Tumor and Stem Cell Biology

Targeting Pioneering Factor and Hormone Receptor Cooperative Pathways to Suppress Tumor Progression

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Abstract

Nuclear receptors and pioneer factors drive the development and progression of prostate cancer. In this disease, aggressive disease phenotypes and hormone therapy failures result from resurgent activity of androgen receptor (AR) and the upregulation of coactivator protein p300 and pioneer factors (e.g., GATA2 and FOXA1). Thus, a major emphasis in the field is to identify mechanisms by which castrate-resistant AR activity and pioneer factor function can be combinatorially suppressed. Here we show that the turmeric spice isoflavone curcumin suppresses p300 and CBP occupancy at sites of AR function. Curcumin reduced the association of histone acetylation and pioneer factors, thereby suppressing AR residence and downstream target gene expression. Histone deacetylase inhibitors reversed the effects of curcumin on AR activity, further underscoring the impact of curcumin on altering the chromatin landscape. These functions precluded pioneer factor occupancy, leading ultimately to a suppression of ligand-dependent and ligand-independent AR residence on chromatin. Moreover, these functions were conserved even in cells with heightened pioneer factor activity, thus identifying a potential strategy to manage this subclass of tumors. Biological relevance was further identified using in vivo xenograft models mimicking disease progression. Curcumin cooperated in vivo with androgen deprivation as indicated by a reduction in tumor growth and delay to the onset of castrate-resistant disease. Together, our results show the combinatorial impact of targeting AR and histone modification in prostate cancer, thus setting the stage for further development of curcumin as a novel agent to target AR signaling.

Introduction

Steroid hormone receptors are critical targets for therapeutic intervention in hormone-dependent cancers, including tumors of the breast and prostate. Although current strategies to suppress receptor activity entail use of agents that deplete ligand or compete for the ligand binding domains of steroid receptors [e.g., estrogen receptor (ERs) in breast cancer and the androgen receptor (AR) in prostate cancer], tumor cells develop sophisticated mechanisms to bypass receptor-directed therapeutics (1, 2). In prostate cancer, a major mechanism of therapeutic failure and progression to advanced disease is inappropriate reactivation of AR (2). This stage of disease is referred to as castrate-resistant prostate cancer (CRPC), and a plethora of clinical and preclinical studies strongly support the contention that AR remains essential for growth and survival in CRPC (3, 4). Thus, development of novel treatments that can act in concert with AR-directed therapeutics would be of benefit.

Activated nuclear receptors function as ligand-dependent transcription factors. Hence, receptor activity largely depends on access to binding sites on chromatin, facilitated in part by histone-modifying enzymes (which directly promote a chromatin landscape favorable for transcriptional activation) and pioneer factors such as FOXA1 and GATA2 (which promote open chromatin structure, subsequent nuclear receptor binding, and resultant initiation of context-specific transcriptional programs; refs. 5–7). Histone acetyl transferases (HAT) such as p300 and CBP promote AR-mediated transcription (8) and harbor protumorigenic activity. Notably, human prostate tumors expressing high levels of p300 show aggressive phenotypes accompanied by increased proliferation and poor prognosis (9). p300 and CBP also promote transcriptional activity of selected pioneer factors (e.g., GATA2), which play critical roles in AR-dependent transcription (10) and are elevated in human disease (1, 11). Resistance to treatment mediated by upregulation of pioneer factors is attributed, in part, through the ability to interact with AR and increase transcriptional activity (12, 13); consonantly, FOXA1 can promote CRPC development (13). Collectively, these observations suggest that disrupting pioneer factor binding and/or activity may be advantageous.

Here, using xenograft models mimicking androgen deprivation therapy (ADT)-sensitive and CRPC prostate cancer, it is shown that the isoflavone curcumin suppresses both CBP/p300 activity and pioneer factor function, thereby attenuating both ligand-dependent and castrate-resistant AR activity.
Remarkably, these effects were conserved in both ADT-sensitive and CRPC model systems. Substantial in vitro and in vivo analyses further show that curcumin cooperates with hormone therapy to suppress AR-dependent cell proliferation, tumor growth, and the transition to castration resistance. The findings presented herein suggest a new paradigm for nuclear receptor inhibition that may be relevant for a multitude of nuclear receptor–dependent cancers.

Materials and Methods

Cell culture, reagents, and cell-based assays

LNCaP, LAPC4, VCaP, C4-2, 22Rv1 cells were cultured in androgen ablative condition using charcoal dextran–treated (CDT) FBS as described (14). Prostatic epithelial benign cells (BHPRe1) were cultured in presence of androgen as described (15). Curcumin (C7727), TSA (T8552), and DHT were obtained from Sigma-Aldrich. All experiments were conducted with at least 3 independent biological replicates. Statistical significance was determined using Student t test, *, P < 0.05. Proliferation assays were done as previously described (14). Cells cultured in androgen deprivation were transiently transfected using Lipofectin (Invitrogen) reagent and treated with vehicle or curcumin (2.5 μmol/L) for 24 hours. Plasmids encoding wild-type AR (pSG5-AR) and GATA2 construct have been previously described (11, 16). mRNA quantitation was done as described in Supplementary Data.

Immunoblot

Cells were treated with vehicle or curcumin and subjected to SDS-PAGE for immunoblotting as previously described (14), using antisera described in the Supplementary Data.

Chromatin immunoprecipitation and qPCR

Cells seeded in androgen deprivation for 72 hours were treated with ethanol or curcumin (8 μmol/L). For ADT-sensitive cells, DHT (1 nMol/L) was added 1 hour subsequent to curcumin treatment. At selected time points, cells were fixed and chromatin immunoprecipitation (ChIP) analyses were done as described (14). Antibodies and primers used for ChIP are listed in Supplementary Tables S1 and S2. For RNA analyses, cells in absence of androgen were treated with either vehicle or curcumin (2.5 μmol/L) for 24 hours. TRIzol reagent extracted RNA was used to generate cDNA using the superscript reverse transcription PCR system (Invitrogen). Quantitative PCR (qPCR) was conducted using Power SYBR-Green reagent (Applied Biosystems). Primer sequences are provided in Supplementary Table S1.

Xenograft analyses

Xenografts were developed as described (17). Mice were subjected to bilateral orchectomy when tumors reached 200 to 250 mm³. Tumor-bearing animals were randomized into control group (receiving 100 μL vehicle IP/48 hours) or a treatment group (receiving 50 mg/kg/d curcumin IP; ref. 18). Tumor volumes were monitored weekly using calipers, and animals were followed for 5 weeks postcastration. Bromodeoxyuridine (BrdUrd) analysis and immunoblotting was done as described (17).

Results

Curcumin cooperates with hormone ablation to suppress cell growth and survival in vitro

Nuclear receptor–directed therapeutics typically entail agents that act through the ligand binding domain to modulate receptor signaling in disseminated prostate cancer (19, 20). However, recurrent tumors arise through aberrant reactivation of the receptor (2, 14, 20–22). Thus, development of strategies for suppression of aberrant receptor activity could confer significant benefit. The isoflavone curcumin has been suggested in preclinical models of prostate cancer to inhibit receptor function and decrease cell number, albeit using doses that may not be pharmacologically attainable (23). Here, these findings held true using physiologically attainable doses of curcumin (1–2.5 μmol/L, Supplementary Fig. S1; ref. 24). Because curcumin can suppress cell survival through multiple mechanisms, it was hypothesized that curcumin may act in concert with existing prostate cancer therapeutics. As ADT is the first line of therapeutic intervention for nonorgan confined tumors (2, 20), cells were subjected to hormone deprivation in the presence and absence of curcumin. As expected, all ADT-sensitive model systems exhibited a cytostatic effect upon hormone withdrawal used to mimic ADT. Remarkably, curcumin (1–2.5 μmol/L) augmented the effects of ADT (Fig. 1A) and reduced cell number compared with androgen deprivation alone (Fig. 1A). Consistently, high concentration curcumin (5 μmol/L) induced reduction in cell number (Supplementary Fig. S1B). Moreover, BrdUrd incorporation assays showed that S-phase progression was significantly inhibited by curcumin with hormone withdrawal (Supplementary Fig. S2A), thus implicating curcumin as a potent inhibitor of both cell cycle and survival in prostate cancer cells.

Next, the effect of curcumin on CRPC cells was studied under conditions of androgen deprivation. As expected, CRPC cells sustained proliferative capacity under androgen deprivation, but curcumin (1 μmol/L) significantly increased the doubling time for CRPC cells (Fig. 1B). Interestingly, in both the CRPC and ADT-sensitive cells, enrichment of cleaved PARP was observed only at high concentrations (Supplementary Fig. S2B), thus indicating that, lower doses curcumin (1–2.5 μmol/L) exhibit antiproliferative effects, whereas higher doses (5–10 μmol/L) result in cell death. By contrast, combinations of curcumin with ADT did not significantly affect the survival of nontransformed prostatic epithelial cells (BHPRe1, Fig. 1C, left panel), consistent with previous reports suggesting that curcumin shows little activity in nonneoplastic cells (25). Finally, because cells expressing selected tumor-derived mutants of AR (e.g., T877A) can bypass ADT therapy by utilizing endocrine-disrupting compounds as agonists, the impact of curcumin on mutant AR was tested using cells treated with an endocrine-disrupting compound (DDE; ref. 14). In cells cultured in the absence of androgen and stimulated with DDE...
(Fig. 1C, right panel), curcumin inhibited cell proliferation. Together, these data indicated that curcumin enhances the cellular response to ADT in both ADT-sensitive as well as castrate-resistant cell types and suppresses cell survival in concert with ADT.

Curcumin influences AR activity without affecting AR protein accumulation in the castrate condition

To assess the means by which curcumin regulates castrate-resistant AR activity, the impact on AR accumulation was explored. Consistent with prior reports, the agent reduces AR protein levels in the presence of androgen (Supplementary Fig. S1C; ref. 26). However, under castrate conditions, curcumin did not alter AR mRNA (Supplementary Fig. S3A) or AR protein levels in both ADT-sensitive and CRPC cells (Fig. 2A). Prolonged curcumin exposure (48 and 96 hours) failed to alter AR protein levels in all cells tested, except in 22Rv1 cells (data not shown and Supplementary Fig. S3B), wherein both full-length AR and an AR splice variant (AR-SV) were modestly reduced at high curcumin concentrations. These data indicated that low dose curcumin can suppress growth of prostate cancer cells without affecting AR levels. In addition, AR localization was not affected by curcumin treatment (Supplementary Fig. S3, panel C and D).

Because, curcumin yielded no effect on AR protein levels, yet cooperated with ADT to suppress cell growth (Fig. 1), the impact of curcumin on AR activity was examined under castrate conditions. To accomplish this, cells were cultured in the absence of androgen, treated with 2.5 μmol/L curcumin, and relative levels of AR target genes (PSA and TMPRSS2) were determined. ADT treatment alone significantly reduced AR target gene expression as expected (data not shown). As shown, curcumin further inhibited AR target gene expression in ADT-sensitive cells (LNCaP and LAPC-4, Fig. 2B). As AR reactivation is known to be responsible for CRPC development, the impact of curcumin on AR activity in CRPC cells was assessed under castrate conditions. Curcumin curtailed AR target gene expression in CRPC cells (C4-2 and 22Rv1, Fig. 2B). Moreover, curcumin inhibited gene expression of additional AR targets, especially important for AR-dependent G2-M progression in CRPC cells (UBE2C, CDK1, and CDC20; ref. 13; Supplementary Fig. S4A). Consistent with previous studies linking these genes to AR regulation, the direct AR antagonist Casodex further suppressed gene expression under conditions of hormone ablation (Supplementary Fig. S4B). These findings revealed new functions for curcumin, wherein this agent suppresses castrate-resistant AR activity through mechanisms distinct from alteration of AR levels. Overall, these data indicated that

Figure 1. Curcumin cooperates with hormone ablation to suppress cell growth and survival in vitro. ADT-sensitive cells (LNCaP, LAPC-4, or VCaP; A) or CRPC cells (C4-2 and 22Rv1; B) were cultured in androgen deprivation condition for 24 hours before treatment with vehicle or increasing concentrations of curcumin (1, 2.5, and 5 μmol/L). Cells were counted using trypan blue exclusion. C, prostatic epithelial cells (BHPrE1, left) were cultured with vehicle or curcumin (2.5 μmol/L) and effect on cell number monitored. LNCaP cells (right) cultured under androgen deprivation were treated with DDE alone or in combination of curcumin and cell number determined. P < 0.05.
Curcumin impacts AR activity, without affecting AR expression in hormone depletion.

Previous research has indicated that curcumin can also attenuate NF-κB activity. Consistent with the established connection between DHT and NF-κB (27), ADT alone significantly reduced NF-κB activity (data not shown); however, addition of curcumin did not further suppress NF-κB target gene expression (COX2 and IL-6) in ADT-sensitive cells or CRPC cells (Fig. 2C). To further investigate the specificity of curcumin action on other transcriptional regulators known to foster protumorigenic functions in prostate cancer, Myc target gene (CAD1 and JPO1) expression was assessed. Neither CAD1 nor JPO1 levels were altered by curcumin treatment alone (Fig. 2C), indicating that under androgen depletion, pharmacologically attainable doses of curcumin (2.5 μmol/L) preferentially inhibit AR-mediated transcriptional regulation.

Curcumin alters histone modification to suppress AR recruitment and activity

Chromatin binding of AR is known to regulate target gene induction via direct binding to regulatory loci. To determine whether AR occupancy is affected by curcumin at the regulatory loci of genes with known prostate cancer relevance, chromatin immunoprecipitation (ChIP) analysis was done.
For ADT-sensitive models, cells were initially steroid deprived, then stimulated with androgen. AR recruitment in steroid-deprived cells was not consistently above background, consistent with the necessity of ligand in these cells to induce AR occupancy to levels reliably detected by ChIP assay (Supplementary Fig. S4C). At the KLK3/PSA enhancer, DHT-induced AR recruitment was unaffected by curcumin at the earliest time points (1.75 hours post-DHT, LNCaP), however, over time curcumin suppressed AR residence on chromatin (4 and 12 hours, Fig. 3A). Similar effects were observed in the castrate setting (CRPC models), wherein the effects of curcumin on AR residence were observed 4 hours post-DHT treatment at KLK3/PSA locus and continued over time (C4-2, Fig. 3A, middle panel). Similarly, AR occupancy at enhancer regions of other castrate-resistant model (22Rv1) was also reduced by curcumin (Fig. 3A, right panel). At the TMPRSS2 locus, modest effects were observed at the earliest time points (LNCaP, Fig. 3A, bottom panel), but these effects were enhanced over time. In CRPC cells also, AR occupancy was significantly reduced at TMPRSS2 locus only at extended period (Fig. 3A, middle and right panels). Together, these data suggested that within 4 hours, curcumin can alter AR occupancy at sites of transcriptional regulation and impact target gene expression (data not shown).

Because transcriptionally active regions at chromatin are marked by increased acetylation of histones, and curcumin is known to affect HAT proteins, the impact of curcumin on histone modifications was studied. In contrast to the delayed effects on AR occupancy, curcumin treatment significantly reduced histone H4 acetylation at the KLK3/PSA enhancer with rapid kinetics (1.75 hours; Fig. 3B, left panel), and these effects were enhanced over time (4 and 12 hours). Similarly, in CRPC cells, from the shortest time point (1.25 hours), curcumin significantly inhibited histone acetylation at the AR regulatory region at the KLK3/PSA enhancer (C4-2, Fig. 3B, middle panel); and sustained the effects for longer periods (4 and 12 hours). Other CRPC cells (22Rv1) also showed similar reduction in acetyl-H4 at the KLK3/PSA enhancer. A second AR target gene

![Figure 3](image_url)

Figure 3. Curcumin suppresses histone acetylation and AR recruitment at AR-regulated loci. A, ADT-sensitive cells (LNCaP) cultured under androgen deprivation were treated with DHT (1 nmol/L) alone or in combination with curcumin (8 μmol/L) for select time points (1.75, 4, or 12 hours), whereas CRPC cells (C4-2 and 22Rv1) under androgen deprivation were treated with vehicle or curcumin (8 μmol/L) alone for selected time points (1.25, 4, and 12 hours). The cells were processed for ChIP analysis to determine AR occupancy at the enhancer region of KLK3/PSA (top) and TMPRSS2 (bottom; primer sequences, Supplementary Table S1). B, acetylated histone levels at KLK3/PSA (top) or TMPRSS2 (bottom) enhancer region were determined by ChIP analysis. *, P < 0.05.
locus, TMPRSS2, also showed significant reduction in histone acetylation upon curcumin treatment (1.75 hours, LNCaP, Fig. 3B, bottom panel) and continued to repress acetylation after extended periods (4 and 12 hours). Even in CRPC cells, histone H4-acetylation was significantly reduced at TMPRSS2 locus from the earliest time point (C4-2, 1.25 hours) and continued for extended periods. Other models of CRPC (22Rv1) also showed reduced histone acetylation at TMPRSS2 region. Investigation of another AR target gene locus (FKBP5) revealed similar effects (Supplementary Fig. S5A), and the effects observed were conserved with lower doses of curcumin (2.5 μmol/L; Supplementary Fig. S5B). These observations suggested that curcumin significantly impacts histone acetylation and that this event precedes observed changes in AR occupancy.

Curcumin impinges on histone-modifying enzymes to suppress AR activity

Key components of the AR transcriptional complex include histone acetyl transferases p300 and CBP, and ChIP analyses...
herein revealed that curcumin treatment significantly suppressed p300 recruitment (KLK3/PSA, Fig. 4A, left panel). Extended curcumin exposure resulted in significant reduction in recruitment of both p300 and CBP proteins at KLK3/PSA enhancer, thus indicating that curcumin can ultimately impinge on both cofactors. Interestingly, in CRPC cells curcumin significantly inhibited recruitment of these proteins at PSA locus from the earliest time point (Fig. 4A, right panel) and sustained these effects on both proteins over extended time points. Interestingly, no changes in p300 or CBP protein levels were observed at the earliest time point in the conditions used for ChIP analyses (Supplementary Fig. S6A, LNCaP, left panel), thus indicating that curcumin primarily affects recruitment without altering protein levels; longer exposure to curcumin did reduce p300 and CBP protein expression in both ADT-sensitive and CRPC cells (Supplementary Fig. S6A, right panel). No changes were observed in histone methylation in both ADT-sensitive and CRPC cells (Supplementary Fig. S6B), at the time when CBP/p300 occupancy and histone acetylation was suppressed. Interestingly, histone acetylation was also inhibited at the promoter regions of genes not affected by curcumin (IL-6 and COX2, Supplementary Fig. S6C), indicating that the effect of curcumin on higher acetylation status is not always sufficient to alter gene expression. Thus, although these data indicated that a primary effect of curcumin is to suppress CBP/p300 occupancy at chromatin, the consequence of this function of curcumin required further consideration.

To functionally challenge the impact of curcumin-mediated histone alterations, cells were treated with the histone deacetylase (HDAC) inhibitor (HDACI) trichostatin (TSA) to result in net gain of acetylated histones. Consistent with previous reports (28), TSA treatment alone increased PSA mRNA levels in ADT-sensitive cells (Fig. 4B, left panel) but not in CRPC cells. However, in both cell types, TSA reversed curcumin-mediated inhibition of PSA mRNA (Fig. 4B). Similar effects were also observed with another HDACI SAHA (data not shown). TSA reversed the ability of curcumin to inhibit cell survival (Fig. 4B, left panel), but these effects were nullified by curcumin (8 μmol/L; Fig. 4B, right panel). These collective findings suggested that the ability of curcumin to suppress AR activity is dependent on alteration of histone acetylation. These effects seemed to be specific to curcumin, as other isoforms of known inhibitory effects on prostate cancer and AR activity, Indole-3-Carbinol (13C; refs. 29, 30), failed to show additive effects on AR activity, recruitment, or histone acetylation under androgen depletion (Supplementary Fig. S7A and S7B).

Curcumin suppresses occupancy of pioneering factors at enhancers of AR target genes

Although early response to curcumin under castrate conditions involves displacement of CBP/p300 and reduced histone acetylation at sites of AR activity, the observation that AR occupancy was ultimately reduced indicated that other events likely cooperate with the effects of curcumin on CBP/p300. Recent findings have shown that AR function is influenced by prior recruitment of pioneer factors (GATA2 and FOXA1) that act as placeholders for AR recruitment. Interestingly, several reports have indicated that transcriptional activity of pioneering factors is influenced by CBP or p300 function (10, 31). Conversely, GATA2 is necessary for AR recruitment (13), whereas FOXA1 cooperates with AR function to stimulate expression of G2-M phase genes (5, 12, 13). Notably, mRNA levels of UBE2C, CDC20, and CDK1 were suppressed by curcumin in both ADT-sensitive (Supplementary Fig. S4A) and CRPC cells (Supplementary Fig. S4B), thus indicating that curcumin may impinge on pioneer factor function. ChIP analyses showed that GATA2 recruitment was not consistently altered at AR target gene loci in the early time points (1.75 hours, Fig. 5A and Supplementary Fig. S5A, right panel). In CRPC cells (C4-2 and 22Rv1) no significant displacement of GATA2 was observed at shortest time point (1.25 hours; C4-2, KLK3/PSA and TMPRSS2, Fig. 5A, middle panel). However, by 4 hours a significant reduction of GATA2 occupancy in ADT-sensitive cells was consistently observed (Fig. 5A and Supplementary Fig. S5A, right panel) and sustained at the 12-hour time points. Similar analysis of FOXA1 indicated that although FOXA1 occupancy was significantly inhibited at KLK3/PSA regulatory region (Fig. 5B) in the ADT-sensitive (LNCaP) and CRPC cells, FOXA1 recruitment was not affected consistently at TMPRSS2 in CRPC cells. Protein levels of pioneer factors were not affected by curcumin (Supplementary Fig. S8A), indicating that consonant to p300/CBP, curcumin seems to modulate GATA2 residence at sites of AR activity. As pioneer factors are critical for AR-dependent transcription of genes of prostate cancer relevance, it was therefore not surprising that Pol-II recruitment was ultimately suppressed (Supplementary Fig. S8B).

Curcumin suppresses GATA2-mediated, castrate-resistant AR activity

The effect of curcumin on GATA2 occupancy at sites of AR activity are of potentially strong clinical interest, as tumors showing GATA2 overexpression are associated with poor prognosis (11). Moreover, GATA2 binding at the promoter region of the AR gene can enhance overall production of AR, an event known to be associated with CRPC development (11). To determine whether curcumin can inhibit castrate resistance induced by either pioneering factors or AR itself, AR or GATA2 was transiently transfected in ADT-sensitive cells and the impact on AR activity assessed. As expected, PSA mRNA induction was observed under castrate conditions upon AR, as well as after ectopic GATA2 reexpression, but curcumin treatment significantly inhibited PSA induction under both conditions (Fig. 6A). Similarly, in CRPC cells curcumin significantly inhibited both AR and GATA2-mediated enhanced PSA expression (Fig. 6B). These findings further underscored the potential importance of curcumin as a means to dampen GATA2-mediated AR activity in select tumors.

Curcumin reduces castrate-resistant tumor growth in vivo

Given the potent effects of curcumin as a means to suppress GATA2- and p300/CBP-dependent AR activity, in vivo efficacy of curcumin was assessed. For these studies, immunocompromised mice harboring established human tumor xenografts were castrated (to mimic ADT) and randomized into cohorts treated with curcumin or control. As shown in Fig. 7A,
postcastration ADT-sensitive tumors showed delayed growth kinetics. Tumor volume as well as tumor wet weight analyses indicated that curcumin induced significant growth retardation (Fig. 7A). Similarly for the aggressive CRPC xenograft tumors (22Rv1), curcumin treatment significantly reduced tumor growth kinetics and tumor mass (Fig. 7B). These data showed for the first time that curcumin not only hampers the transition of ADT-sensitive disease to castration resistance but is also effective in blocking the growth of established CRPC tumors in vivo.

To determine the impact of curcumin on AR activity, AR target gene expression was analyzed. As shown, KLK3/PSA and TMPRSS2 mRNA levels were reduced in both tumor types by curcumin (Fig. 7C and supplementary Fig. S9A). Serum PSA quantification further confirmed that curcumin inhibits AR activity in vivo (data not shown). Immunohistochemistry (IHC) and immunoblotting of tumor tissues showed that, similar to in vitro data, curcumin exposure did not alter AR expression under conditions of androgen deprivation (Fig. 7C, right panels). However, congruent with the in vitro data, in presence of constitutively active AR splice variants, curcumin inhibited mRNA and protein expression of full-length AR as well as AR-SV (Supplementary Fig. S9B and S9C).

Curcumin significantly inhibited the in vivo cell proliferation of both ADT-sensitive and CRPC tumors, as tested by BrdUrd incorporation (Fig. 7D). Detection of apoptotic cells using caspase-3 detection as well as terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling assay failed to show any significant changes in xenograft tissues (data not shown). These data indicated that curcumin largely inhibits tumor cell proliferation and that a combination of curcumin with currently used ADT treatment may suppress ligand-dependent as well as castrate-resistant AR activity, thus providing a novel means to target hormone receptor signaling in CRPC.

Discussion

Resurgent AR activity is the underlying cause of therapeutic failure and progression to the incurable castrate-resistant state (2, 14, 20–22). Here, it was shown that curcumin cooperates...
with androgen deprivation to suppress AR activity on chromatin. Functional analyses revealed that a primary and critical action of curcumin is to inhibit CBP/p300 recruitment to sites of AR activity and displace pioneer factors governing AR recruitment, thus suppressing AR-dependent cell survival. These functions of curcumin were conserved in ADT-sensitive cells as well as in CRPC model systems. Consequently, curcumin could act in concert with androgen deprivation to delay the transition to castration resistance in ADT-sensitive tumors and was able to slow the growth of castrate-resistant tumors. These collective findings identify curcumin as a novel means to thwart p300/CBP and GATA2-dependent AR activity and resultant tumor growth.

Curcumin effectively suppressed CBP/p300 recruitment and acetylation of histones as a primary response and thus manifest the effects through alteration of the chromatin environment. Curcumin has been suggested to alter CBP/p300 autoacetylation activity required for chromatin recruitment (albeit at high concentration, 100 μmol/L; ref. 32) and inhibit p300 HAT function (33). Specifically, curcumin forms covalent bonds with p300 proteins, thus inhibiting the catalytic activity required for acetylation function (33). Moreover, HDAC inhibitors reversed the effects of curcumin on AR-target gene expression, thus indicating that curcumin-mediated effects on histone acetylation are necessary for curcumin action. To our knowledge, this study is the first to show that: (i) curcumin-mediated changes in CBP/p300 activity are attainable at physiologically attainable concentrations, and (ii) inhibition of CBP/p300 function by curcumin ultimately impinges on recruitment of pioneer factors and AR (34, 35). These findings are of potentially strong clinical relevance, as CBP/p300 support ligand-independent AR activity (22) and progression to CRPC (8). As a result, recent attempt have been made to therapeutically target CBP/p300 for prostate cancer (36). Moreover, p300/CBP function is also pivotal for transcriptional function of the pioneer factor GATA2 (16, 37). Concordantly, curcumin resulted in reduced occupancy of GATA2 to regulatory loci. These observations are of relevance, as GATA2 is a major effector of AR activity and is associated with poor prognosis in advanced prostate cancer (13, 38). Although curcumin dramatically altered GATA2 occupancy in both ADT-sensitive and CRPC cells, these effects were delayed as compared with the reduction in CBP/p300 occupancy (Fig. 4, 5A and Fig. 6A), further implicating the role of CBP/p300 in GATA2 displacement. To date, this study provides one of the first reports to identify achievable means for suppressing GATA2 function in prostate cancer. Moreover, curcumin reduced expression of G2-M genes regulated by pioneer factors in CRPC, thus suggesting a positive outcome for different stages of prostate cancer. Although the ability of curcumin to suppress pioneer factor activity was unexpected, it suggests that combinatorial targeting of CBP/p300 HAT function and AR activity could be of clinical benefit.

Together, the in vitro and in vivo data presented suggest a complex hierarchy for curcumin, wherein the agent suppresses early events required for AR function (i.e., histone acetylation and pioneer factor binding), thus resulting in loss of AR function, critical for tumor growth and progression to castrate resistance. Although previous reports indicate that curcumin inhibits both AR gene and protein expression in prostate cancer cells (validated in Supplementary Fig. S1C), under castrate conditions no changes were detected in AR expression (Fig. 2 and Supplementary Fig. S3; with an exception of cells expressing AR splice variants, wherein differential AR regulation is reported). Interestingly, androgen itself affects AR regulation by increasing protein expression and stabilizing AR.
protein. Also, androgen-bound AR can either induce or inhibit AR mRNA expression in prostate cancer. Therefore the changes in culture condition (i.e., androgen status) may explain the difference in AR regulation by curcumin. Nonetheless, although effects of curcumin on relative AR levels seem to be variant depending on context and cell type, the inhibitor effect on AR activity is conserved across these model systems.

Overall, the study likely has implications far beyond prostate cancer, as CBP/p300 and pioneer factor activity is important in multiple nuclear receptor–dependent malignancies (39–41). For example, CBP promotes treatment resistance by facilitating ligand-independent activation of ERs and the oncogenic coactivator AIB1 in breast cancer (41, 42). Similarly, GATA2 and FOXA1 function as pioneer factors for multiple nuclear receptors and are associated with poor prognosis (1, 43–45). In tumors wherein CBP function is known to be necessary for GATA2 activity (37), curcumin may prove promising therapeutic agent. Conversely, in tumors wherein GATA2 function is repressed by HDAC3 (46), curcumin-mediated CBP repression may increase response to HDAC3-targeted therapies. Similarly, FOXA1, a putative oncogene is essential in breast cancer initiation and has been speculated to be a potential therapeutic target for both ERα-positive and ERα-negative apocrine breast tumors (1, 47). Because curcumin significantly reduced both...
GATA2 and FOXA1 residence on chromatin, it is enticing to speculate that this agent may show heightened anticancer activities in tumors with high FOXA1 and/or GATA. Although the data herein focused on the significant effects of curcumin on resultant AR activity, involvement of other pathways were also studied (Akt, NF-κB, and Myc), but no significant changes were observed, thus indicating that under androgen depletion, curcumin-mediated inhibition of CBP/p300 predominantly affects AR regulatory functions. This discrepancy in susceptibility of transcription factor function to curcumin could be due to changes in curcumin concentration used here, or due to dependency of certain loci on pioneering factors, or CBP/p300 compensation by other factors (e.g., GCN5, PCAF).

Finally, the translational relevance of the findings should be further addressed, as this agent increases the efficacy of AR-targeted strategies and delays the onset of CRPC. Interestingly, curcumin has been assessed in multiple clinical trials (60–62) and showed little toxicity. Moreover, curcumin effectively curtailed serum PSA expression in a clinical trial involving patients with increased PSA (but no prostate cancer; ref. 48). In this preclinical study, it is noteworthy that curcumin was effective in models that achieved ligand-independent AR activity through heightened pioneer factor expression (GATA2, Fig. 6) in tumors that sustain castration-resistant phenotypes (22Rv1) and in model systems wherein the means by which castration-resistance was attained remains scanty defined (C4-2). On the basis of these findings, it is reasonable to hypothesize that curcumin may cooperate with the second generation of AR antagonists (MDV3100 or EPI-001; refs. 49, 50).

In summary, this study shows that curcumin impinges on CBP/p300 and pioneer factor function, thus altering the chromatin landscape so as to act in concert with hormone deprivation. Most critically, these effects were sufficient to delay the onset of tumor progression and provided novel means to control advanced tumor growth in vivo. These studies lay the foundation for further development of agents that combinatorially target factors requisite for nuclear receptor activity on chromatin and hold significant implications for the management of hormone-dependent cancers.

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

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