Enzalutamide-Induced Feed-Forward Signaling Loop Promotes Therapy-Resistant Prostate Cancer Growth Providing an Exploitable Molecular Target for Jak2 Inhibitors

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ABSTRACT

The second-generation antiandrogen, enzalutamide, is approved for castrate-resistant prostate cancer (CRPC) and targets androgen receptor (AR) activity in CRPC. Despite initial clinical activity, acquired resistance to enzalutamide arises rapidly and most patients develop terminal disease. Previous work has established Stat5 as a potent inducer of prostate cancer growth. Here, we investigated the significance of Jak2–Stat5 signaling in resistance of prostate cancer to enzalutamide. The levels of Jak2 and Stat5 mRNA, proteins and activation were evaluated in prostate cancer cells, xenograft tumors, and clinical prostate cancers before and after enzalutamide therapy. Jak2 and Stat5 were suppressed by genetic knockdown using lentiviral shRNA or pharmacologic inhibitors. Responsiveness of primary and enzalutamide-resistant prostate cancer to pharmacologic inhibitors of Jak2–Stat5 signaling was assessed in vivo in mice bearing prostate cancer xenograft tumors. Patient-derived prostate cancers were tested for responsiveness to Stat5 blockade as second-line treatment after enzalutamide ex vivo in tumor explant cultures. Enzalutamide-ligated AR induces sustained Jak2–Stat5 phosphorylation in prostate cancer leading to the formation of a positive feed-forward loop, where activated Stat5, in turn, induces Jak2 mRNA and protein levels contributing to further Jak2 activation. Mechanistically, enzalutamide-ligated AR induced Jak2 phosphorylation through a process involving Jak2-specific phosphatases. Stat5 promoted prostate cancer growth during enzalutamide treatment. Jak2–Stat5 inhibition induced death of prostate cancer cells and patient-derived prostate cancers surviving enzalutamide treatment and blocked enzalutamide-resistant prostate cancer growth in mice. This work introduces a novel concept of a pivotal role of hyperactivated Jak2–Stat5 signaling in enzalutamide-resistant prostate cancer, which is readily targetable by Jak2 inhibitors in clinical development.

Introduction

The standard treatment of locally advanced or metastatic prostate cancer is androgen deprivation therapy (ADT; refs. 1, 2). Response to ADT is limited, and prostate cancer becomes castrate-resistant (CR), a stage for which there is no cure (1–4). ADT targets androgen receptor (AR), which translocates from cytoplasm to nucleus upon ligand binding followed by chromatin engagement (5). ADT is carried out by luteinizing hormone–releasing hormone (LHRH) agonists/antagonists or by antiandrogens, which block the AR activity at the tissue level (1–5). The first-generation antiandrogen, bicalutamide, competitively inhibits androgen binding to AR (1–6) and recruitment of AR co-repressors (6). The recently developed second-generation antiandrogen, enzalutamide (ENZ), has gained increasing dominance in the clinical space and is currently FDA approved as a monotherapy in both pre- and postchemotherapy settings (7–10). Enzalutamide is a more effective competitive inhibitor of steroid binding to the androgen-binding pocket on the ligand-binding domain of the AR and retains AR more effective in the cytoplasmic compartment of prostate cancer cells (11). However, despite its initial promise, enzalutamide provides an improvement in patient survival only by 4–6 months due to rapid development of resistance (8, 10, 12, 13).

Mechanisms underlying prostate cancer resistance to enzalutamide are incompletely understood. The current proposed mechanisms include emergence of AR splice variants (14, 15), glucocorticoid
receptor expression (16), a ligand-binding domain mutation F876L in the AR that promotes an antagonist-to-agonist switch of enzalutamide (17, 18), and neuroendocrine differentiation (NE; ref. 19). Moreover, emergence of an AR-null, NE-null prostate cancer phenotype driven by MAPK signaling pathway was recently reported (20). However, no single mechanism has been reliably shown to completely account for progress to enzalutamide resistance in prostate cancer in experimental models or in patients, and it is likely that additional mechanisms are involved.

Stat5a/b (Stat5), which is both a signaling protein and a nuclear transcription factor (21, 22), sustains viability of prostate cancer (23–29). Blockade of Stat5 signaling induces apoptotic death of hormone-naïve prostate cancer cells, suppresses growth of both xenografted and autochthonous prostate cancer tumors as well as clinical patient-derived prostate cancers ex vivo in culture (23–28, 30). Conversely, overexpression of active Stat5 has been shown to induce proliferation of prostate cancer cells in vitro and growth of prostate cancer tumors in mice (31). Stat5 induces metastatic progression of prostate cancer, as evidenced by Stat5 promotion of metastasis formation in vivo, and induction of hallmark signs of EMT and stem-like cancer cell properties through induction of Twist1 and BMI1 expression in prostate cancer (30, 32). In 30%–40% of advanced CRPCs, the chromosome 17 locus encompassing STATA5a and STATA5b genes undergoes amplification resulting in increased Stat5 protein levels (31). Notably, high nuclear Stat5 protein expression at the time of the initial prostate cancer treatment predicted recurrence of the disease in three independent cohorts totaling 1,035 patients (33, 34). The predictive role of active Stat5 for clinical prostate cancer progression to lethal CR state (33, 34) is supported by a higher rate of overexpression of Stat5a/b in prostate cancer tumor xenograft growth of enzalutamide-resistant cell lines (31).

Activation of Stat5 occurs in the cytoplasm through inducible phosphorylation of a conserved C-terminal tyrosine residue (21). Phosphorylated Stat5 (pY694/699) forms functional dimers that translocate to the nucleus and bind to specific DNA response elements to regulate transcription (21). In prostate cancer, Stat5 is activated predominantly by the Jak2 tyrosine kinase (35), a member of Jak family (36). The binding of cytokines, hormones, and growth factors to the specific receptor–Jak2 complex leads to a conformational change in Jak2, which results in autophosphorylation and kinase activation. Phosphorylation of amino acid residues Tyr1007/1008 in the activation loop of the kinase domain is required for full catalytic activity of Jak2 (36). Besides canonical cytokine-activated activation, Jak2 phosphorylation in nonhematopoietic tissues is regulated by a set of Jak2–specific phosphatases, which include SHP-2 (37), PTP1B (38), and PTB (39, 40). In addition, Jak2 phosphorylation state is affected by the levels of Jak2 protein in cells where overexpression of Jak2 leads to Jak2 autoprophosphorylation in the absence of cytokine stimulation (41).

The findings of Stat5 as a prostate cancer growth promoter and a predictor of prostate cancer recurrence implies involvement of Stat5 in the development of CRPC, which led us to hypothesize that Jak2–Stat5 signaling sustains viability of prostate cancer cells following disruption of AR signaling by enzalutamide. We demonstrate, for the first time, that enzalutamide induces a robust increase in Stat5 activation in prostate cancer cells in vitro, in xenograft tumors in vivo, and in patient-derived prostate cancers during enzalutamide treatment. Mechanistically, enzalutamide-liganded AR induces rapid and sustained Jak2 phosphorylation in prostate cancer cells via Jak2-specific phosphatases PTPe and SHP2. Enzalutamide-induced Jak2 activation leads to Stat5 phosphorylation, and the formation of a feed-forward loop in prostate cancer cells where active Stat5 increases Jak2 mRNA and protein levels. We further demonstrate that inhibition of Stat5 as a second-line treatment induces extensive death of prostate cancer cells surviving enzalutamide treatment. Stat5 blockade inhibited CR growth of prostate cancer xenograft tumors after enzalutamide resistance developed and induced further death after enzalutamide treatment in patient-derived prostate cancer s ex vivo in tumor explant cultures.

In summary, this work supports the new concept of a critical role of a hyperactive Jak2–Stat5 signaling loop in promoting resistance of prostate cancer to enzalutamide. Pharmacologic Jak2-Stat5 inhibition may provide an effective therapy for Stat5-positive advanced prostate cancer in combination with enzalutamide or after enzalutamide fails.

Materials and Methods

Prostate cancers from enzalutamide-treated patients

Paraffin-embedded tissues sections of prostate cancers from enzalutamide-treated patients were obtained from the pathology archives at Helsinki University Hospital (Helsinki, Finland) and Medical College of Wisconsin (MCW, Milwaukee, WI). In addition, tissue sections of hormone-naive prostate cancers of corresponding histologic grades were from patients treated by radical prostatectomy (RP) without adjuvant hormone therapy (Helsinki University Hospital and MCW). Patient demographics and clinicopathologic data are presented in Supplementary Table S1A. The study protocol for the samples obtained from archives in Finland was approved by the Ethical Committee of the University of Helsinki, and the National Data Protection Ombudsman was notified about the collection of the information. Tissue sections of prostate cancers from patients before and after enzalutamide treatment were obtained from MCW and the patient demographics and clinicopathologic data are presented in Supplementary Table S1B. These samples were deidentified archival tissues and were granted an exemption from the MCW Institutional Review Board and is in compliance with federal regulations governing research [45 CFR 46102(f)]. Prostate cancer specimens for 3D explant cultures were obtained from patients with localized or locally advanced prostate cancer undergoing RP at MCW (Supplementary Table S2) and were deidentified excess tissue available for research purposes granted an exemption by the MCW Institutional Review Board. All the studies were conducted with the guidelines of Declaration of Helsinki and U.S. Common Rule.

Ex vivo tumor explant cultures of clinical prostate cancers

Detailed information is provided in Supplementary Materials and Methods.

Cell lines and reagents

Human prostate cancer cell lines LNCaP, PC-3, DU145 (from ATCC), and CWR22P (42) were cultured in RPMI1640 growth media (Mediatech) containing 10% FBS (Quality Biological) and penicillin/streptomycin (50 IU/mL and 50 μg/mL, respectively; Mediatech). LAPC-4 cells (from Dr. Charles Sawyers, Memorial Sloan-Kettering Cancer Center, New York, NY) were cultured under the same conditions of RPMI1640 for Iscore’s modified Dulbecco medium (Mediatech). LNCaP, LAPC-4, and CWR22P cells were cultured in the presence of dexamethasone (DHT) (Sigma-Aldrich; LNCaP: 0.5 nmol/L, LAPC-4: 1 nmol/L, CWR22P: 0.8 nmol/L). CWR22P subline expressing AR-F876L (from Dr. Charles Sawyers, Memorial Sloan-Kettering Cancer Center, New York, NY) was cultured in RPMI1640 supplemented with enzalutamide (10 μmol/L). All cell lines were regularly authenticated by observation of cell morphology, androgen responsiveness, and expression of cell
line–specific markers and tested for Mycoplasma contamination (PCR Mycoplasma Detection Set; Takara Bio Inc.) every 3 months. Enzalutamide and AZD1480 were purchased from MedChem Express, MG132, and cyclohexamide from Calbiochem. LFA102 (43) from Novartis, sodium orthovanadate from Sigma-Aldrich, and IST5-002 was provided by Fox Chase Chemical Diversity Center (Doylestown, PA). Recombinant human prolactin (Prl) was obtained from NIDDK Hormone and Peptide Program (Torrance, CA).

**shRNA and cDNA constructs and lentiviral shRNA production**

Detailed information is provided in Supplementary Materials and Methods.

**Adenovirus generation**

Detailed information is provided in Supplementary Materials and Methods.

**Protein solubilization, immunoprecipitation, and immunoblotting**

Detailed information is provided in Supplementary Materials and Methods and antibodies used are listed in Supplementary Table S3.

**Quantitative real-time RT-PCR**

Detailed information is provided in Supplementary Materials and Methods.

**Cell viability analysis**

Detailed information is provided in Supplementary Materials and Methods.

**IHC**

Detailed information is provided in Supplementary Materials and Methods.

**Scoring of cell viability and nuclear Stat5a/b**

Detailed information is provided in Supplementary Materials and Methods.

**Immunofluorescence cytochemistry**

Detailed information is provided in Supplementary Materials and Methods.

**Human prostate cancer xenograft tumor studies**

All the animal studies have been conducted in accordance with and approved Institutional Animal Care and Use Committee (IACUC). Castrated male athymic nude mice (Taconic) were cared for according to the institutional guidelines. Mice were implanted with sustained release DHT pellets (60-day release, 1 pellet/mouse, Innovative Research of America) 7 days prior to prostate cancer cell inoculation. Briefly, 1.5 × 10^7 CWR22Pc cells were mixed with 0.2 mL of Matrigel (BD Biosciences) and inoculated subcutaneously into flanks of nude mice (one tumor/mouse) as described previously (24, 29). Bicalutamide and enzalutamide were dissolved in 0.5% Tween 80 (Sigma-Aldrich)/PBS, AZD1480 in 0.1% Tween 80/0.5% hydroxypropyl methyl cellulose (HPMC, KM4 prep, Dow Chemical)/H_2O_2, and IST5-002 (IST5) in 0.3% hydroxypropyl cellulose (HPC, Sigma-Aldrich)/H_2O.

For the monotherapy study (Fig. 6A), mice (5 mice/group) were treated daily for 32 days by oral gavage with vehicle (0.5% Tween 80/PBS), bicalutamide (30 mg/kg) or enzalutamide (30 mg/kg), or by intraperitoneal injection with IST5-002 (50 mg/kg), DHT pellets were removed from the mice in the castration group concurrently with the start of the treatment period. Tumor dimensions were measured using Vernier calipers three times per week and tumor volumes calculated using the following formula: (3.14 × length × width × depth)/6. Mice were sacrificed, and the tumor tissues were harvested at the end of the 32-day treatment period, or prior to this endpoint if tumor sizes reached 15–20 mm in diameter, and tumor tissues were harvested. Tumor growth rates were calculated from the beginning of drug treatment and are presented as fold changes in tumor volume of each group.

For the sequential therapy study (Fig. 6B and C), mice (10 mice/treatment group) were treated daily for 13 days (phase I) by oral gavage with vehicle (0.5% Tween 80/PBS) or enzalutamide (30 mg/kg) as the first-line therapy. On day 15, mice were randomly distributed into the indicated second-line therapy groups and treated daily for an additional 18 days (phase II) by oral gavage with vehicle, enzalutamide (30 mg/kg), AZD1480 (30 mg/kg), or by intraperitoneal injection with IST5-002 (50 mg/kg), or by combination of enzalutamide (30 mg/kg) and IST5-002 (50 mg/kg). Tumor dimensions were measured twice per week and tumor volumes calculated as described for the monotherapy study. Mice were sacrificed when tumor sizes reached 15–20 mm in diameter in the vehicle-treated group (day 31), and the tumor tissues were harvested.

**Statistical analyses**

Detailed information is provided in Supplementary Materials and Methods.

**Results**

**Enzalutamide-liganded AR induces phosphorylation and activation of Stat5 in prostate cancer**

IHC analyses of the activation status of Stat5 in prostate cancers of patients treated with enzalutamide show that the levels of nuclear active Stat5 were significantly (P < 0.0001) elevated in biopsies of enzalutamide-treated prostate cancers when compared with hormone-naïve prostate cancers of corresponding histologic grades (Fig. 1A; Supplementary Table S1A). In paired prostate cancer samples from patients before and after enzalutamide treatment (Supplementary Table S1B), active Stat5 levels were robustly elevated in the biopsies after enzalutamide treatment compared with the biopsies taken prior to enzalutamide treatment (Fig. 1B). In CWR22Pc and LAPC4 cell lines, enzalutamide induced a rapid increase in Stat5 phosphorylation at 6 hours that was sustained and further increased at day 7 (Fig. 1C).

At the same time, phosphorylation of Stat3 was not induced by enzalutamide in the same cell lines (Fig. 1C). In a CWR22Pc cell subline expressing AR-F876L (CWR22Pc-ENZ-R; ref. 17), which emerges in CWR22Pc cells surviving extended treatment with enzalutamide (>6 months; ref. 18), active Stat5 levels were markedly higher compared with the parental CWR22Pc cells (Fig. 1C). Enzalutamide induction of Stat5 phosphorylation resulted in nuclear translocation of Stat5 to an extent comparable with cytokine-induced Stat5 nuclear translocation (Supplementary Fig. S1), indicating that enzalutamide induces formation of Stat5 dimers capable of nuclear localization in prostate cancer cells.

To investigate whether AR is required for enzalutamide induction of Stat5 signaling, we first analyzed enzalutamide activation of Stat5 in DU145 prostate cancer cell line which is AR-negative. In a parallel set of experiments, we inhibited AR expression in CWR22Pc and LAPC4 cells by genetic knockdown using lentiviral expression of AR shRNA. In the absence of AR, enzalutamide failed to induce Stat5 activation.
Enzalutamide-induced Stat5 activation in prostate cancer cells occurs via Jak2

To assess whether enzalutamide-induced Stat5 activation in prostate cancer cells is dependent on Jak2 signaling, Jak2 activity was pharmacologically inhibited by Jak2 inhibitor AZD1480 (44) during enzalutamide treatment. AZD1480 blocked enzalutamide-induced Stat5 phosphorylation in CWR22Pc and LAPC4 cells (Fig. 2A). To further assess the involvement of Jak2 in enzalutamide-induced Stat5 activation, Jak2 was genetically knocked down by lentiviral expression of Jak2 shRNA (Fig. 1E). In the absence of Jak2, enzalutamide failed to induce Stat5 phosphorylation in both cell lines. Unexpectedly, the protein levels of Jak2 on day 7 were robustly increased in enzalutamide-treated CWR22Pc and LAPC4 cells compared with nontreated cells (Fig. 2B). Collectively, these results indicate that enzalutamide-induced Stat5 activation in prostate cancer cells requires Jak2.

We next investigated whether enzalutamide-ligated AR influences the phosphorylation status of Jak2 in prostate cancer cells. Enzalutamide induced a rapid phosphorylation of Jak2 already at 6 hours in both CWR22Pc and LAPC4 cells to almost the same extent as stimulation of the cells with the cytokine prolactin (Prl; Fig. 2C). Enzalutamide-induced Jak2 phosphorylation was sustained at 12 days and accompanied with a strong induction of Stat5 phosphorylation in both CWR22Pc and LAPC4 cells (Figs. 2C). To further examine whether AR is required for enzalutamide induction of Jak2 activation, AR was suppressed by lentiviral AR shRNA in CWR22Pc cells, which blocked the enzalutamide-induced Jak2 activation (Fig. 2D). In addition, AR agonists were not able to increase Jak2 phosphorylation in prostate cancer cells, because supplementation of culture medium with additional DHT (1.5 μmol/L) for 6 hours, which blocked enzalutamide-induced Jak2 phosphorylation (Fig. 2D). Using lentiviral expression of shRNA, we depleted each of the three Jak2 phosphatases for 3 days prior to enzalutamide treatment (6 hours) of CWR22Pc and LAPC4 cells (Figs. 2C). In both prostate cancer cell lines, enzalutamide-induced Jak2 phosphorylation was significantly attenuated by depletion of either SHP-2 or PTPε. Genetic knockdown of PTPγ resulted in generally elevated levels of Jak2 phosphorylation, which indicates that PTPγ is a crucial negative regulator of Jak2 phosphorylation in prostate cancer cells. Importantly, when PTPε was genetically depleted in prostate cancer cells, there was no difference between enzalutamide treatment versus nontreated cells suggesting that the mechanism of action of enzalutamide on Jak2 phosphorylation involves suppression of PTPε activity (Fig. 2G, left). At the same time, genetic depletion of SHP-2 decreased enzalutamide induction of Jak2 phosphorylation while not increasing the general phosphorylation levels of Jak2 (Fig. 2G, middle). This, in turn, suggests that SHP-2 is a positive regulator of enzalutamide-induced Jak2–Stat5 signaling and is required for enzalutamide induction of Jak2 phosphorylation in prostate cancer cells.
Enzalutamide (ENZ)-liganded androgen receptor induces Jak2 phosphorylation in prostate cancer cells. A, CWR22Pc, LAPC4, and ENZ-R cells were cultured with enzalutamide or Jak2 inhibitor AZD1480 alone or in combination for 12 days at indicated concentrations. Expression levels of active Stat5 were determined by immunoprecipitation (IP) of Stat5 followed by immunoblotting (WB) for pStat5a/b and total Stat5. Whole cell lysates (WCL) were immunoblotted for pStat3, Stat3 and actin. B, Genetic knockdown of Jak2 blocks enzalutamide-induced Stat5 phosphorylation in prostate cancer cells. Jak2 was suppressed by lentiviral Jak2 shRNA versus shCtrl in prostate cancer cells for 3 days followed by enzalutamide or vehicle for 7 days. Active Stat5, Stat5, and Jak2 levels were evaluated by IP and Western blot analysis, as depicted. C, CWR22Pc and LAPC-4 cells were treated with enzalutamide or vehicle for 6 hours at the indicated concentrations. Control cells were stimulated with prolactin (Prl; 10 nmol/L) for 20 minutes as control for cytokine-induced Jak2 phosphorylation. Alternatively, prostate cancer cells were treated with enzalutamide or vehicle for 12 days or stimulated with Prl for 20 minutes at the indicated concentrations. Stat5 and Jak2 were immunoprecipitated and immunoblotted (WB) for pStat5, pJak2, total Stat5, and total Jak2. WCLs were immunoblotted for actin. D, AR is required for enzalutamide induction of Jak2 activation. AR was suppressed in CWR22Pc cells by lentiviral AR shRNA (shAR) versus shCtrl in prostate cancer cells for 3 days followed by enzalutamide or vehicle for 7 days. Enzalutamide induction of Jak2–Stat5 activation requires a cytokine receptor shown by treatment of CWR22Pc cells with enzalutamide alone or in combination with a prolactin receptor antagonist LFA102 for 6 hours or 7 days at the indicated concentrations. (Continued on the following page.)
phosphorylation in prostate cancer cells. A positive regulatory role of SHP-2 for Jak2–Stat5 signaling has been previously reported for several different cell types (45, 46). Genetic knockdown of PTP1B did not affect Jak2 phosphorylation levels nor enzalutamide induction of Jak2 phosphorylation (Fig. 2G, right). In conclusion, these data indicate involvement of Jak2 phosphatases SHP-2 and PTP1B in enzalutamide-induced Jak2 phosphorylation in prostate cancer cells.

Enzalutamide-induced Stat5 activation increases Jak2 mRNA and protein levels and a formation of a positive feed-forward loop in prostate cancer cells

The finding demonstrating that enzalutamide may increase Jak2 protein levels in CWR22Rc and LAPC4 cells (Fig. 2B), led us to evaluate the effects of enzalutamide on phosphorylation, protein levels, and mRNA levels of Jak2 and Stat5 during a time-course ranging from 2 hours to 6 days in CWR22Rc and LAPC4 cells (Fig. 3A and B). Enzalutamide increased phosphorylation of Jak2 in both CWR22Rc and LAPC4 cells at 6 hours, which was accompanied by an increase in Stat5 phosphorylation. Activation of Stat5 reached maximum at 12–16 hours and was sustained at 6 days of exposure to enzalutamide (Fig. 3A). The levels of Jak2 protein, but not Stat5, increased starting at approximately 16 hours (CWR22Rc) and 6 hours (LAPC4) after initiation of the enzalutamide treatment. Concomitant with enzalutamide-induced activation of Jak2 and Stat5, Jak2 mRNA levels were increased by 70% at the 6-hour time-point of enzalutamide treatment, which was sustained at the day 6 (Fig. 3B). In parallel experiments, prostate cancer cells resistant to enzalutamide (CWR22Rc-ENZ-R) displayed markedly higher levels of Jak2 protein (Fig. 3A) and mRNA (Fig. 3B) in comparison with the parental CWR22Rc cells. While inducing Jak2 levels, enzalutamide did not induce Jak1 levels in CWR22Rc or LAPC4 cells indicating specificity of enzalutamide regulation of the phosphorylation state of Jak2 (Fig. 3C).

To further understand the mechanisms underlying enzalutamide induction of Jak2 mRNA levels, we analyzed the significance of Stat5 in this process by genetic knockdown of Stat5 by lentiviral shRNA in CWR22Rc and LAPC4 cells. In the absence of enzalutamide, enzalutamide failed to induce Jak2 mRNA and protein expression and Jak2 mRNA levels were generally decreased by more than 90% (Fig. 3D). This finding suggests that Stat5 is critical for enzalutamide-induced upregulation of Jak2 mRNA expression in prostate cancer cells. Because Stat5 has not been shown to upregulate Jak2 mRNA expression in other cell types, we investigated this in prostate cancer cells in more detail. First, we suppressed Stat5 by lentiviral Stat5 shRNA in CWR22Rc and LAPC4 cells for 3 days and assessed Jak2 mRNA levels by qRT-PCR. Depletion of Stat5 led to a 60%–80% decrease in Jak2 mRNA levels in both CWR22Rc and LAPC4 cells (Fig. 3E, left). In parallel experiments, lentiviral expression of constitutively active (CA) Stat5 (32) in CWR22Rc and LAPC4 cells increased Jak2 mRNA expression by 120%–160%, which was also reflected at Jak2 protein levels (Fig. 3E, middle). In addition, Prl-induced Stat5 activation increased Jak2 mRNA levels by 80%–120% in prostate cancer cells, which was blocked by genetic knockdown of Stat5 by shRNA (Fig. 3E, right). In summary, these results demonstrate that enzalutamide induces Jak2 mRNA and protein levels by Stat5-driven mechanisms in prostate cancer. Collectively, these findings indicate that enzalutamide induces a positive Jak2–Stat5 feed-forward loop in prostate cancer, depicted in Fig. 3F. In this proposed scheme, enzalutamide-liganded AR induces Jak2 phosphorylation in prostate cancer cells through regulation of the activity and function of Jak2 phosphatases. Activated Jak2 leads to phosphorylation of Stat5 which, in turn, increases Jak2 mRNA and protein levels. It is known that elevated Jak2 protein levels result in Jak2 autophosphorylation (41). In addition, increased levels of Jak2 are likely to result in upregulation of Jak2–Stat5 activation in prostate cancer cells induced by enzalutamide.

Stat5 promotes viability of prostate cancer cells surviving enzalutamide treatment

Having established that enzalutamide induces Jak2–Stat5 signaling in prostate cancer, we investigated whether active Stat5 promotes CRPC growth during enzalutamide treatment. First, constitutively active (CA) Stat5 (32) was expressed in CWR22Rc and LAPC4 cells using lentivirus during treatment of the cells with increasing doses of enzalutamide ranging from 5 to 40 μmol/L for 6 days. Expression of CAStat5 increased the fraction of viable CWR22Rc and LAPC4 cells at all concentrations of enzalutamide with the maximum increase of 60% (P < 0.0001) and 45% (P < 0.0001) in CWR22Rc and LAPC4 cells (Fig. 4A, B, D, and E). The efficacy of lentiviral CAStat5 expression in prostate cancer cells was confirmed by immunoblotting (Fig. 4C and F).

To investigate the efficacy of inhibition of Jak2–Stat5 signaling in enzalutamide-resistant prostate cancer cells that survive enzalutamide treatment, we used a specific Stat5 inhibitor IST5-002 (47) or Jak2 inhibitor AZD1480 (44). Prostate cancer cells were exposed to enzalutamide for 5 days followed by treatment with IST5-002 or AZD1480 for 5 or 10 days (Fig. 5A). Control cells were grown in culture medium (MOCK), in the presence of vehicle (VEH), enzalutamide, IST5-002 (ISTS), AZD1480, or enzalutamide combined with IST5-002 (ENZ + IST5) or AZD1480 (ENZ + AZD1480) for 5, 10, or 15 days, as depicted in Fig. 5A. In comparison with the control group (enzalutamide followed by vehicle: ENZ > vehicle), CWR22Rc cells treated at the start of the experiment with enzalutamide and later switched to IST5-002 after 5 days (ENZ > IST5-002) incurred additional loss of viability at both time points with the maximal reduction of 50% at 10 days of enzalutamide treatment (15-day time-point, P < 0.0001; Fig. 5B). Notably, IST5-002 alone or in combination with enzalutamide was more efficacious (P < 0.001) in reducing viability of CWR22Rc cells than enzalutamide applied as a single treatment (Fig. 5B). This may be due to enzalutamide induction of hyperactive Jak2–Stat5 signaling loop, which leads to Stat5 promotion of prostate cancer cell viability and growth. Moreover, when the cells were pretreated with enzalutamide, inhibition of Stat5 was less efficient in reducing prostate cancer cell viability compared with Stat5 inhibition monotherapy or combination treatment of the cells with both IST5-002 and enzalutamide (Fig. 5B). Similarly, this may be explained by enzalutamide activation of positive Jak2–Stat5 feed-forward loop, which sets a barrier for a second-line...
therapy to overcome. Viability of CWR22Pc in each group was visualized and quantified by cell-cycle analysis and crystal violet staining (Fig. 5C and D). The fractions of viable cells were determined by counting three separate fields from each of the three parallel wells, and the averages are indicated by a line graph with SDs. Crystal violet staining (B and E) and immunoblotting of whole cell lysates (WCL) for Stat5 and actin on day 6 (C and F).

Given that enzalutamide induces a marked increase in Jak2 phosphorylation in prostate cancer cells, we next assessed the efficacy of a sequential application of enzalutamide followed by AZD1480 (44) as a potential treatment strategy. Figure 3.

**Figure 3.** Enzalutamide (ENZ) induces a formation of a positive Jak2-Stat5 feed-forward loop in prostate cancer. A, Enzalutamide-induced Jak2 phosphorylation is accompanied by an increase in Stat5 phosphorylation and Jak2 protein levels in prostate cancer cells. CWR22Pc, LAPC-4, and ENZ-R cells were treated with enzalutamide or vehicle during a time-course at the indicated concentrations. Prostate cancer cells were stimulated with Prl (10 nmol/L) as control for Jak2 and Stat5 phosphorylation. The phosphorylation status of Stat5 and Jak2 was determined by IP of Stat5 and Jak2 followed by immunoblotting for pStat5, total Stat5, pJak2, and total Jak2. WCLs were immunoblotted for actin. B, Enzalutamide induces Jak2 mRNA levels in prostate cancer cells. CWR22Pc and LAPC-4 cells were treated with enzalutamide for indicated periods of time or infected with lentiviral Jak2 shRNA (shJak2) versus shCtrl (Jak2 mRNA control; 3 days) followed by qRT-PCR. C, Jak2, but not Jak1, levels are induced by enzalutamide. Prostate cancer cells were treated with enzalutamide or vehicle for 12 days. Jak1 and Jak2 were immunoprecipitated and blotted for Jak1 and Jak2. WCLs were immunoblotted for actin. D, Enzalutamide increases Jak2 protein levels by Stat5-driven upregulation of Jak2 mRNA expression in prostate cancer cells. When Stat5 was depleted in prostate cancer cells by lentiviral Stat5 shRNA (shStat5) or shCtrl for 3 days followed by enzalutamide or vehicle for 7 days, enzalutamide failed to increase Jak2 mRNA levels. For Jak2 mRNA control, Jak2 was suppressed by lentiviral Jak2 shRNA (shJak2) or shCtrl (left) for 3 days. Jak2 mRNA levels were determined by qPCR and protein levels by IP and Western blot analysis (WB) as described in A, E, CWR22Pc and LAPC-4 cells were infected with lentiviral Stat5 shRNA (shStat5), shCtrl (top and bottom panels), constitutively active (CA) Stat5 or GFP for 3 days (middle). After 3 days, prostate cancer cells were treated with Prl for 3 days (bottom). IP, immunoblotting (WB), and qPCR were conducted as described in A and B, F. Schematic representation of the proposed enzalutamide induction of hyperactivated Jak2-Stat5 feed-forward loop in prostate cancer.

**Figure 4.** Stat5 promotes viability of prostate cancer cells during enzalutamide (ENZ) treatment. Constitutively active (CA) Stat5 or GFP was lentivirally expressed in CWR22Pc and LAPC-4 cells for 2 days followed by enzalutamide treatment (5, 10, 20, 30, and 40 μmol/L) or vehicle for 6 days (A and D). The fractions of viable cells were determined by counting three separate fields from each of the three parallel wells, and the averages are indicated by a line graph with SDs. Crystal violet staining (B and E) and immunoblotting of whole cell lysates (WCL) for Stat5 and actin on day 6 (C and F).
representative of Jak2 inhibitors. Compared with control (ENZ > vehicle), CWR22Pc cells initially receiving enzalutamide at the start of the experiment for 5 days and switched to AZD1480 for 5 or 10 days (ENZ > AZD1480) exhibited additional loss of 15%–20% of cell viability at both time points (P < 0.05; Fig. 5E–G). Stat5 inhibition was verified by immunoblotting in cells receiving AZD1480, combined enzalutamide and AZD1480, or enzalutamide followed by AZD1480 (Fig. 5E). In summary, Jak2 inhibition by AZD1480 induced extensive death of CWR22Pc cells that survived enzalutamide treatment.

To investigate whether prostate cancer cells encompassing AR with the mutation conferring enzalutamide resistance are sensitive to inhibition of Jak2–Stat5 signaling with cell viability as the endpoint, Jak2–Stat5a/b signaling was inhibited in both parental CWR22Pc and ENZ-R (AR-F876L) cells by AZD1480 or IST5-002. While parental CWR22Pc cells remained sensitive to enzalutamide, viability of enzalutamide-resistant (AR-F876L) CWR22Pc cells was unaffected by enzalutamide, as expected (Fig. 5H). Viability of both parental and enzalutamide-resistant CWR22Pc cells was decreased by both IST5-002 (12.5 μmol/L; P < 0.001) or AZD1480 (P < 0.001; 0.8 μmol/L) at 10 days, indicating that enzalutamide-resistant CWR22Pc cells remained sensitive to Stat5a/b inhibition (Fig. 5H). Enzalutamide-resistant cells displayed uniformly high levels of active Stat5 (Fig. 5H). Collectively, these findings support the concept of an emergence of sustained upregulation of Jak2–Stat5 activation during long-term enzalutamide treatment. To summarize, these data demonstrate that active Stat5 increases viability and growth of prostate cancer cells during enzalutamide treatment, and pharmacologic inhibition of Jak2–Stat5 signaling induces death of prostate cancer cells surviving enzalutamide treatment.

**Stat5 inhibition decreases growth of enzalutamide-resistant prostate cancer xenograft tumors and patient-derived prostate cancers in tumor explant cultures *ex vivo***

Given that Stat5a inhibition potently induced apoptotic death of prostate cancer cells depleted of AR signaling, we sought to extend our *in vitro* findings to prostate cancer xenograft tumor growth *in vivo*. Castrated athymic nude mice, implanted with sustained-release DHT pellets to normalize circulating androgen levels, were inoculated with CWR22Pc cells. CWR22Pc tumors are known to initially display androgen-sensitive growth and regress upon castration, but later recur as castrate-resistant tumors (42). The mice were treated with bicalutamide, enzalutamide, or IST5-002 daily for 32 days with vehicle or surgical castration as control groups (Fig. 5I). Tumors in the castration group rapidly resumed the same growth rate as tumors in the vehicle-treated mice, suggesting the presence of adrenal and/or intracrine androgen synthesis. As expected, enzalutamide was more effective than bicalutamide in blocking androgen-sensitive CWR22Pc tumor growth (P < 0.001; Fig. 6A). In line with the higher efficacy of IST5-002 than enzalutamide in inducing death of prostate cancer cells *in vitro* shown in Fig. 5, IST5-002 was superior to enzalutamide (P < 0.05) at suppressing *in vivo* androgen-sensitive growth of CWR22Pc xenograft tumors in the presence of circulating androgens (Fig. 6A). IHC analyses showed decreased nuclear Stat5 content within tumor cells treated with IST5-002, while nuclear Stat5 levels were elevated in tumors treated with enzalutamide (Supplementary Fig. S2A). Collectively, these results indicate that Stat5 inhibition by IST5-002 blocked CWR22Pc xenograft tumor growth equally or more effectively than enzalutamide.

To assess therapeutic efficacy of sequential targeting of AR and Stat5 in prostate cancer tumors *in vivo*, we designed a two-phase *in vivo* experiment, depicted in Fig. 6B. Mice received first-line therapy of vehicle or enzalutamide alone until emergence of resistance to enzalutamide occurred (day 13), followed by randomization and switch to a second-line therapy (vehicle, enzalutamide, AZD1480, IST5-002, or ENZ + IST5-002) daily for additional 18 days. Tumors in mice receiving vehicle during the first phase represent androgen-sensitive prostate cancer growth (Fig. 6C), and final fold changes in tumor volume (volume at day 31/day 0 ± SEM) at the end of the second phase (day 31) were as follows: vehicle > vehicle, 41.6 ± 1.9; vehicle > ENZ, 29.8 ± 2.5; vehicle > AZD1480, 23.7 ± 0.9; vehicle > IST5-002, 18.9 ± 2.0; vehicle > ENZ + IST5-002, 14.6 ± 2.0 (Fig. 6C). As expected, enzalutamide reduced androgen-sensitive CWR22Pc xenograft tumor growth significantly compared with vehicle-treated tumors (P < 0.05). At the same time, Stat5 inhibition by IST5-002 alone suppressed androgen-sensitive prostate cancer xenograft tumor growth equally effectively as enzalutamide when compared with the control group (vehicle; P < 0.01). However, the growth of androgen-sensitive prostate cancer tumors was suppressed more effectively by combination of IST5-002 with enzalutamide when compared with enzalutamide alone (P = 0.02; Fig. 6C). In conclusion, while enzalutamide alone suppressed growth in the presence of androgens as expected, combination of enzalutamide with IST5-002 was remarkably more effective than enzalutamide alone.

Prostate cancer tumors in mice receiving enzalutamide during the first phase developed resistance as evidenced by regrowth of tumors in enzalutamide-treated mice starting on day 13. At the end of the second-line treatment on day 31, final fold changes in enzalutamide-resistant tumor volumes were as follows: ENZ > vehicle, 22.8 ± 2.3; ENZ > ENZ, 13.5 ± 1.5; ENZ > AZD1480, 8.9 ± 1.3; ENZ > IST5-002, 7.3 ± 0.9; ENZ > ENZ + IST5-002, 4.9 ± 0.6 (Fig. 6D).
Importantly, IST5-002 alone suppressed enzalutamide-resistant prostate cancer tumor growth. At the same time, combined enzalutamide with IST5-002 was most effective in suppressing enzalutamide-resistant CRPC tumor growth when compared with combined enzalutamide treatment (P < 0.01). IHC analysis of Stat5 demonstrated that enzalutamide increased nuclear Stat5 in both androgen-induced and castrate-resistant tumors (Supplementary Fig. S2). In conclusion, growth of enzalutamide-resistant CRPC xenograft tumors was most effectively suppressed by a second-line therapy of combined enzalutamide and IST5-002.

To evaluate the efficacy of combined enzalutamide and IST5-002 as a second-line therapy in clinical patient-derived prostate cancers, we utilized an ex vivo 3D tumor explant culture system of patient-derived prostate cancers, which we have established and characterized previously (25, 32, 47–49). Crucial interactions between prostate epithelium and stroma are retained in tumor explant cultures and, therefore, it is considered a more physiologic model of prostate cancer growth than cell lines or primary cell cultures. Prostate cancers from seven individual patients (Supplementary Table S2) were cultured ex vivo in tumor explant cultures with enzalutamide or vehicle alone for 8 days as control groups. Prostate cancers receiving combined enzalutamide and IST5-002 as the second-line therapy demonstrated extensive loss of viable epithelium at the end of the culture period, with only 15% viable cells at the end of the treatment period compared with enzalutamide monotherapy (30%; P < 0.05) or vehicle (80%; P < 0.0001; Fig. 6E). Nuclear levels of active Stat5 evaluated by IHC, were increased in the explants treated with enzalutamide compared with vehicle-treated explants (P = 0.05). At the same time, treatment of the explants with the combination of enzalutamide and IST5-002 as a second-line therapy reduced the nuclear active Stat5 levels compared with enzalutamide (P = 0.05; Fig. 6F). Overall, these data suggest that second-line therapy with combined enzalutamide and IST5-002 was more effective than enzalutamide alone in inducing epithelial cell death in patient-derived clinical prostate cancers ex vivo.

**Discussion**

Prostate cancer cells require androgen receptor (AR) signaling for growth and survival, a dependency exploited by androgen deprivation therapy (ADT) for locally advanced, recurrent, or metastatic prostate cancer. A more potent second-generation AR antagonist, enzalutamide, was developed to re-target the persistent AR activity in CRPC tumors and has become standard-of-care in this setting (7–9). Enzalutamide is approved as first-line therapy for metastatic and nonmetastatic CRPC (8, 10). However, despite these advances, CRPC remains a uniformly lethal disease due to rapid development of resistance. Identification of molecular mechanisms and pathways that drive growth of enzalutamide-resistant prostate cancer cells holds potential for development of effective first-line combination therapies that suppress development of enzalutamide resistance or second-line therapies once enzalutamide fails. In this study, we show, for the first time, that enzalutamide induces a hyperactive Jak2–Stat5 signaling loop in prostate cancer through mechanisms that involve Jak2 phosphatases PTPr and SHP-2. Active Stat5 increases prostate cancer cell survival during enzalutamide treatment and, conversely, Stat5 inhibition blocked growth of prostate cancer cells surviving enzalutamide treatment. Pharmacologic inhibition of Stat5 suppressed enzalutamide-resistant growth of xenograft tumors that emerged during enzalutamide treatment and reduced cell viability in patient-derived clinical prostate cancers cultured ex vivo in tumor explant cultures. Pharmacologic inhibitors of Jak2–Stat5 signaling may be effective agents to overcome enzalutamide-resistant CRPCs driven by Jak2–Stat5.

One of the key results of the work presented here is that enzalutamide induced a hyperactive Jak2–Stat5 feed-forward signaling loop in prostate cancer, as demonstrated in prostate cancer cell lines, prostate cancer xenograft tumors, and clinical prostate cancers from patients treated with enzalutamide. Eight of 8 enzalutamide-treated clinical prostate cancer specimens examined showed high Stat5 signaling. This finding has high translational significance because Stat5 is a potent inducer of prostate cancer growth and a therapeutically targetable survival factor in prostate cancer. Pharmacologic inhibition of Stat5 suppresses enzalutamide-resistant growth of both androgen-dependent and enzalutamide (ENZ)-resistant xenograft tumors in nude mice and in patient-derived prostate cancers ex vivo in tumor explant cultures. A. Inhibition of Stat5 by IST5-002 suppressed androgen-sensitive CWR22Pc xenograft tumor growth more effectively than bicalutamide (BIC) or enzalutamide, as shown by tumor growth curves (top) and representative tumor images (bottom). CWR22Pc cells were inoculated subcutaneously (s.c.) into flanks of castrated athymic nude mice supplied with sustained release DHT pellets. Mice were surgically castrated or vehicle alone at the start of the experiment. Mice were treated with enzalutamide (50 mg/kg), enzalutamide-resistant (right) (ENZ; 50 mg/kg), enzalutamide (30 mg/kg), or IST5-002 (IST5; 50 mg/kg), and tumor growth rates were calculated for each treatment group and are presented as fold changes in tumor volume (volume at timepoint/volume at treatment start). B. Experimental design for the sequential therapy of the xenograft tumors in vivo. A two-phase in vivo experiment using vehicle or enzalutamide as first-line therapy (phase I, 15 days) and vehicle, enzalutamide, AZD1480, IST5-002, or ENZ + IST5-002 as second-line therapy (phase II, 18 days). On day 31, mice were sacriﬁced and CWR22Pc xenograft tumors collected for analyses. Sequential, second-line therapy with IST5-002 (IST5) or ENZ + IST5-002 (IST5) effectively suppressed the growth of androgen-sensitive (left, C) and enzalutamide-resistant (right, D) CWR22Pc xenograft tumors, as shown by tumor growth curves (top) and representative tumor images (bottom). CWR22Pc cells were inoculated subcutaneously into flanks of castrated athymic nude mice supplied with sustained release DHT pellets. Mice were treated with vehicle or enzalutamide (50 mg/kg) for 13 days. On day 13, mice were randomly distributed into subgroups and treated daily with an additional 18 days with vehicle, enzalutamide (50 mg/kg), AZD1480 (30 mg/kg), IST5-002 (IST5; 50 mg/kg), or enzalutamide (30 mg/kg) + IST5-002 (IST5; 50 mg/kg). Tumor growth curves are calculated and presented as described in A. E, To test the efficacy of a second-line therapy with combined enzalutamide and IST5-002 (IST5) versus enzalutamide monotherapy in clinical prostate cancers, we utilized an ex vivo 3D tumor explant culture system of patient-derived prostate cancers obtained from radical prostatectomies cultured ex vivo in tumor explant cultures. Seven localized prostate cancers obtained from radical prostatectomies were cultured ex vivo in tumor explant cultures in the presence of vehicle (8 days), enzalutamide (40 μM/L) alone (8 days), or enzalutamide (40 μM/L) as first-line therapy (4 days) followed by enzalutamide (40 μM/L) and IST5-002 (IST5; 25 μM/L) as a second-line therapy (4 days). Second-line therapy with combined enzalutamide and IST5-002 (IST5) induced cell death in clinical prostate cancer more effectively than enzalutamide alone, as demonstrated by extensive loss of viable acinar epithelium. The number of viable epithelial cells in the explants at the end of the culture period was counted and presented as percentage per epithelial cells prior to culture. Representative histologies are shown (image magnification 40×; scale bar, 50 μm). F, Levels of nuclear Stat5 in the prostate cancer explants were determined by immunostaining using biotin-streptavidin-amplified peroxidase-antiperoxidase immunodetection at the end of the cultures and expressed as percentages of Stat5-positive cells per 100 epithelial cells in explants for each treatment group (12–20 explants; image magnification 40×; scale bar, 50 μm).
cancer (23–28, 32). We show here that the initial step in the hyperactive Jak2–Stat5 signaling in enzalutamide-treated prostate cancer cells was induction of Jak2 phosphorylation by enzalutamide-liganded AR. Enzalutamide-induced Jak2 activation was accompanied by simultaneous and robust upregulation of Stat5 phosphorylation in prostate cancer. In addition, the levels of both Jak2 mRNA and protein were increased by exposure of prostate cancer cells to enzalutamide, as demonstrated by parallel evaluation of Stat5 and Jak2 phosphorylation, protein levels, and Jak2 mRNA expression in two prostate cancer cell lines during a time-course ranging from 2 hours to 6 days. Enzalutamide induction of Jak2 mRNA levels preceded enzalutamide induction of Jak2 protein expression, which suggests that Jak2 mRNA levels are predominantly induced by enzalutamide and lead to increased Jak2 protein levels in prostate cancer. When Stat5 was genetically suppressed, enzalutamide failed to induce Jak2 mRNA and protein levels. This demonstrates that enzalutamide-induced Stat5 activation is crucial for enzalutamide-induced upregulation of Jak2 in prostate cancer. Separate evaluation of Stat5 regulation of Jak2 mRNA and protein expression by genetic knockdown or overexpression approaches revealed that Jak2 mRNA levels are tightly regulated by Stat5 in prostate cancer. Future work will need to determine whether Stat5 induces Jak2 mRNA stability and/or the transcription of the Jak2 gene in prostate cancer and the associated enhancer elements and their genomic loci.

Jak2 phosphorylation triggered by enzalutamide in prostate cancer cells was rapid occurring already at 6 hours and was further increased over the 12-day period tested here. The fact that ongoing protein synthesis was required for enzalutamide induction of Jak2 phosphorylation and that a broad-spectrum phosphatase inhibitor blocked enzalutamide-induced Jak2 phosphorylation both suggest mechanistic involvement of Jak2 phosphatases in this process. Genetic AR knockdown or androgen depletion failed to increase Jak2–Stat5 signaling, which indicates that phosphorylation of Jak2 is not triggered by cellular stress or androgen pathway suppression in general. We further showed that genetic depletion of PTPe, but not SHP-2 or PTP1B, resulted in elevated levels of Jak2 phosphorylation, suggesting that PTPe is a key phosphatase and a negative regulator of Jak2 phosphorylation in prostate cancer. PTPe knockdown abolished the difference in Jak2 phosphorylation levels in enzalutamide-treated versus nontreated cells, which implies that enzalutamide-ligated AR directly or indirectly suppresses the activity of PTPe in prostate cancer cells. At the same time, depletion of SHP-2 levels decreased enzalutamide induction of Jak2 phosphorylation. This is consistent with a previously reported role of SHP-2 as a positive regulator of Jak2 activation (45, 46) and is required for enzalutamide induction of Jak2 phosphorylation in prostate cancer cells. The finding that AR liganded by DHT did not increase phosphorylation of either Stat5 or Jak2 implies that enzalutamide induction of Jak2 phosphorylation may be caused by cytoplasmic actions of the AR. Future studies will need to establish the mechanisms underlying enzalutamide regulation of enzymatic activity of PTPe as well as the mechanistic role of SHP-2 in enzalutamide-enhanced coupling of Jak2–Stat5 signaling in prostate cancer.

Active Stat5 increased the viability of prostate cancer cells during enzalutamide treatment and, conversely, Stat5 inhibition induced death of the residual prostate cancer cells surviving enzalutamide therapy. In addition, pharmacologic inhibition of Stat5 in combination with enzalutamide displayed greater efficacy in suppressing growth of prostate cancer in vitro and in vivo than enzalutamide alone in both androgen-sensitive and castrate-resistant settings. Combined enzalutamide and IST5-002 as second-line therapy was more effective than enzalutamide monotherapy in suppressing in vivo growth of CRPC xenograft tumors and ex vivo growth of clinical patient-derived prostate cancers in tumor explant cultures. Importantly, Stat3 has been implicated in enzalutamide-resistant CRPC growth in different preclinical models than those used in this study (50). Evaluation of the efficacy of Jak2 inhibitors that are currently in the clinical development for myeloproliferative disorders for blocking enzalutamide-resistant prostate cancer tumor growth will be essential for transition to phase 1/II studies in prostate cancer and may provide efficacious second-line treatment for enzalutamide-resistant prostate cancer. Clinical evaluation of the efficacy of Jak2–Stat5 inhibitors in enzalutamide-resistant prostate cancers should utilize positive Stat5 activation status as a biomarker for patient selection for accrual.

In summary, this work introduces a concept of a hyperactive Jak2–Stat5 signaling loop as a mechanism mediating resistance of prostate cancer to enzalutamide. The Jak2–Stat5 pathway provides an attractive therapeutic target for enzalutamide-resistant prostate cancer as a second-line treatment or for first-line therapy in combination with enzalutamide. Future work will need to evaluate the efficacy of Jak2 inhibitors in the treatment of enzalutamide-resistant prostate cancer with positive status for active Stat5 as one of the inclusion criteria.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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