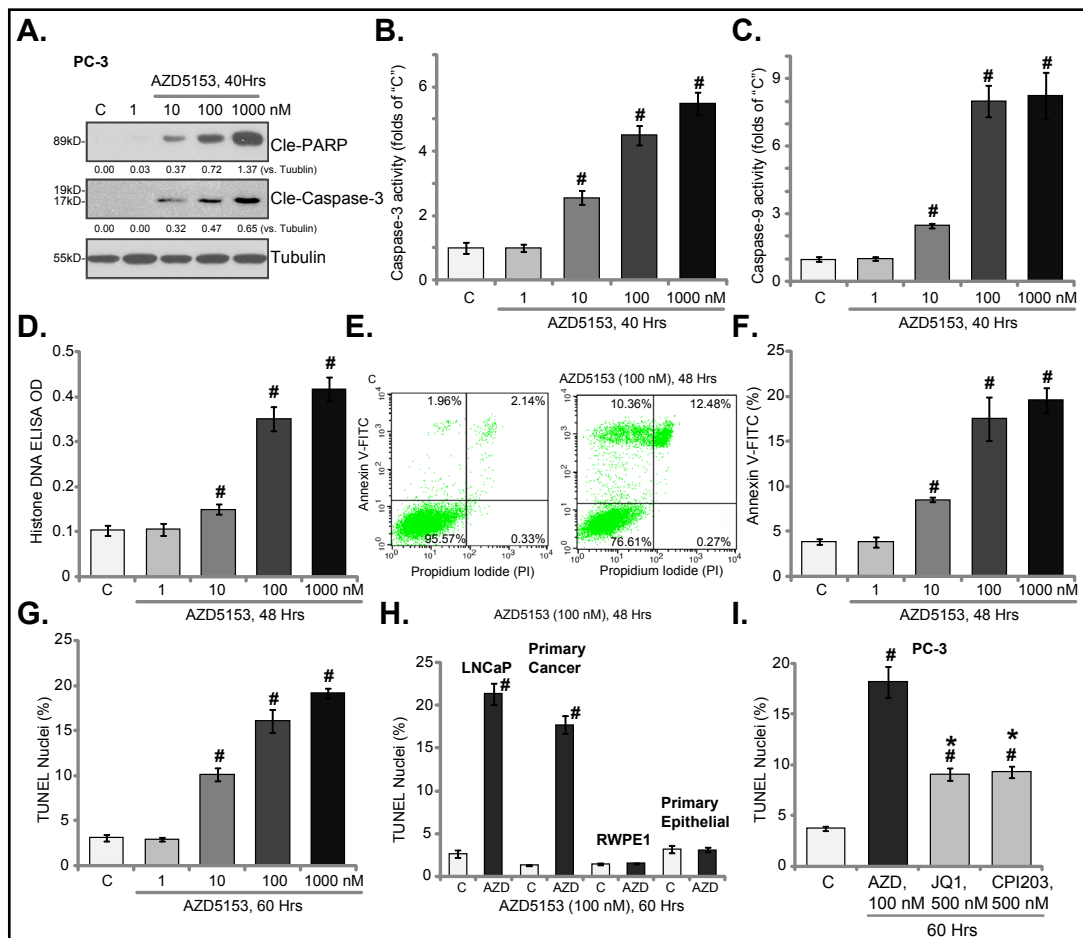


Fig. 2. AZD5153 induces apoptosis activation in prostate cancer cells. The established (LC-3 and LNCaP lines) or the primary human prostate cancer cells, the prostate epithelial RWPE1 cells or the primary human prostate epithelial cells were cultured in complete medium, and cells were treated with applied concentration of AZD5153 (A-I), JQ1 (500 nM) (I) or CPI203 (500 nM) (I) for indicated time, cell apoptosis was tested by the assays mentioned in the text. Cleaved-Caspase-3 and cleaved-PARP expression were quantified (A). "C" stands for cells without treatment. For each assay, n=5. Data are mean ± SD. # P<0.05 vs. "C" cells. * P<0.05 vs. AZD5153 treatment (I). Experiments in this Fig. were repeated four times, and similar results were observed.

AZD5153 was more potent in inhibiting PC-3 cell survival than higher concentration of other BRD4 inhibitors, including JQ1 (500 nM) [16, 33] and CPI203 (500 nM) [34] (Fig. 1F). These results demonstrate that AZD5153 inhibits prostate cancer cell survival and proliferation in vitro.

AZD5153 induces apoptosis activation in prostate cancer cells

The potential effect of AZD5153 on cell apoptosis was tested. Western blotting assay results in Fig. 2A demonstrate that AZD5153 dose-dependently induced cleavages of caspae-3 and its substrate poly (ADP-ribose) polymerase (PARP) in PC-3 cells. Activities of caspase-3 (Fig. 2B) and caspase-9 (Fig. 2C) were significantly increased as well, followed by the increased content of Histone-bound DNA (Fig. 2D). Additionally, the percentage of PC-3 cells with positive Annexin V-positive staining (“apoptotic cells”) was increased after AZD5153 treatment (Fig. 2E and F).

Further studies show that 10-1000 nM of AZD5153 increased the percentage of PC-3 cells with TUNEL staining (Fig. 2G). AZD5153-induced apoptosis activation was dose-dependent (Fig. 2A-G). TUNEL staining assay results in Fig. 2H suggest that AZD5153 (100 nM) induced apoptosis in LNCaP and primary human prostate cancer cells. Conversely, after AZD5153 treatment, TUNEL ratio was unchanged in RWPE1 and primary prostate epithelial cells (Fig. 2H). Significantly, TUNEL assay results in Fig. 2I demonstrate that AZD5153 (100 nM) was more efficient in inducing PC-3 cell apoptosis than JQ-1 and CPI203 (at 500 nM). Together, AZD5153 induces apoptosis activation in prostate cancer cells.

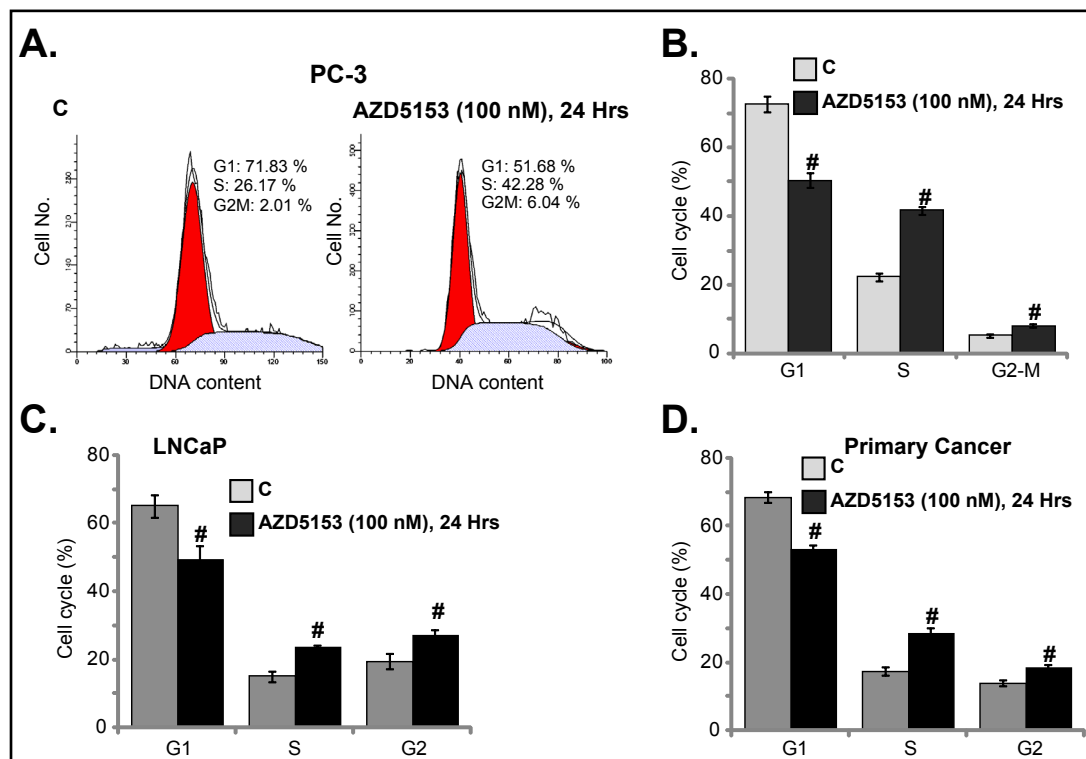


Fig. 3. AZD5153 induces cell cycle arrest in prostate cancer cells. PC-3 cells, LNCaP cells and primary human prostate cancer cells (“Primary Cancer”) were cultured in complete medium, and cells were treated with 100 nM of AZD5153 for 24 hours, cell cycle distribution was tested by the PI-FACS assay, representative FACS images were shown (A) and data were quantified (B-D). “C” stands for cells without treatment. For each assay, n=5. Data are mean ± SD. # P<0.05 vs. “C” cells. Experiments in this Fig. were repeated four times, and similar results were observed.

AZD5153 induces cell cycle arrest in prostate cancer cells

As discussed, BRD4 regulates activation of P-TEFb and RNA polymerase II, both are essential for cell cycle progression [9]. PI FACS assay results in Fig. 3A demonstrate that AZD5153 (100 nM, 24 hours) significantly decreased G1-phase PC-3 cells, yet increasing S- and G2-M phase cells. Results in Fig. 3B confirm that AZD5153-induced cell cycle arrest was significant. The similar results were also observed in AZD5153-treated LNCaP cells (Fig. 3C) and primary human prostate cancer cells (Fig. 3D). Thus, AZD5153 induces cell cycle arrest in prostate cancer cells.

AZD5153 downregulates BRD4 targets in prostate cancer cells

BRD4 is required for the expression of several key oncogenes [12, 13, 15, 35, 36]. Western blotting assay results show that treatment with AZD5153 (100 nM, 24 hours) induced downregulation of multiple BRD4 targets, including cyclin D1, Myc and Bcl-2 (Fig. 4A and B) as well as FOSL1 and CDK4 (Fig. 4A and B) in PC-3 cells. BRD4 protein expression and AKT expression/activation were not affected by AZD5153 (Fig. 4A and C). The similar results were also obtained in the primary human prostate cancer cells, where AZD5153 (100 nM, 24 hours) efficiently downregulated the BRD4 targets (Fig. 4D and E), while leaving BRD4 expression and AKT unaffected (Fig. 4D and F). These results suggest that AZD5153 inhibits BRD4 in prostate cancer cells.

AKT is a primary resistance factor of AZD5153 in prostate cancer cells

One aim of this study is to identify the possible resistance factors of AZD5153. A very recent study by Zhang et al., has implied that AKT activation might be important for the resistance of BRD4 inhibitors [18]. First, two known AKT inhibitors, AKTi-1/2 [24] and MK-2206 [37], were utilized. As shown in Fig. 5A, AKTi-1/2 or MK-2206 blocked phosphorylation of AKT (at Ser-473) and S6K1 (at Thr-389) [38] in PC-3 cells. BRD4 protein

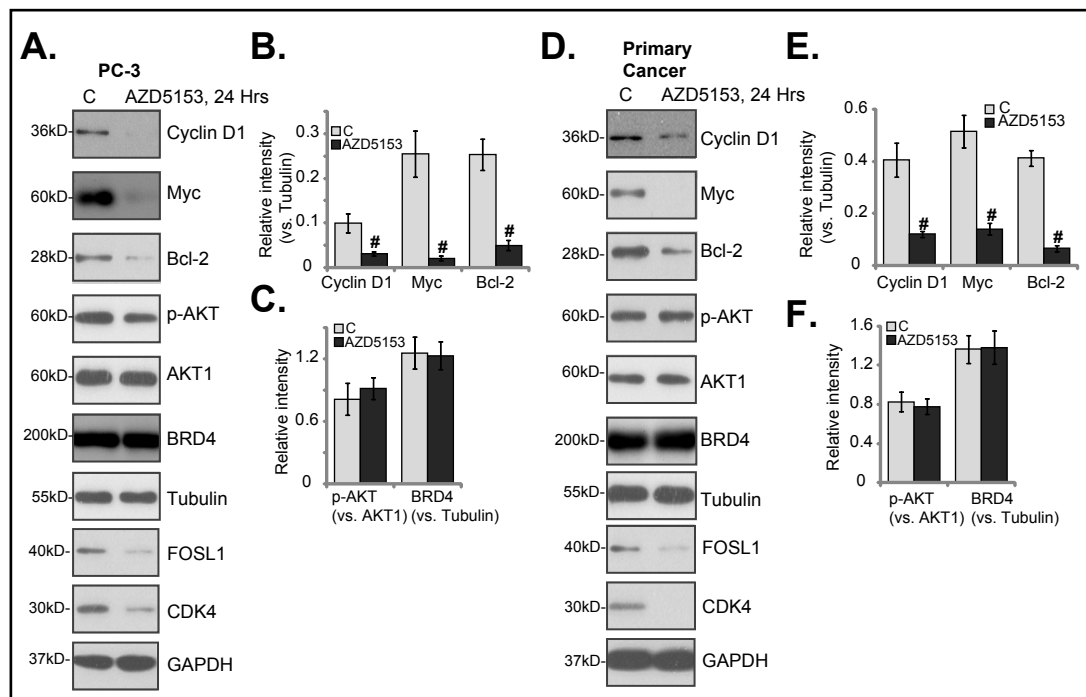


Fig. 4. AZD5153 downregulates BRD4 targets in prostate cancer cells. PC-3 cells (A-C) or the primary human (patient-derived) prostate cancer cells (D-F) were treated with 100 nM of AZD5153 for 24 hours, expression of listed proteins in total cell lysates were tested by the Western blotting assay (A and D); The total gray of each band was quantified, which was normalized to the corresponding loading control (B, C, E and F). "C" stands for cells without treatment. Data are mean ± SD. # P<0.05 vs. "C" cells (ANOVA test). Experiments in this Fig. were repeated five times, and similar results were observed.

and mRNA expression were downregulated as well by the AKT inhibitors (Fig. 5A and B). AZD5153-induced Myc downregulation was however not affected by the AKT inhibitors (Fig. 5A). Significantly, AZD5153 (100 nM)-induced viability reduction (MTT assay, Fig. 5C) and apoptosis activation (TUNEL assay, Fig. 5D) were potentiated by the AKT inhibitors. The CalcuSyn software was applied to calculate Combination Index (CI) using the Chou-Talalay method [39]. CI values between AZD5153 and the AKT inhibitors were less than one, indicating a possible synergism. The AKT inhibitors alone only induced minor cytotoxicity in PC-3 cells (Fig. 5C and D).

To exclude the possible off-target toxicities by the AKT inhibitors, genetic strategies were applied. AKT1 shRNA lentivirus were added to PC-3 cells, resulting in significant AKT1 knockdown (Fig. 5E). Further, CRISPR-Cas-9 gene-editing method was utilized to knockout AKT1. AKT1 expression was depleted in stable PC-3 cells with the CRISPR-Cas-9-AKT1 KO plasmid ("AKT1-KO" cells, Fig. 5E). Significantly, expression BRD4 protein and mRNA were reduced by AKT1 shRNA or knockout (Fig. 5E and F). As a result, AZD5153-induced viability reduction (Fig. 5G) and apoptosis (Fig. 5H) were significantly potentiated. The pharmacological and genetic evidence suggest that AKT is a primary resistance factor of AZD5153 in prostate cancer cells.

AZD5153 oral administration inhibits PC-3 xenograft growth in nude mice

The potential effect of AZD5153 in vivo was tested. PC-3 cells were inoculated s.c. to the nude mice, treatment was started when tumor volume was around 100 mm³. Tumor growth curve results in Fig. 6A demonstrate that oral administration of AZD5153 (10 mg/kg body weight, daily, for 18 days) significantly inhibited PC-3 xenograft growth. When analyzing estimated daily tumor growth, which was calculated by (volume at Day-35 — volume at Day-0)/35, we show again that AZD5153 administration inhibited PC-3 xenograft growth (Fig. 6B). At Day-35, tumors of each group were isolated. AZD5153-treated PC-3 tumors weighted much lower than vehicle control tumors (Fig. 6C). Thus, AZD5153 oral administration inhibited PC-3 xenograft growth in vivo.

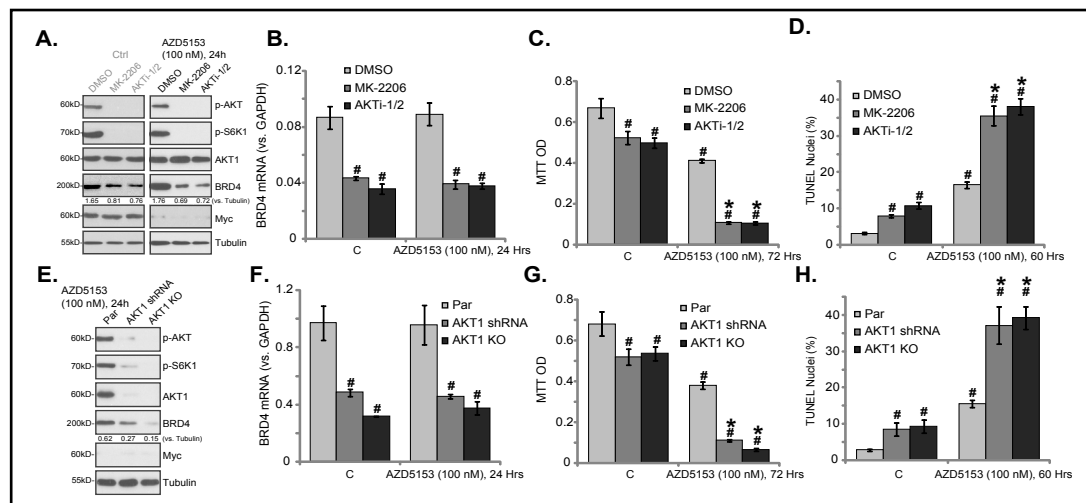


Fig. 5. AKT is a primary resistance factor of AZD5153 in prostate cancer cells. PC-3 cells were treated with 100 nM of AZD5153, with/out AKTi-1/2 (10 μ M) or MK-2206 (10 μ M), expression of listed proteins and BRD4 mRNA were shown (A and B); Cell survival (MTT OD, C) and apoptosis (TUNEL-nuclei ratio, D) were tested. The stable PC-3 cells, with AKT1 shRNA or CRISPR-Cas9-AKT1 ("AKT1 KO"), as well as the parental control PC-3 cells ("Par"), were treated with 100 nM of AZD5153, listed proteins and BRD4 mRNA (E and F) were shown. Cell survival (G) and apoptosis (H) were tested. "C" stands for cells without treatment. BRD4 protein expression was quantified (A and E). Data are mean \pm SD. # P<0.05 vs. "C" cells. * P<0.05 vs. AZD5153 treatment in DMSO (0.1%, C and D) or "Par" cells (G and H). Experiments in this Fig. were repeated five times, and similar results were observed.

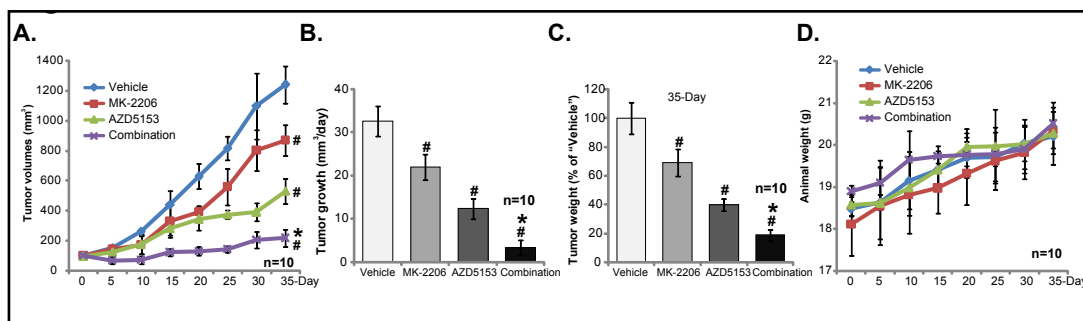


Fig. 6. AZD5153 oral administration inhibits PC-3 xenograft growth in nude mice. PC-3 tumor-bearing nude mice were administrated with AZD5153 (10 mg/kg body weight, daily, p.o.) and/or MK-2206 (5 mg/kg body weight, daily, i.p.) for a total of 18 consecutive days, tumor volume (A) and the mice body weight (D) were recorded every 5 days for a total of 35 days; Estimated daily tumor growth (in mm³ per day) was calculated as described (B). At Day-35, tumors of each group were isolated and weighted (C). For each assay, n= 10 mice. Data are mean ± SD. # P<0.05 vs. "Vehicle" group (ANOVA test) (A-C). * P<0.05 vs. AZD5153 only treatment (A-C).

PC-3 xenograft-bearing nude mice were also co-administrated with MK-2206. As demonstrated, MK-2206 (5 mg/kg body weight, daily, i.p., for 18 days) co-treatment potentiated AZD5153-induced inhibition on PC-3 xenografts (Fig. 6A-C). MK-2206 administration alone only induced relatively weak tumor inhibition (Fig. 6A-C). The combined activity was more potent than each single treatment (Fig. 6A-C). Experimental animals were well-tolerated to the treatment regimens. We failed to detect any significant body weight differences among the mice (Fig. 6D). Therefore, MK-2206 co-administration sensitizes AZD5153-induced anti-tumor activity *in vivo*.

Discussion

BRD4 regulates transcription and expression of several key oncogenes, including cyclin D1, c-Myc, Bcl-2 and androgen receptor (AR) [15]. Recent studies have proposed BRD4 as an important oncotarget protein for prostate cancer [12-14]. Here, we show that AZD5153, a novel bivalent BRD4 inhibitor [21-23], inhibited survival and proliferation of established (PC-3 and LNCaP lines) and primary human prostate cancer cells. Further, AZD5153 induced apoptosis activation and cell cycle arrest in prostate cancer cells. AZD5153-induced anti-prostate cancer cell activity *in vitro* was more potent than other known BRD4 inhibitors (JQ1 and CPI203). *In vivo*, AZD5153 oral administration inhibited PC-3 xenograft tumor growth in nude mice.

Resistances to BET inhibitors have been well-documented, the molecular mechanisms of acquired resistance of BRD4 inhibitors are largely unknown until recently. The gene encoding the E3 ubiquitin ligase substrate-binding adaptor speckle-type POZ protein (SPOP) is commonly mutated in human prostate cancers [40]. Cancer-associated SPOP mutations are observed in the meprin and TRAF (Tumor necrosis factor receptor-associated factor) homology (MATH) domain, inhibiting substrate binding [40]. Recent studies have demonstrated that BET proteins, including BRD2, BRD3 and BRD4, are the targets of SPOP [18-20]. Wild-type SPOP binds to the a degron motif in the BET proteins, causing their ubiquitination and proteasomal degradation [18-20]. Conversely, prostate cancer-associated SPOP mutants are not able to bind to BET proteins, which will lead to impaired BET proteins proteasomal degradation [18-20].

A very recent study by Zhang *et al.*, has suggested that activation of AKT-mTORC1 signaling could be a consequence of BRD4 stabilization in human prostate cancer cells [18]. In this study, we show that inhibition of BRD4 by AZD5153 failed to affect AKT-mTORC1 signaling in prostate cancer cells. Rather, AKT activation could be a major resistance factor

of AZD5153. AKT activation is important for BRD4 expression in prostate cancer cells. Pharmacological inhibition (MK-2206 or AKTi1-2) or genetic depletion (by shRNA/CRISPR-Cas9 method) of AKT induced BRD4 downregulation, which significantly potentiated AZD5153-induced cytotoxicity in prostate cancer cells. *In vivo*, AZD5153-induced anti-tumor activity was sensitized with co-treatment of MK-2206. Thus, AKT inhibition could efficiently sensitize AZD5153 in prostate cancer cells. Further studies will be needed to explore the underlying mechanism of AZD5153 sensitization by AKT inhibition.

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Disclosure Statement

The authors have no conflict of interest to declare.

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