Therapeutic Targeting of the CBP/p300 Bromodomain Blocks the Growth of Castration-Resistant Prostate Cancer

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Abstract

Resistance invariably develops to antiandrogen therapies used to treat newly diagnosed prostate cancers, but effective treatments for castration-resistant disease remain elusive. Here, we report that the transcriptional coactivator CBP/p300 is required to maintain the growth of castration-resistant prostate cancer. To exploit this vulnerability, we developed a novel small-molecule inhibitor of the CBP/p300 bromodomain that blocks prostate cancer growth in vitro and in vivo. Molecular dissection of the consequences of drug treatment revealed a critical role for CBP/p300 in histone acetylation required for the transcriptional activity of the androgen receptor and its target gene expression. Our findings offer a preclinical proof of concept for small-molecule therapies to target the CBP/p300 bromodomain as a strategy to treat castration-resistant prostate cancer. Cancer Res; 77(20); 5564–75. ©2017 AACR.

Introduction

Prostate cancer cell growth is driven by activity of the androgen receptor (AR), and therapies that target the androgen signaling axis have been highly successful in treatment of the disease (1, 2). Despite the success of these agents in slowing disease progression, prostate cancer remains one of the leading causes of cancer-related deaths in men, with approximately 26,000 deaths in 2016 (3). The majority of patients who progress on antiandrogen therapies [a state termed “castration-resistant prostate cancer” (CRPC)] retain activity of the AR signaling pathway, as evidenced by maintained AR and PSA levels (4–8). Preclinical models have identified a number of mechanisms by which AR activity can be maintained in the presence of therapy. These include alterations to androgen synthesis pathways, AR amplification, point mutations in the AR ligand–binding domain (LBDmut), and expression of AR splice variants lacking the LBD (ALBD; refs. 7, 9–12). Many of these alterations have also been observed in clinical samples (13, 14).

Current prostate cancer therapies that target the androgen signaling axis focus on inhibition of AR or its ligand. Thus, resistance mechanisms commonly center on reestablishment of AR activity via the mechanisms described above. However, to coordinate gene expression, AR must act in concert with numerous coactivator proteins (15). The formation of AR–coactivator complexes promotes the opening of chromatin at AR-binding sites and the recruitment of transcriptional machinery to target genes. We hypothesized that this dependence on coactivator proteins for the functional output of AR activation may present a potential point of intervention in CRPC. The highly homologous histone acetyltransferases CBP [cAMP response element binding protein (CREB) binding protein] and p300 are known coactivators of AR and have been implicated in enhancing the response to androgen (16–18). Consistent with this, CBP/p300 has been proposed to play an oncogenic role in prostate cancer, and upregulation of both proteins has been observed in tumors (19–21).

In the current study, we use a novel small-molecule inhibitor of the CBP/p300 bromodomain to demonstrate a key role for this domain in the coactivator functions of CBP and p300. We describe significant dose-dependent inhibition of AR signaling and prostate cancer proliferation in vitro and in vivo. Taken together, these data strongly support CBP/p300 bromodomain inhibition as a therapeutic strategy in CRPC.

Materials and Methods

Inhibitor characterization and use

Enzalutamide and JQ1 were obtained from Selleckchem, dissolved in DMSO, and used at the indicated concentrations. See Supplementary Note S1 for the detailed experimental synthesis procedures for GNE-049. For cellular experiments,
GNE-049 was dissolved in DMSO and used at the indicated concentrations.

GNE-049 potency and selectivity were evaluated in a panel of biochemical bromodomain binding assays. All biochemical assay protocols were carried out as described previously (22–24). Binding of biotinylated small-molecule ligands to recombinant His-tagged bromodomains was assessed by time-resolved fluorescence resonance energy transfer (TR-FRET). Test compounds that compete with the biotinylated ligand for bromodomain binding reduce the TR-FRET signal. A bioluminescence resonance energy transfer (BRET) assay was used to measure cellular engagement of CBP by GNE-049 through disruption of the interaction between a tagged histone H3 construct and a CBP-halolucase construct in transfected HEK293 cells. The assay was carried out as described previously (22). To determine the inhibition of MYC expression, MV-4;11 cells were plated at 10,000 cells per well in 96-well plates in RPMI1640 media supplemented with 10% FBS and 2 mmol/L l-glutamine. Test compounds diluted in DMSO were transferred to the cell plates, keeping final DMSO concentration constant at 0.1%, and incubated for 4 hours at 37°C. Lysis and analysis for MYC expression were carried out using QuantiGene 2.0 reagents (Affymetrix/eBioscience, probe set catalog number SA-50182) and following the manufacturer's instructions. Luminescence was read using an EnVision plate reader (PerkinElmer) and EC50s generated in Genedata Screener using a 4-parameter nonlinear regression fit.

Pharmacokinetic properties of GNE-049 were determined in mice at WuXi AppTec. Three SCID.Beige mice were obtained from SLK Laboratory Animal Co., Ltd. All animals were female, 6 to 9 weeks old at the time of study, and weighed between 15 and 25 g. The mice were dosed with GNE-049 30 mg/kg orally (suspended in 0.5% w/v methylcellulose, 0.2% w/v Tween 80). Food and water were available ad libitum to all animals. Serial blood samples (15 μL) were collected by tail nick at 0.083, 0.25, 0.5, 1, 3, 8, and 24 hours after the oral administration. All blood samples were diluted with 60 μL water containing 1.7 mg/mL EDTA and kept at −80°C until analysis. All animal studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and were approved by the Institutional Animal Care and Use Committee.

Concentrations of GNE-049 were determined by a non-GLP LC/MS-MS assay. The diluted blood samples were prepared for analysis by placing a 20 μL aliquot into a 96-well plate, followed by the addition of 200 μL of acetonitrile containing an internal standard mixture (0.1 μg/mL diclofenac). The samples were vortexed and centrifuged at 4,000 rpm for 20 minutes at 4°C; 70 μL of the supernatant was diluted with 140 μL of 0.1% formic acid (FA) in water, and 10 μL of the solution was injected onto an analytic column. An ACQUITY UPLC System (Waters) coupled with an API 4000 mass spectrometer (AB Sciex) was used for sample analysis. The mobile phases were 0.3% FA and 2 mmol/L NH4OAc in water/ACN (v:v, 95:5) (A) and 0.3% FA and 5 mmol/L NH4OAc in ACN/water (v:v, 95:5) (B). The gradient was started at 20% B and increased to 90% B for 1.2 minutes, maintained at 90% B for 0.4 minutes, and then decreased to 20% B within 0.1 minute. The total flow rate was 0.55 mL/minute, and samples were injected onto an ACQUITY BEH C8 (100 × 2.1 mm, 1.7 μm) analytic column, with a total runtime of 1.7 minutes. Data were acquired using multiple reactions monitoring (MRM) in positive ion electrospray mode with an operating source temperature of 550°C. The MRM transition was m/z 511.2 → 471.2 for GNE-049 and 296.0 → 214.0 for diclofenac. The lower and upper limits of quantitation of the assay for GNE-049 were 0.005 and 10 μmol/L, respectively.

Cell culture and proliferation assays

All prostate cancer cell lines were obtained from ATCC, authenticated by STR, and tested to ensure mycoplasma free within 3 months of use. All lines were cultured in RPMI1640 media supplemented with 10% FBS and 2 mmol/L glutamine, and used for experimentation within 1 month of thawing from frozen stock. Androgen deprivation was achieved by culturing cells in RPMI media supplemented with 10% charcoal-stripped serum (CCS; Sigma-Aldrich) for at least 5 days. Androgen stimulation was then achieved by addition of 0.1 nmol/L Methyltrienolone (R1881; Sigma-Aldrich) for at least 24 hours. Two-dimensional (2D) cell proliferation was evaluated in a 384-well format using CellTiter-Glo reagent (Promega) according to the manufacturer's instructions. Three-dimensional (3D) growth was assessed by culture in soft agar in a 96-well format. Growth was assessed by colony number and area counts using an optical plate reader.

For assessment of drug combination effects, the Bliss independence model was used (25). The Bliss expectation was determined with the equation (A + B)−λ·A·B, in which A and B are the fractional growth inhibitions induced by agents A and B at a given dose. The difference between the Bliss expectation and the observed growth inhibition with agents A and B used at the same dose is determined to be the Bliss excess. A Bliss sum score was calculated by summing the Bliss excess values of all dose combinations. To determine whether positive Bliss sum scores represented robust synergy, we ranked our data compared with previous datasets generated in the same manner (26, 27).

Stable cell line generation

Stable LNCaP cell lines overexpressing wild-type (WT) or mutant AR were generated by lentiviral delivery of a plasmid encoding N-terminally FLAG-tagged AR. Briefly, lentivirus was generated by cotransfection in 293T cells with plasmid Lv102-AR [GeneCopoeia, Inc.; WT or F877L introduced by site-directed mutagenesis (QuickChange II; Agilent)], envelop plasmid VSVG, and packaging plasmid deltat89 at a molar ratio of 1:2:3:0.2. Forty-eight hours after transfection, lentiviral supernatant was collected, filtered through a 0.45-μm syringe, and added to LNCaP cells. Cells were centrifuged at 1,800 rpm for 45 minutes at room temperature and incubated at 37°C for 3 days before adding puromycin at a final concentration of 2 μg/mL. Three weeks after puromycin selection, LNCaP cells stably expressing WT or mutant AR were used for subsequent analysis.

Cell transfection and RNAi

Knockdown of AR, CBP, and p300 was accomplished by RNAi. For each, a pool of four commercially available siRNAs was used (Dharmacon - SMARTpool ON-TARGETPlus. AR: L-003400-00-0005, CREBBP: LI-003477-00-0002, EP300: L-003489-10-0005). siRNA (100 nmol) was transfected using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's instructions. Gene expression was evaluated 72 hours after siRNA transfection, and cell viability was assessed 5 days after transfection.

Western blot analysis and immunoprecipitation

Cells were lysed in RIPA buffer and separated on a denaturing polyacrylamide gel (Invitrogen) according to the manufacturer's instructions.
instructions. Proteins were transferred to nitrocellulose mem-
brane using the iBlot system (Invitrogen) and blocked for
2 hours in 5% nonfat milk solution. Membranes were incubated
overnight with the following antibodies: AR (Santa Cruz
Biotechnology), CBP (Santa Cruz Biotechnology), p300
(Bethyl Laboratories), and tubulin (DM1a; Abcam). Signal
was detected using fluorescently labeled secondary antibodies
(IRDye; LI-COR) and the Odyssey CL-x imager (LI-COR). FLAG-tagged
AR was immunoprecipitated from LNCaP nuclear extract,
obtained by sequential extraction with hypotonic buffer
(10 mmol/L HEPES pH 7.9, 10 mmol/L KCl, 1.5 mmol/L MgCl2,
0.34 mol/L sucrose, 10% glycerol, Triton X 0.1%, 1 mmol/L DTT,
and protease inhibitor) and nuclear lysis buffer (20 mmol/L Tris
pH8, 10% glycerol, 1% NP40, 2 mmol/L EDTA, 400 mmol/L
NaCl, 1 mmol/L DTT, and protease inhibitor). Extracts were
incubated with anti-FLAG antibody (Sigma-Aldrich) overnight,
before precipitation with protein G magnetic beads (Dynabeads;
Thermo Fisher Scientific). Immune precipitates were washed three
times with PBS plus 1% NP40, washed once with PBS, and boiled
in SDS sample buffer.

Gene expression analysis
RNA was purified from cells using the RNeasy kit (Qiagen)
according to the manufacturer’s instructions. Quantitative RT-
PCR was performed using the TaqMan assay (Thermo Fisher
Scientific) on the ABI QuantStudio 7 Flex Real-Time PCR System.
For whole-transcriptome RNA sequencing, RNA libraries were
made using TruSeq RNA Sample Preparation Kit v2 (Illumina).
Size of the libraries was confirmed using Fragment Analyzer
(Advanced Analytical Technologies), and their concentration was
determined by a qPCR-based method using Library Quanti-
cation Kit (KAPA). The libraries were multiplexed and then
sequenced on HiSeq2500 (Illumina) to generate 30M of single-
end 50 base-pair reads. Data have been made publicly available at
the European Nucleotide Archive (ENA) under project ID
PRJEB20821. Gene set enrichment analysis (GSEA) was per-
formed using Broad Institute software (http://software.broadin
stitute.org/gsea/index.jsp; ref. 27). For total mRNA quantification,
polyadenylated mRNA was isolated from total RNA using the
Oligotex Mini Kit (Qiagen) according to the manufacturer’s
instructions and quantitated using a NanoDrop spectrophotom-
eter (Thermo Fisher Scientific).

Chromatin immunoprecipitation and sequencing
After appropriate drug treatment, cells were cross-linked by
15-minute incubation in 1% formaldehyde and then quenched
for 5 minutes in 125 mmol/L glycine. Subsequent steps were
performed at Active Motif. Cells were lysed and chromatin
sheared by sonication to an average length of 300 to 500 bp.
Genomic DNA (Input) was prepared by treating aliquots of
chromatin with RNase, proteinase K, and heat for deccross-
linking, followed by ethanol precipitation. Pellets were resus-
pended, and the resulting DNA was quanti-
ted using a NanoDrop spectrophotom-
erometer. Chromatin immunoprecipitation
was performed using Broad Institute software (http://software.broadin
stitute.org/gsea/index.jsp; ref. 27). For total mRNA quantification,
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chromatin with RNase, proteinase K, and heat for deccross-
linking, followed by ethanol precipitation. Pellets were resus-
pended, and the resulting DNA was quantified on a NanoDrop
spectrophotometer. Extrapolation to the original chromatin
volume allowed quantitation of the total chromatin yield.
Thirty micrograms of chromatin was precleared with protein
A agarose beads (Invitrogen). Immunoprecipitation was then
carried out using the following antibodies: anti-H3K27Ac
(Active Motif), anti-AR (Santa Cruz Biotechnology), and
anti-p300 (Santa Cruz Biotechnology). Reactions were spiked
with Drosophila chromatin (Active Motif, catalog number
53083) and a Drosophila-specific antibody (Active Motif, cata-
log number 61686) as a mechanism for normalization. Com-
plexes were washed, eluted from the beads with SDS buffer,
and subjected to RNase and proteinase K treatment. Cross-links
were reversed by incubation overnight at 65°C, and chromatin
immunoprecipitation (ChIP) DNA was purified by phenol–
chloroform extraction and ethanol precipitation.

ChIP and input DNAs were prepared for amplification by
converting overhangs into phosphorylated blunt ends and adding
an adenine to the 3’ ends. Illumina genomic adapters were ligated,
and the sample was size fractionated (200–300 bp) on an agarose
gel. After a final PCR amplification step (15 cycles), the resulting
DNA libraries were quantified and sequenced on Illumina
NextSeq 500 (75 nt reads, single end).

Reads were aligned to the human genome (GRCh37) using the
GSNAP algorithm (version 2013-10-10) with the following
settings: “-M 2 -n 10 -B 2 -i 1 -pairmax-dna=1000 -terminal-
threshold=1000 -gmap-mode=None -clip-overlap.” Fragment
length was determined by the strand cross-correlation method.
Reads were extended to this fragment length before coverage
was calculated at a per-nucleotide level using uniquely map-
ning reads.

These coverage values were normalized using the coverage
from uniquely mapping reads for the Drosophila melanogaster
genome as follows. All reads were also aligned to the Drosophila
melanogaster genome (version BDGP5.25.64 from Ensembl)
using the above GSNAP settings and version. The GRCh37
uniquely mapped coverage values (H) were normalized by the
Drosophila uniquely mapped coverage values (D) to produce
scaled coverage values (Hs) with the following formula: Hs
= (H/D) * 1e6. MACS (PMID: 22936215 version 2.0.10) was used
to identify coverage peaks using the default settings. Data have
been made publicly available at the ENA under project ID
PRJEB20981.

Enhancers regions were identified by processing the
H3K27Ac ChIP sequence reads and associated MACS 2.0 peaks
with the ROSE program (PMID:23582322) using the settings
“-g HG19 -t 0.” For the comparison of enhancer and transcrip-
tion start sites (TSS), these enhancers were scaled to a common
width of 5 kb. TSS regions were centered on the TSS of each
NCBI RefSeq transcript (release 53) and set to a common width
of 5 kb. Coverage, normalized to sample library size, was
counted in these regions, for each sample, using 45 bins.

In vivo studies
All procedures were approved by and conformed to the guide-
lines and principles set by the Institutional Animal Care and Use
Committee of Genentech, Inc. and were carried out in an Associ-
ation for Assessment and Accreditation of Laboratory Animal
Care–accredited facility. Prostate cancer patient-derived xenograft
(PDX) tumors were established by passage of tumor fragments (~1
mm3) subcutaneously into 6- to 8-week-old recipient mice. One to
3 days prior to inoculation with TM00298, female NSG mice (The
Jackson Laboratory) were inoculated subcutaneously with 12.5 mg
90-day slow release DHT pellets (Innovative Research of America).
TM00298 primary tumor fragments (between passages 2–9) were
implanted subcutaneously in the hind flank into DHT-pellet
inoculated NSG mice. For the LuCAP77 tumors, male NSG mice
(The Jackson Laboratory) aged 6 to 7 weeks old were transplanted
in the right flank subcutaneously with tumor fragments without
the addition of DHT pellets. For LuCAP.96.1 tumors, male C.B-17

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SCID/bg mice were inoculated as above. For the LuCAP-35V model, castrated male C.B-17 SCID/bg mice were inoculated as above, without the addition of DIHT pellets. Tumors were monitored until they reached a mean tumor volume of approximately 150 to 350 mm³, and then, animals were randomized into one of four treatment groups [pharmacodynamic analyses (n = 5–9 per group); efficacy studies (n = 10/group for LuCAP-96.1 and LuCAP-77, 5/group for LuCAP-35V); (i) vehicle controls (0.5% methylcellulose/0.2% Tween80 in water and 1% carboxymethylcellulose/0.1% Tween80/5%DMSO in water (100 µl total), dosed twice daily via oral gavage; (ii) GNE-049 (25 mg/kg for pharmacodynamic study, 30 mg/kg for efficacy studies (100 µl total), dosed twice daily, orally; (iii) enzalutamide 10 mg/kg (100 µl total) orally, once daily; and (iv) GNE-049 plus enzalutamide at these same doses and schedules]. For pharmacodynamic studies, animals were dosed for 7 days, with tumors being collected 3 to 4 hours after last dose. Tumor size and body weight measurements were made twice per week. Tumor volumes were measured in two dimensions (length and width) using Ulta Cal IV calipers (model 54 – 10 – 111; Fred V. Fowler Co.) and analyzed using Excel, version 11.2 (Microsoft), or Prism 6 (GraphPad Software, Inc.). The tumor volume was calculated with the following formula: tumor size (mm³) = (longer measurement × shorter measurement³) × 0.5. Animal body weights were measured using an Adventurer Pro AV812 scale (Ohaus Corporation). Percent weight change was calculated using the following formula: group percent weight change = [(new weight – initial weight)/initial weight] × 100.

To analyze the repeated measurement of tumor volumes from the same animals over time, a mixed modeling approach was used to address both repeated measurements and modest dropouts due to any nontreatment-related removal (28). This approach addresses both repeated measurements and modest dropouts due to any nontreatment-related removal of animals before the end of study. Cubic regression splines were used to fit a nonlinear profile to the time courses of log2 tumor volume at each dose level. The nonlinear profiles were then related to dose within the mixed model. Tumor growth inhibition (TGI) as a percentage of vehicle was calculated using the following formula: %TGI = 100 × [1 – (AUCCdose per day / AUCCvehicle per day)].

Results

AR-driven prostate cancer cell lines are sensitive to CBP/p300 bromodomain inhibition

To investigate the role of CBP/p300 in prostate cancer biology, we selected a panel of cell line models in which to evaluate their contribution to cell proliferation (Fig. 1A). We identified six cell lines with varying AR expression: LNCaP and MDA-PCa2b (AR WT), VCaP (AR overexpressing), 22Rv1 [AR splice variant lacking LBD (∆LBD); ref. 29], PC3 and DU145 (AR absent). We confirmed that AR-expressing cell lines were dependent on AR for proliferation by siRNA knockdown, using an N-terminally targeted siRNA that downregulated both full-length and ∆LBD AR (Fig. 1B; Supplementary Fig. S1A). We also confirmed that cell lines were dependent on CBP/p300 for proliferation by siRNA knockdown (Fig. 1C; Supplementary Fig. S1B; ref. 30). CBP and p300 co-depletion inhibited proliferation even in cell lines lacking AR, consistent with a core role for these coactivators in transcriptional regulation.

To probe the potential for therapeutic targeting of CBP/p300 in prostate cancer, we generated a small-molecule inhibitor (GNE-049) targeting the chemically tractable bromodomain of CBP/p300 (Fig. 1D). We and others have previously shown that CBP/p300 bromodomain inhibition to have antitumor activity in hematologic cancer models (31–33). GNE-049 is a potent inhibitor of both the CBP (IC₅₀ = 1.1 nmol/L) and p300 (IC₅₀ = 2.3 nmol/L) bromodomains in a biochemical bromodomain-binding assay (Supplementary Fig. S1C). This compound displayed excellent potency (12 nmol/L) in a BRET cellular assay and inhibited the expression of MYC with an EC₅₀ of 14 nmol/L in MV-4-11 cells (Supplementary Fig. S1D and S1E). Importantly, this compound is exquisitely selective for CBP/p300 in general and remarkably selective (3,850-fold) for CBP/p300 over BRD4 (IC₅₀ = 4,240 nmol/L; Supplementary Table S1). The compound afforded a good balance of cell potency, selectivity, and favorable pharmacokinetic properties (Supplementary Fig. S1F) and was therefore selected for additional studies.

We evaluated the effect of CBP/p300 bromodomain inhibition by GNE-049 on prostate cancer growth in both a 2D setting with growth of cells on plastic and a 3D setting with growth in soft agar. In all models, proliferation of AR-expressing prostate cancer cell lines was significantly impacted by GNE-049 treatment to an extent greater than enzalutamide. This effect was observable under both 2D and 3D growth conditions (Fig. 1E). However, viability of AR-negative PC3 and DU145 cells was unaffected by GNE-049 treatment under any conditions.

The CBP/p300 bromodomain is required for AR target gene expression

To investigate in more detail the selective effect of GNE-049 only on AR-expressing prostate cancer cells, we evaluated the impact of CBP/p300 knockdown and GNE-049 on expression of select AR target genes (Fig. 2A and B). Although GNE-049 did not impact AR levels (Supplementary Fig. S2A), it repressed AR target gene expression in a dose-dependent manner in all three AR-expressing cell lines (Fig. 2B), as well as Myc expression in a subset of lines (Supplementary Fig. S2B). Consistent with modulation of the AR signaling axis by GNE-049, enzalutamide was not strongly synergistic with GNE-049 in LNCaP cells, in both gene expression and cell viability assays (Supplementary Fig. S2C and S2D). In a cell viability assay, GNE-049 was moderately synergistic with the BET inhibitor JQ1, as reported previously (Supplementary Fig. S2E; ref. 32). In all cell lines, the extent of AR target gene repression with GNE-049 was greater than that with enzalutamide. In particular, we noted significant repression of AR target genes in 22Rv1 cells, which remain insensitive to enzalutamide due to expression of AR∆LBD. This result suggests that even in the absence of ligand dependence, the coactivator function of CBP/p300 is required for AR-driven gene expression. Consistent with published data, this was recapitulated by CBP and p300 co-depletion by siRNA, which also significantly repressed AR target gene expression (Fig. 2A; ref. 30). To confirm that GNE-049 impacted the full repertoire of AR-regulated genes, we conducted whole-transcriptome RNA sequencing (RNA-Seq) of cell lines after 24-hour treatment with 1 µmol/L GNE-049 (Fig. 2C). Although we observed no global repression of transcription upon drug treatment (Supplementary Fig. S2F), GSEA indicated that a hallmark set of AR target genes was downregulated by GNE-049 treatment, implying a significant impact on the transcriptional output of AR (Fig. 2C; ref. 34).
Figure 1.
AR-expressing prostate cancer cell lines are sensitive to CBP/p300 bromodomain inhibition. A, Western blot showing AR expression levels in prostate cancer cell lines. B, Prostate cancer cell line viability measured by CellTiter-Glo 6 days after transfection with AR-targeted siRNAs or nontargeted control (NTC) siRNAs. Data represent mean ± SD. n = 4. n. term, N terminus. C, Prostate cancer cell line viability measured by CellTiter-Glo 6 days after transfection with CBP- or p300-targeted siRNAs. Data represent mean ± SD. n = 4. D, Chemical structure of the CBP/p300 bromodomain inhibitor (GNE-049). E, Prostate cancer cell lines were cultured under 2D or 3D (soft agar) growth conditions and treated with the indicated concentrations of compound. For 2D growth conditions, viability was measured by CellTiter-Glo after 6 days of treatment. For 3D growth conditions, colony size was measured after 21 days of treatment. Data represent mean ± SD. n = 4. conc., concentration.
Figure 2.
The CBP/p300 bromodomain is required for AR target gene expression. A, RNA levels of the indicated AR target genes were determined by RT-PCR 3 days after LNCaP cells were treated with CBP- and p300-targeted siRNAs. B, LNCaP, VCaP, and 22RV1 cells were treated for 24 hours with a 2-fold dilution series of the indicated compounds, starting at 1 μmol/L. Expression of the indicated AR target genes was then measured by RT-PCR. Data, mean ± SD. n = 3. C, GSEA of RNA-Seq data after the indicated cell lines were treated for 24 hours with 1 μmol/L GNE-049 or DMSO control. n = 3.
The CBP/p300 bromodomain is required for the response to androgen.

The observation that GNE-049 treatment reduced expression of known AR target genes suggests that GNE-049 may directly impact the ability of prostate cancer cells to respond to androgen stimulation. To test this, we starved cells of androgen by growth in CSS for 5 days and then assessed gene expression via RNA-Seq after 24 hours of stimulation with 0.1 nmol/L R1881 (synthetic androgen). We identified a set of 292 genes induced by androgen in LNCaP cells (Fig. 3A; Supplementary Table S2). GSEA confirmed that this gene set significantly overlapped with previously identified AR target gene signatures (Fig. 3B; ref. 35). Cotreatment with 1 µmol/L of GNE-049 in parallel with R1881 stimulation prevented induction of this gene set in a manner similar to enzalutamide cotreatment (Fig. 3A). Consistent with this analysis, RT-PCR of the KLK3 gene (encoding PSA) indicated that GNE-049 could block R1881-induced KLK3 expression in a dose-dependent manner in LNCaP cells (Fig. 3C). GNE-049 treatment also blocked androgen-induced proliferation when cells were stimulated with R1881 in the presence of 1 µmol/L GNE-049 for 6 days (Fig. 3D). In particular, we noted significant reduction in proliferation of R1881-stimulated 22RV1 cells in the presence of GNE-049, even though these cells remained unresponsive to enzalutamide in this assay. Taken together, these data suggest that the CBP/p300 bromodomain is required for prostate cancer response to androgen and imply a direct role for the bromodomain of CBP/p300 in mediating the coactivator function for AR.

Inhibition of the CBP/p300 bromodomain prevents AR coactivator function.

We sought to better understand the mechanism by which CBP/p300 functionally regulates the AR transcriptional response by investigating AR and CBP/p300 coassociation and localization on chromatin. Consistent with an AR coactivator function, p300 could be communoprecipitated with AR in nuclear extract from LNCaP cells expressing FLAG-tagged AR (Fig. 4A). We also confirmed the colocalization of these proteins on chromatin by CHIP and sequencing analysis of LNCaP cells after 24-hour stimulation with R1881 (Fig. 4B and C). Using the set of 292 androgen-induced genes identified by RNA-Seq (Fig. 3A), we defined a set of direct AR target genes, as those R1881-induced genes with AR peaks located at their promoters or nearby androgen response elements (ARE). This represented 117 of the 292 R1881-induced genes (Fig. 4B). Of these genes, 98 (83%) also had overlapping p300 peaks located at their promoters or AREs (Fig. 4B and C). These data confirm that AR and p300 function together on chromatin.

To test the hypothesis that GNE-049 directly inhibits the AR coactivator function of CBP/p300, we evaluated the impact of drug treatment on acetylation of histone 3 (H3) at lysine 27 (H3K27Ac). H3K27Ac is directly mediated by CBP/p300, and modification of this residue promotes an open chromatin structure, allowing for active gene transcription (36). Consistent with a block in coactivator function, GNE-049 treatment markedly reduced H3K27Ac at AR/p300 overlapping peaks (Fig. 4C and D). In addition to changes at specific AR target genes, a reduction in H3K27Ac levels could also be observed globally at enhancers upon GNE-049 treatment (Supplementary Fig. S3A and S3B), indicating a potential impact on other coactivator functions of CBP/p300 in addition to AR. Surprisingly, GNE-049 did not disrupt the interaction between AR and p300 (Fig. 4A), nor did it impact AR or p300 recruitment to chromatin by androgen stimulation (Fig. 4C and D). These data suggest a model wherein GNE-049 inhibits the AR coactivator function of p300, preventing histone acetylation and activation of gene transcription, but not disruption of AR/coactivator complex formation. This implies...
Figure 4.
Inhibition of the CBP/p300 bromodomain prevents its AR coactivator function. A, LNCaP cells stably expressing FLAG-tagged AR were treated with 1 μmol/L GNE-049 for 24 hours. FLAG-tagged AR was then immunoprecipitated (IP) from nuclear extracts with anti-FLAG, or control IgG. Immunoprecipitates were probed for the presence of p300 using anti-p300 antibodies. Four percent total extract is shown as an input control. B, ChIP sequencing with anti-AR, p300, and histone H3K27Ac antibodies was performed on LNCaP cells after 24-hour stimulation with 0.1 nmol/L R1881. Diagram indicates the overlap between AR, p300, and H3K27Ac peaks at genes induced by R1881 treatment (as determined in Fig. 3A). C, Genome browser representation of ChIP sequencing tracks at the KLK3 (PSA) and ALDH1A3 genes. ChIPs with anti-AR, anti-p300, and anti-histone H3K27Ac are shown after 24-hour 0.1 nmol/L R1881 stimulation ± 1 μmol/L GNE-049. y-axis scale shows normalized reads per million per base pairs. D, Summary plots showing p300 and H3K27Ac ChIP sequencing peak size 24 hours after stimulation with R1881 ± GNE-049 for peaks overlapping with AR peaks induced by R1881 treatment (as determined in Fig. 3A). Axes show normalized reads per million per base pairs in each peak.
that the bromodomain, although dispensable for chromatin binding, plays a critical role in the AR coactivator function of CBP/p300.

Endocrine therapy resistance models remain sensitive to CBP/p300 bromodomain inhibition

The observation that GNE-049 can significantly inhibit the proliferation of prostate cancer cells suggests such inhibitors may represent a novel therapeutic strategy in this disease. As CRPC remains a major unmet medical need, we wished to further evaluate the effectiveness of GNE-049 treatment in this context. To better model the progression from castration-sensitive to castration-resistant disease, we sought to generate cell line models that recapitulate some of the common mechanisms of androgen therapy resistance in the same background as a castration-sensitive model. To this end, we generated LNCaP cell lines overexpressing either WT AR or F877L mutant AR (ARmut), a mutation identified in patients and known to confer resistance to enzalutamide (Supplementary Fig. S4A; refs. 11, 37, 38). As expected, expression of ARmut in LNCaP cells decreased sensitivity to enzalutamide (Fig. 5A). However, ARmut protein still co-immunoprecipitated with p300, suggesting a continued requirement for p300 coactivator activity (Supplementary Fig. S4B). Consistent with this, ARmut-expressing cells remained highly sensitive to GNE-049, with significant inhibition of cell proliferation and AR target gene expression (Fig. 5A and B). These data suggest GNE-049 could have significant therapeutic impact in the context of androgen therapy-resistant disease.

CBP/p300 bromodomain inhibitors show antitumor activity in vivo

Although cell line models provide an essential tool to dissect the role of CBP/p300 in prostate cancer, their long-term propagation in vitro can lead to adaptation and alterations that may no longer fully recapitulate the clinical disease. To better model the clinical utility of CBP/p300 inhibitors for the treatment of prostate cancer, we examined GNE-049 activity in vivo in PDX models of prostate cancer. These models have been continually propagated in vivo and may therefore better reflect the human disease state (39). qPCR analysis of tumors after 7 days of treatment with GNE-049, enzalutamide, or the combination of both drugs indicated significant suppression of AR target genes KLK3 (PSA), TMPRSS2, and Myc.
Figure 6. CBP/p300 bromodomain inhibitors show antitumor activity in vivo. A, TM00298 PDX prostate tumor-bearing mice were randomized into four groups. Mice were treated by oral gavage (PO) with vehicle or the indicated compounds twice (BID; GNE-049) or once daily (QD; enzalutamide). After 7 days, tumors were harvested 4 hours after the last dose and expression of the indicated AR target genes assessed by RT-PCR. Each point represents data from an individual mouse. B, TM00298 PDX prostate tumor-bearing mice were randomized into four groups, with 9 or 10 mice per group. Mice were treated orally with vehicle or the indicated compounds twice daily (GNE-049) or once daily (enzalutamide). Tumor volume (left) and body weight (right) were monitored over 18 days of continuous treatment with the indicated compounds. Data, mean ± SD. C, LuCaP-96 PDX prostate tumor-bearing mice were randomized into four groups, with 9 or 10 mice per group. Mice were treated orally with vehicle or the indicated compounds twice daily (GNE-049) or once daily (enzalutamide). Tumor volume (top) and body weight (bottom) were monitored over 21 days of continuous treatment with the indicated compounds. Data, mean ± SD. D, LuCaP-77 PDX prostate tumor-bearing mice were randomized into four groups, with 9 or 10 mice per group. Mice were treated orally with vehicle or the indicated compounds twice daily (GNE-049) or once daily (enzalutamide). Tumor volume (top) and body weight (bottom) were monitored over 21 days of continuous treatment with the indicated compounds. Data, mean ± SD. E, LuCaP-35V PDX prostate tumor-bearing mice were randomized into four groups, with 5 mice per group. Mice were treated orally with vehicle or the indicated compounds twice daily (GNE-049) or once daily (enzalutamide). Tumor volume (top) and body weight (bottom) were monitored over 21 days of continuous treatment with the indicated compounds. Data, mean ± SD.

Enza, enzalutamide.
TMPRSS2, FKB5, and MYC in the AR-expressing Tm00298 PDX model (Fig. 6A). Consistent with these findings, GNE-049 treatment significantly impaired the growth of Tm00298 tumors, resulting in 55% TGI, relative to 21% TGI with enzalutamide after 18 days (Fig. 6B). The utility of GNE-049 was further validated in three additional AR-positive prostate cancer PDX models. Activity was observed in LuCaP-77, LuCaP-96.1, and LuCAP-35V, both as single agents (86%, 75%, and 91% TGI, respectively) and when combined with enzalutamide (106%, 118%, and 105% TGI, respectively) over 21 days (Fig. 6C–E). As enzalutamide demonstrated some single-agent activity in the LuCaP-77 and LuCaP-96.1 models (53% and 39% TGI, respectively), the combination antitumor activity was deemed to be additive, consistent with in vitro data (Fig. 6C and D). LuCAP-77 and LuCAP-35V are well-documented castration-resistant models (40, 41), confirming the activity of GNE-049 in the CRPC setting. Treatments were tolerated over the course of the studies, although tumor-related cachexia contributed to progressive animal body weight loss in all groups (Fig. 6B–E). Taken together, these data indicate a significant role for the CBP/p300 bromodomain in vivo in the response to androgen and demonstrate that pharmacologic inhibition of the CBP/p300 bromodomain can inhibit prostate cancer tumor growth.

Discussion

Although androgen-directed therapies have successfully extended the lifespan of patients with prostate cancer, many ultimately still succumb to their disease, and CRPC remains a significant unmet medical need. The common reemergence of AR signaling in CRPC patients highlights a critical dependence on this pathway and the need for additional strategies for its inhibition. Reemergence of AR signaling leads to restoration of transcriptional output and increased expression of AR target genes, including cell-cycle regulators responsible for driving proliferation. This transcriptional activity of AR requires the interaction with essential cofactors, which act to open chromatin and recruit the basal transcription machinery. As such, AR cofactors represent a potential point of intervention in prostate cancer. Cofactor inhibitors would have the hypothesized advantage that as they are “downstream” of AR, resistance cannot easily be brought about by upregulation of AR or its ligand, making the case for cofactor inhibitor use up-front in combination with traditional androgens to enhance efficacy and block potential resistance mechanisms. Advances in technologies to interrogate chromatin have resulted in the identification of a number of essential AR coactivators and their proposal as potential drug targets (30, 42–45). Historically, such cofactors and epigenetic regulators have proved notoriously difficult targets for the design of small-molecule inhibitors, hindering progress in this area. However, recent advances in the design of small-molecule bromodomain inhibitors have provided the opportunity to evaluate the potential of AR cofactor inhibitors (46).

In this study, we describe a CBP/p300 bromodomain inhibitor with properties allowing direct in vivo assessment of the role of these coactivators in prostate cancer biology. Using the potent and selective inhibitor GNE-049, we demonstrate the requirement for CBP/p300 bromodomain activity for the response to androgen in prostate cancer, and for the growth of prostate cancer models both in vitro and in vivo. Specifically, we show that inhibition of the CBP/p300 bromodomain prevents its coactivator function at AR by preventing the acetylation of H3K27 normally associated with androgen-induced recruitment of AR to chromatin. As we observe no reduction in p300 or AR recruitment to chromatin in response to drug inhibition, the exact mechanism by which the CBP/p300 bromodomain contributes to histone acetylation remains an exciting open question and a point for further study. Using GNE-049 to probe the role of this domain in prostate cancer biology, we demonstrate significant inhibition of AR target gene as well as Myc expression in several CRPC models, leading to a block in proliferation. These models include those with clinically relevant mechanisms of resistance to current therapies.

On the basis of our findings, we propose a model wherein CBP/p300 bromodomain inhibition is sufficient to prevent AR activity at chromatin, thereby blocking expression of key proliferative gene signatures and tumor growth, and demonstrating the potential for therapeutic inhibition of CBP/p300 for the treatment of prostate cancer.

Disclosure of Potential Conflicts of Interest

L. Jin is a senior research associate at Genentech. C. de la Cruz is a senior research associate at Genentech. M. Merchant is the senior scientist at Genentech and has ownership interest (including patents) in Roche. K.E. Gascoigne is a scientist at Genentech. No potential conflicts of interest were disclosed by the other authors.

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