CRISPR screening identifies CDK12 as a conservative vulnerability of prostate cancer

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Androgen receptor (AR) signaling inhibitors provide limited survival benefits to patients with prostate cancer (PCa), and worse, few feasible genomic lesions restrict targeted treatment to PCa. Thus, a better understanding of the critical dependencies of PCa may enable more feasible therapeutic approaches to the dilemma. We performed a kinome-scale CRISPR/Cas9 screen and identified cyclin-dependent kinase 12 (CDK12) as being conservatively required for PCa cell survival. Suppression of CDK12 by the covalent inhibitor THZ531 led to an obvious anti-PCa effect. Mechanistically, THZ531 downregulated AR signaling and preferentially repressed a distinct class of CDK12 inhibition-sensitive transcripts (CDK12-ISTS), including prostate lineage-specific genes, and contributed to cellular survival processes. Integration of the super-enhancer (SE) landscape and CDK12-ISTS indicated a group of potential PCa oncogenes, further conferring the sensitivity of PCa cells to CDK12 inhibition. Importantly, THZ531 strikingly synergized with multiple AR antagonists. The synergistic effect may be driven by attenuated H3K27ac signaling on AR targets and an intensive SE-associated apoptosis pathway. In conclusion, we highlight the validity of CDK12 as a druggable target in PCa. The synergy of THZ531 and AR antagonists suggests a potential combination therapy for PCa.

Cell Death and Disease (2021) 12:740; https://doi.org/10.1038/s41419-021-04027-6

INTRODUCTION

The primary therapy for prostate cancer (PCa) is targeting androgen receptor (AR) signaling, while the disease inevitably progresses to castration-resistant prostate cancer (CRPC). Next-generation AR signaling inhibitors have significantly improved the survival of patients with CRPC, but further resistance remains an issue [1–3]. For targeted therapy, large-scale genomic analyses have discovered genetic drivers (ETS fusions, CDKN2A loss, PTEN, RB1, and SPOP mutations, etc.) and delineated distinct molecular subtypes of PCa [4–6], while few genetic abnormalities are being actively translated into promisingly druggable targets. Therefore, a better understanding of the critical dependencies of PCa may enable more feasible therapeutic approaches to the dilemma.

Compared with shRNA- or siRNA-based genetic dependency screens, CRISPR/Cas9 technology minimizes off-target effects, maintains higher efficiency and identifies more fitness genes [7, 8]. Current genome-scale CRISPR/Cas9 screens have been successfully used to identify genes required for cancer cell survival as candidate targets [9, 10], while their further synergy with existing medicine has been less studied. Relatively, studies used CRISPR/Cas9 screens and focused on specific genes of “synthetic lethality” for clinical drugs, but neglected cancer dependencies under medicated stress [11, 12]. This screening strategy provides potential targets for synergy, while attenuates the applicable value for monotherapy to a certain extent. To our knowledge, we first identified cyclin-dependent kinase 12 (CDK12) as conservatively required for PCa cells under both normal and AR antagonism stress conditions, suggesting that CDK12 inhibition may confer synergistic anti-PCa and AR antagonism properties.

CDK12 primarily regulates transcription elongation by phosphorylating serine-2 (S2) of the C-terminal domain (CTD) of RNA polymerase II (RNAPII) [13] and plays an essential role in DNA damage repair (DDR), especially homologous recombination (HR) [14, 15]. Notably, a CDK12 somatic loss mutation induces a novel, genetically unstable subtype of advanced CRPC [19–21]. Therefore, the role of CDK12 in PCa requires further elaboration.

Here, we report that CDK12 is a conservative target of PCa. The underlying mechanisms that mediate the conservative vulnerability of CDK12 may be driven by its preferential repression of basic survival pathways and super-enhancer (SE) associated oncogenes. Furthermore, we validated the striking synergy between CDK12 inhibition and AR antagonism. H3K27ac alteration on AR signaling may account for the synergistic effect.
MATERIALS AND METHODS

Pooled CRISPR screen

For the design of the kinome CRISPR library, 5157 gRNAs targeting 507 human kinases were selected. Then, oligo gRNA sequences with flanking adaptors were synthesized by Symbio Technologies (Monmouth Junction, NJ, USA). The oligo pool was amplified via PCR using primers with the lentiviral CRISPR V2 vector, and then the product was subsequently inserted into the lentiviral CRISPR V2 vector using the Gibson Assembly.

The genome CRISPR library was introduced into C4–2 cells by lentiviral transduction. After 7 days of puromycin selection, all remaining cells were divided into input, normal (DMSO), and AR antagonism (enantitamivir 10–25 μM) groups. PCa cells were then collected for DNA extraction on the 21st and 28th days. Changes in library representation were determined by quantification of the barcode identifiers present in each gRNA vector through next-generation sequencing by Novogene using the Illumina NovaSeq 6000 platform. Raw read count data were acquired and processed with the model-based analysis of genome-wide CRISPR/Cas9 knockout (MAGeCK) software to prioritize gRNAs and genes, and the results are presented as robust rank aggregation (RRA) scores.

Human cell lines

The cell lines used in this study were maintained in a 37 °C and 5% CO2 incubator. LNCaP, C4–2, 22Rv1, and DU145 cells were cultured in RPMI-1640 medium (Gibco), while PC3 cells were cultured in DMEM (Gibco). All media were supplemented with 10% fetal bovine serum (FBS) (Gibco), glucose, and penicillin/streptomycin. Mycoplasma contamination was excluded via a PCR-based method.

Compounds and antibodies

THZ531 (HY-103618), enzalutamide (HY-70002), apalutamide (HY-16600), and bicalutamide (HY-14249) were purchased from MCE. Antibodies against CDK12 (ab37014) were purchased from Abcam. Additional antibodies against CDK12 (PAB39156) were purchased from Bioswamp. Antibodies against phospho-CTD-RNAPII-S2 (Cat# 041571-1, lot# 3023013), RBPJ (Cat# 05-623–25UG, lot# Q2925497), AR (Cat# 5153T), and H3K27ac (Cat# 8173T, lot# 6) were purchased from Cell Signaling Technology. Antibodies against GRIN3A (Cat# bs12100R) were purchased from Bioss.

Bioinformatics analysis tools

We downloaded datasets for PCA cell lines (LNCaP, DU145, and 22Rv1) from the Project Score database (https://score.depmap.sanger.ac.uk/) and analyzed them in terms of the whole genome. Raw read count data were acquired and processed with MAGeCK to prioritize gRNAs and genes, and the results are presented as beta scores, whose values are <0 and represent fitness effects. We obtained datasets regarding SE-associated associations of disease-free survival (DFS), recurrence and PCa status with mutations in LNCaP cells treated with R1881 that had been processed in LNCaP cells treated with R1881 that had been processed by SEdb (http://www.licpathway.net/sedb/, GSE73994). The genes were processed with the following websites:

CANCERTOOL: http://web.bioinformatics.cigiobune.es/CANCERTOOL/index.html,
THPA: https://www.proteinatlas.org, GEPCA: http://gepca.cancer-pku.cn/.

Tissue microarray (TMA) analysis

A commercially available human PCA TMA (HProA150CS01, Outdo Biotech, Shanghai, China) including samples from 100 patients (150 PCa and 50 normal prostate tissue specimens) who underwent radical prostatectomy was used for photographing. Two pathologists without knowledge of patient characteristics independently assessed the immunohistochemistry (IHC) score. The immunostaining score was calculated as the percentage score × the intensity score.

Individual plasmid construction and virus production

The lentiviral CRISPR V2 vector was used to generate the sgCDK12 and sgGRIN3A constructs. The sgHT control construct served as the empty control vector.

The sequences of the gRNAs are listed below:

lentiCRISPRv2-sgCDK12#1-F: CAACCCGCAAAGAGCAGCAGGAGA
lentiCRISPRv2-sgCDK12#1-R: AAAGAACTCTGCCTGCATCTGGC
lentiCRISPRv2-sgCDK12#2-F: CAACCCGCAAGCTGATGACATGGG
lentiCRISPRv2-sgCDK12#2-R: AAATCTCCATAGCTACAGCTCC

lentiCRISPRv2-sgGRIN3A#1-F: CACCCGCAAGATTTTGCTAGCGG
lentiCRISPRv2-sgGRIN3A#1-R: AACCCGGTGACAGAAACTCTTC
lentiCRISPRv2-sgGRIN3A#2-F: CACCCGCAAGCTGATGACATGGG
lentiCRISPRv2-sgGRIN3A#2-R: AAATCTCCATAGCTACAGCTCC

Protein lystate preparation and western blotting

Cells were washed with PBS (Gibco) and lysed with RIPA buffer (50 mmol/L Tris pH 7.4, 150 mmol/L NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, and 1 mmol/L PMSF). After being quantified by Bradford assay, protein samples were separated using a 10% TGX Stain-Free™ FastCast™ Acrylamide Kit and blotted to Immobilon® NC membranes. The membranes were blocked (5% BSA in TBS-T) and incubated with primary and secondary antibodies (suggested concentrations in 5% skimmed milk) in sequence. Photographs were taken for chemiluminescence using a ChemiDoc MP Imaging System (Bio-Rad).

Colony formation assays

Cells were seeded into six-well plates (0.5–1.0 × 10⁵ cells per well) and cultured in the presence of the indicated intervention. For each cell line, cells cultured under different conditions were fixed with 4% paraformaldehyde (in PBS) at the same time. Afterward, cells were stained with 0.1% crystal violet (in water). A ChemiDoc MP Imaging System (Bio-Rad) was used for photographing.

CCK-8 cell viability assay

Cells were seeded and seeded into 96-well plates at a density of 500–1000 cells per well (24 h later, drugs were added at the indicated concentrations). Ten microliters of CCK-8 solution (Yeasen Biotech) were added to each well (100 µL), and then the cells were incubated for 1–4 h at 37 °C. Cell viability was assessed by measuring the fluorescence emission at 450 nm.

FxCycle™ propidium iodide (PI) /RNAse staining

PCa cells were harvested and fixed with 70% ethanol for 8–10 h at 4 °C. Then, the cells were washed with PBS, and all fixatives was removed. Each prepared flow cytometry sample contained at least 0.5 × 10⁷ cells in suspension. Then, 400 µL FxCycle™ PI/RNase Staining Solution (0.5 mL; lot#: 2165130, Invitrogen) was added to each flow cytometry sample and mixed well, and the sample was incubated for 15–30 min at room temperature. Cell cycle arrest in the form of a PI signal was detected via NovoCyte Flow Cytometer Systems (ACEA Biosciences).

Annexin V (AV)–FITC apoptosis detection assay

For the detection of cell apoptosis, cells were harvested by trypsin without ethylenediaminetetraacetic acid (EDTA) and washed with PBS. Then, 380 µL of 1× binding buffer was added to each sample containing at least 0.5 × 10⁷ cells. Then, AV-FITC apoptosis detection assay reagent (lot#: 185174000, Invitrogen; 10 µL of FITC annexin V and 5 µL of the PI working solution) was added to each sample for 15–30 min at room temperature. Apoptotic cells were analyzed based on AV and PI signals via NovoCyte Flow Cytometer Systems (ACEA Biosciences).

EU staining

Cells were treated with DMSO or THZ531 for 6 h and cultured in RPMI-1640 medium containing 500 µM EU for 2 h at 37 °C before fixation. EU was detected with a Cell-Light EU Apollo 488 instrument (Cat# C10318–3; lot# S1106, Ribo Biotech) according to the manufacturer’s protocol.

Sphere-formation assay

Cells were seeded into six-well nonadherent plates (1000 cells well per well) and cultured in the indicated dose of THZ531 with DMEM/F-12 (Gibco) containing 50 mg/mL insulin (Sigma), 2.5 mg/mL hydrocortisone (Topscience), 2% B27 (Invitrogen), 0.5 mg/mL epidermal growth factor (EGF), and 0.1 mg/mL basic fibroblast growth factor (BFGF) (2.0 mL per well) at 37 °C. Cultures were added 300 µL of 1× binding buffer was added to each sample containing at least 0.5 × 10⁷ cells. Then, AV-FITC apoptosis detection assay reagent (lot#: 185174000, Invitrogen; 10 µL of FITC annexin V and 5 µL of the PI working solution) was added to each sample for 15–30 min at room temperature. Apoptotic cells were analyzed based on AV and PI signals via NovoCyte Flow Cytometer Systems (ACEA Biosciences).

RNA-Sequencing (RNA-Seq) assay

LNCaP cells (1.0 × 10⁶) were cultured with regular RPMI-1640 medium containing 10% FBS. After being treated with DMSO, 0.2, 0.5, and 1.0 µM
Cleavage under targets and targetting (CUT&Tag) assay

The CUT&Tag assay was performed using the NovoNGS® CUT&Tag 2.0 High-Sensitivity Kit (NovoProtein, N259-YH01). 5.0 × 10^7 LNCaP cells were washed twice with 1.5 mL of wash buffer and then mixed with activated concanavalin A beads. After successive incubations with the primary antibody (HK272Ac, 4 °C, 16 h) and secondary antibody (RT, 1 h), the cells were washed and incubated with pAch-TnS for 1 h. Then, MgCl2 was added to activate tagmentation for 1 h. The tagmentation reaction was stopped, and the chromatin complex was digested with a solution containing 10 µL of 0.5 M EDTA, 3 µL of 10% SDS and 2.5 µL of 20 mg/mL Proteinase K at 55 °C for 2 h. The transposed DNA fragments were purified using a Qiagen MinElute PCR Purification Kit and amplified using NEBNext Ultra II Q5 Master Mix (New England Biolabs, M0544L). The libraries were sequenced by Novogene using the Illumina NovaSeq 6000 platform.

RNA isolation and RT-qPCR

Cells were harvested and extracted for RNA using Hipure RNA Mini Kit (Magen). RNA samples were sequenced using the standard Illumina protocol to create raw sequence files (Fastq files) at LC Sciences. Significant genes were selected based on the following cutoffs: 1 for the log2 fold change and 0.05 for the permutation p value. The GO, KEGG, and GSEA analysis were evaluated by bioinformaticists at LC Sciences.

RESULTS

CRISPR/Cas9 screening identifies CDK12 as a conservative kinase target of PCa

To identify the highly conservative dependencies of CRPC as the most promising target, we performed a CRISPR screen targeting 507 kinases to detect genes critically required for PCa cells under normal conditions or under enzalutamide-treated culture conditions (Fig. 1A). The top 20 candidate genes in each group were commonly enriched in cell cycle, transcription, and DDR regulation (Fig. 1B). The correlation heatmap presented better similarity between the 21st and 28th days of the same intervention groups, suggesting a reasonable screening result (Fig. 1C). We further overlapped the top 20 candidate genes from each group and identified six kinases that were depleted in both normal- and enzalutamide-cultured PCa cells (Fig. 1D). Among them, CDK4, BRD2, AKT1, and PLK1 have been validated as critical kinases of PCa.

We focused on CDK12, a key regulator of transcription elongation and DDR, in follow-up experiments. To exclude the screening bias caused by a single cell line, we also validated the dependencies of LNCaP, 22Rv1, and DU145 cells on CDK12 from the Project Score database (Fig. S1A). To further investigate the function of CDK12, we identified significantly higher CDK12 mRNA levels in PCa than in normal prostate tissues from the PCa database of the Tomlins cohort (Fig. S1B). The Cancer Genome Atlas (TGCA) data showed that PCa patients with lower CDK12 mRNA levels experienced slightly longer DFS (Fig. S1C). Next, we analyzed CDK12 protein levels using a TMA containing 150 PCa specimens by immunohistochemical analysis and found that CDK12 protein was expressed at significantly higher levels in PCa tissues (Fig. 1E, F), indicating the feasibility of CDK12 as a target for PCa therapy.

To further validate our findings, we infected a panel of PCa cells with CDK12 sgRNAs. Genetic depletion of CDK12 (Fig. 1G) inhibited the proliferation and viability of both hormone-sensitive prostate cancer (HSPC) and CRPC cell lines in clone formation (Fig. 1H) and CCK-8 cell viability assays (Fig. 1I), respectively. Together, our data indicate that CDK12 is conservatively required for PCa cells.

CDK12 inhibition shows powerful antineoplastic properties against PCa cells

We further treated the panel of PCa cells with THZ531 (a covalent inhibitor of CDK12) [22]. Colony formation assays demonstrated that THZ531 substantially inhibited cell proliferation (Fig. 2A). CCK-8 assays revealed a concentration- and time-dependent decrease in PCa cells viability upon THZ531 treatment (Fig. 2B, C). To intuitively investigate the effect of CDK12 inhibition on transcription, we treated LNCaP and C4–2 cells with THZ531 and observed a conspicuous reduction in the amount of newly transcribed RNA using fluorescently labeled EU incorporation assays (Fig. 2D). FACs cell cycle assays with escalating doses of THZ531 displayed an increasing number of cells in the sub-G2/M phase (Fig. 2E). We subsequently used flow cytometry to assess AV and PI staining in apoptotic cells and observed dose- and time-dependent enhancement in PI signals of positively stained cells (Fig. 2F), indicating that CDK12 inhibition induces apoptosis of PCa cells. As previously reported [22], a dose-dependent decrease in the phosphorylation level of S2 of the RNAPII CTD was present in THZ531-treated PCa cells (Fig. 2G). In addition, the sphere-formation assays demonstrated the lower sphere-formation efficiency on the THZ531-treated LNCaP and C4–2 cells, including a gradual decrease in the expansion sizes (Fig. 2H, S1D) and the total number (Fig. 2I, S1D) of primary spheres formed over time for a given number of total tumor cells, suggesting the impact of THZ531 on the sphere-forming ability of PCa cells.

CDK12-ISTS contain prostate lineage-specific genes and contribute to the survival processes of PCa cells

To examine the effect of CDK12 inhibition on the transcriptome, we performed RNA-Seq on THZ531-sensitive LNCaP cells. Samples were treated with DMSO or THZ531 at 0.2/0.5/1.0 µM for 6 h, excluding the impact of cell cycle arrest (Fig. S2A). THZ531 resulted in a global- and concentration-dependent reduction in steady-state mRNA (Fig. 3A, B). Notably, the transcriptome alteration mediated by CDK12 inhibition was inconsistent with that of the CDK12 loss mutation [19] (Fig. S2B, C), which reveals underlying mechanism differences between them.

In addition to the expected enrichment of the DDR pathway (Fig. S2D), CDK12 inhibition also suppressed key signaling associated with the progression and drug resistance of PCa, such as the Wnt [23], Hippo [24] and, Notch pathways [25] (Fig. 3C). The gene sets HALLMARK_ANDROGEN_RESPONSE and AR_SIGNATURE [26] were significantly de-enriched upon THZ531 treatment (Fig. 3D). Downregulation was also found in AR score genes [27] (Fig. 3E). We validated the significantly lower mRNA levels of canonical AR targets in THZ531-treated LNCaP cells (Fig. S2E). These findings...
CRISPR/Cas9 screening to identify genes critically required for PCa. A Schematic illustration of CRISPR/Cas9 screening to identify conserved kinases in C4-2 cells cultured with normal or 10–25 μM enzalutamide. B RRA scores for the kinome. The top 20 candidate genes are marked in red. C Heatmap showing the correlation of the kinome among the normal and AR antagonism groups on the 21st and 28th days. D Venn diagram showing the overlap of the top 20 candidate genes from each group. E Typical images of immunostaining of the CDK12 protein in PCa and normal prostate tissues. The scale bars represent 100 μm. F IHC score of the CDK12 protein levels in PCa and normal prostate tissues. G Level of CDK12 knockout in PCa cells, as measured by western blotting. H PCa cell proliferation assessed by the colony formation assay. I PCa cell viability assessed by the CCK-8 cell viability assay.
may partially explain the dependence of PCa cells on CDK12 under AR antagonism.

Prior studies have proven that inhibiting transcription preferentially inactivates lineage-specific or oncogenic genes related to tumor survival [28–32]. Based on the essential transcription function of CDK12, we hypothesized that transcripts sensitive to CDK12 inhibition may also include lineage-specific and oncogenic transcripts and, rather than being random, contribute biological functions in PCa cells.

Fig. 2 CDK12 inhibition induces apoptosis in PCa cells. A PCa cells were seeded and treated with THZ531 in colony formation assays. B, C CCK-8 cell viability assay of PCa cells treated with the indicated time and concentrations of THZ531. D EU incorporation assay of LNCaP and C4–2 cells showing the difference in newly transcribed RNA after treatment with 1.0 µM THZ531 for 6 h. The scale bars represent 100 µm. E FACS analysis of the LNCaP and C4–2 cell cycle after THZ531 treatment for 12 h. F FACS analysis of LNCaP and C4–2 cells stained with AV and PI after THZ531 treatment for the indicated times. G Western blotting showing the level of RNAPII CTD S2 phosphorylation in LNCaP and C4–2 cells treated with THZ531. H The numbers of oncosphere colony in LNCaP and C4–2 cells treated with DMSO or 0.2, 0.5 µM THZ531 for 7 days. I The sizes of oncospheres observed for LNCaP and C4–2 cells treated with DMSO or 0.2, 0.5 µM THZ531 for 7 days.
Fig. 3  CDK12-ISTS contribute to the survival processes of PCa cells.  

A. The numbers of differentially expressed genes (DEGs) induced upon either DMSO or THZ531 treatment in LNCaP cells at 6 h.  

B. Log2 fold changes in DEGs, total downregulated genes (TDGs) and significantly downregulated genes (SDGs) expression induced by THZ531 in LNCaP cells for 6 h.  

C. KEGG analysis of significant DEGs in LNCaP cells treated with THZ531 for 6 h.  

D. GSEA revealed that gene sets of androgen response and AR signature were significantly downregulated in THZ531-treated LNCaP cells.  

E. Heatmap showing the downregulation of AR score genes in THZ531-treated LNCaP cells.  

F. Venn diagram showing the SDGs among different doses of THZ531 as CDK12-ISTS.  

G. Heatmap showing the downregulation of prostate basal/luminal cell genes and AR-regulated genes related to CDK12-ISTS.  

H. Enriched GO functional categories of CDK12-ISTS.  

I. Log2 fold changes in the mRNA abundance of CDK12-ISTS and SDGs.
survival relevance to PCa. Then, we identified a distinct class of 867 transcripts that were significantly downregulated in a dose-independent fashion (Fig. 3F) and termed this group CDK12 inhibition-sensitive transcripts (CDK12-ISTs). As expected, CDK12-ISTs contained a number of prostate lineage-specific genes, including basal/luminal [33] and AR-regulated genes (Fig. 3G), suggesting tissue specificity of CDK12-ISTs. More importantly, the GO analysis revealed the participation of CDK12-ISTs in basic survival processes, such as transcription, DDR, and apoptosis regulation, highlighting the critical dependences of PCa on CDK12 (Fig. 3H). In addition, CDK12-ISTs were actually more sensitive to
THZ531 treatment than other affected transcripts (Fig. 3I), further supporting our hypothesis.

Integrating SE landscapes with CDK12-ISTs indicates potential lineage-specific oncogenes of PCa cells

SEs regulate specific gene expression programs to sustain fundamental cell biology, including key lineage-specific oncogenes that control the cancer cell state [34, 35], which is highly relevant to the characterization of CDK12-ISTs. To characterize SE-associated transcripts in PCa, we performed CUT&Tag assays in LNCaP cells using an antibody recognizing H3K27ac modification (Fig. 4A) and obtained 1443 SE-associated genes involved in the progression and drug resistance of PCa (Fig. 4B, C), including essentially lineage-specific transcription factors, such as FOXA1, GATA2, HOXB13 [36], and NCOA1 [37] (Fig. 4A).

A few SE-driven genes are especially vulnerable to transcriptional defects and thereby serve as (i) the reason confering the reliability of CDK12 as a PCa druggable target.

DISCUSSION

Somatic loss-of-function CDK12 mutations generate an aggressive subtype of CRPC with poor outcomes [19–21]. While we identified CDK12 as critically required for PCa cell survival via a CRISPR screen. Both genetic deletion and functional inhibition suggested that CDK12 accelerates PCa progression to a certain extent, similar to that in other malignancies [16–18]. The inconsistent transcriptome alteration further revealed discrepant mechanisms between CDK12 inhibition and somatic loss-of-function CDK12 mutations in clinical patients. Our experiments demonstrated that HSPC and CRPC cells both respond dramatically to THZ531, highlighting the reliability of CDK12 as a PCa druggable target.

Cancer cells require highly active transcription to maintain their essential and oncogenic biological functions, including rapid proliferation and aggressive invasion [46]. Therefore, it is reasonable that CDK12-IST-associated survival pathways may primarily contribute to the vulnerability of PCa cells to CDK12. To our surprise, CDK12-ISTs consisted of genes related to prostatic identity and AR-regulated genes, implying the cancer-type-specific feature of CDK12-ISTs.

SEs control specific gene expression programs [34, 35], including the expression of a few critical oncogenes, which are
particularly vulnerable to transcription defects [28–32]. By virtue of this theory, studies [28–32] have successfully identified oncogenes by inducing transcription initiation defects. Therefore, we hypothesized that SE-driven CDK12-ISTS may enumerate oncogenes that contribute to the dependencies of PCa cells. Subsequent integrative results of the SE landscape and CDK12-ISTS validated our hypothesis. It is worth noting that many AR-regulated genes can be found in SE-associated genes without androgen. Therefore, we believe that oncogenes under androgen stimulation (similar to the early stage of PCa) may also be suppressed by CDK12 inhibition and thus impact PCa cell survival. For this reason, we integrated CDK12-ISTS and SE-associated genes, which included a cohort stimulated by R1881.

Based on our CRISPR results and the impact of THZ531 on AR signaling, we hypothesized and validated a synergistic effect between THZ531 and AR antagonists. To our excitement, the

Fig. 5 THZ531 synergizes with AR antagonists in PCa cells. A Synergistic relationship between different doses of THZ531 and AR antagonists in the CCK-8 assay. CI values < 1 represent synergy. B PCa cells treated with 0.2 µM THZ531, 25 µM AR antagonists or combination in the colony formation assay. C Heatmap showing the signal intensity of H3K27ac and motif enrichment in treated LNCaP cells. D IGV views of sequencing data at AR target loci under different conditions. E Relative mRNA expression of canonical AR target genes in LNCaP cells treated for 24 h. F Venn diagram with the number of SE-associated genes among treated LNCaP cells and KEGG analysis of SE-associated genes exclusive to the combination group. G Relative mRNA expression of MCL1, BIRC5, and BCL2L1 in LNCaP cells treated for 24 h.
synergetic outcome was not restricted to HSPC or CRPC cells or to first- or next-generation AR antagonists. Mechanistically, the FOXAAR motif was significantly enriched with a decreasing H3K27ac signal after combination treatment, strongly supporting that combination treatment results in hypoactive AR transcription activity, which is consistent with transcriptome alteration.

The KEGG analysis of SE-associated genes in combination treatment demonstrated the most significant enrichment of the apoptosis pathway, which contains important anti-apoptosis genes BIRC5 and BCL2L1. AR binds to agonist-ligated ARBEs to mediate DNA damage and is synergistic with sorafenib treatment in hepatocellular carcinoma. Gut. 2020;69:727–36.

Choi HJ, Jin S, Cho H, Won HY, Ahn HW, Jeong GY, et al. CDK12 drives breast tumor initiation and trastuzumab resistance via WNT and IRS1-ErbB-Pi3K signaling. EMBO Rep. 2019;20:e48058.

Ji J, Zhou C, Wu J, Cai Q, Shi M, Zhang H, et al. Expression pattern of CDK12 protein in gastric cancer and its positive correlation with CD8+ cell density and CCL2 expression. Int J Med Sci. 2019;16:1142–8.

Wu YM, Cieślik M, Lonigro RJ, Vats P, Reimers MA, Cao X, et al. Inactivation of CDK12 delineates a distinct immunogenic class of advanced prostate cancer. Cell. 2018;173:1770–82.e14.

Antonarakis ES, Isaacson Velho W, Fu H, Wang N, Agarwal V, Sacristan Santos V, et al. CDK12-altered prostate cancer: clinical features and therapeutic outcomes to standard systemic therapies, poly (ADP-ribose) polymerase inhibitors, and PD-1 inhibitors. JCO Precis Oncol. 2020;4:370–81.

Ranish EA, Bors J, Janssen AH, Bakker C, De Jong K, Redlich K, et al. Characterizing CDK12-mutated prostate cancers. Clin Cancer Res. 2021;27:566–74.

Wu YM, Cieślik M, Lonigro RJ, Vats P, Reimers MA, Cao X, et al. Inactivation of CDK12 delineates a distinct immunogenic class of advanced prostate cancer. Cell. 2018;173:1770–82.e14.

Bainbridge A, Walker S, Smith J, Patterson K, Dutt A, Ng YM, et al. IKBKE activity enhances AR levels in advanced prostate cancer via modulation of the Hippo pathway. Nucleic Acids Res. 2020;48:856–82.

Farah E, Li C, Cheng L, Kong Y, Lanman NA, Pascuski P, et al. NOTCH signaling is activated in and contributes to resistance in enzalutamide-resistant prostate cancer cells. J Biol Chem. 2019;294:8543–54.

Zhang Z, Karthaus WR, Lee YS, Gao VR, Wu C, Russo JW, et al. Tumor microenvironment-driven NRGI promotes antiangiogenesis resistance in prostate cancer. Cancer Cell. 2020;38:279–96.e8.

Hieronymus H, Lamb J, Ross KN, Peng XP, Clement C, Rodina A, et al. Gene expression signature-based chemical genomic prediction identifies a novel class of HS990 pathway modulators. Cancer Cell. 2006;10:321–30.

Chipumuro E, Marco E, Christensen CL, Kvistbak W, Zhang T, Mathewy CM, et al. CDK7 inhibition suppresses super-enhancer-linked oncogenic transcription in MYCN-driven cancer. Cell. 2014;159:1126–39.

Christensen CL, Kvistbak W, Abraham BB, Al-Shahrour F, Zhang T, et al. Targeting transcriptional addictions in small cell lung cancer with a covalent CDK7 inhibitor. Cancer Cell. 2014;26:909–18.

Zhang N, Karthaus WR, Lee YS, Gao VR, Wu C, Russo JW, et al. Tumor microenvironment-driven NRGI promotes antiangiogenesis resistance in prostate cancer. Cancer Cell. 2020;38:279–96.e8.

Zhang J, Liu W, Zou C, Zhao Z, Lai Y, Shi Z, et al. Targeting super-enhancer-associated oncogenes in osteosarcoma with THZ2, a covalent CDK7 inhibitor. Clin Cancer Res. 2020;26:2681–92.

Jiang YY, Lin DC, Mayakonda A, Hazawa M, Ding LW, Chien WW, et al. Targeting super-enhancer-associated oncogenes in osseous squamous cell carcinoma. Gut. 2017;66:1358–68.

Karthaus WR, Liakapou DJ, Drost J. Identification of multipotent luminal progenitor cells in human prostate organoid cultures. Cell. 2014;159:163–75.

Hnisz D, Abraham BB, Lee TI, Lai A, Sant-André V, Sigova AA, et al. Super-enhancers in the control of cell identity and disease. Cell. 2013;155:934–47.

Niederreiter AR, Vashney A, Parker SC, Martin DM. Super enhancers in cancers, complex disease, and developmental disorders. Genes (Basel). 2015;6:1183–200.

Hankey W, Chen Z, Wang Q. Shaping chromatin states in prostate cancer by pioneer transcription factors. Cancer Res. 2020;80:2427–36.

Lopez SM, Agoulnik AI, Zhang M, Peterson LE, Suarez E, Gandarillas GA, et al. Nuclear receptor corepressor 1 expression and output declines with prostate cancer progression. Clin Cancer Res. 2016;22:3937–49.

Chen T, Xu J, Fu W. EGFRI/FOXO3A/LXR-α axis promotes prostate cancer proliferation and metastasis and dual-targeting LXR-α/EGRF shows synthetic lethality. Front Oncol. 2020;10:1688.

Azemikhalik M, Ashtiani HA, Aghaei M, Rastegar H. Evaluation of discoidin domain receptor-2 (DDR2) expression level in normal, benign, and malignant human prostate tissues. Res Pharm Sci. 2015;10:356–63.

Domińska K, Ochędalski T, Kowalska K, Matysiak-Buzrycka ZE, Plucznik E, Piastowska-Ciesielska AW. Interaction between angiotensin II and relaxin 2 in the...
ACKNOWLEDGEMENTS
The authors would like to thank Quentin Liu’s lab members for their critical comments and technical support.

AUTHOR CONTRIBUTIONS
HL and ZW performed development of methodology and writing, review and revision of the paper, provided analysis and interpretation of data, and statistical analysis; DJ and FL performed development of methodology and writing, review, and revision of the paper; ML and XL provided analysis and interpretation of data, and statistical analysis; YY, BH and MY provided technical support; HH, QL and JP performed study concept and design. All authors read and approved the final paper.

FUNDING STATEMENT
The present study was funded by the National Natural Science Foundation of China (81772754 to JP); the Major Basic Research and Cultivation Program of Natural Science Foundation of Guangdong Province (2017A03038009 to JP); Shenzhen Basic Science Research (JCZY20190809164617205 to JP); the Sanming Project of Medicine in Shenzhen (ZSM202011011 to JP); the research start-up fund of part-time PI, SAHSYSU (ZSQYJZPI202003 to JP); the National Natural Science Foundation of China (81902613 to JD); the National Key R&D Program of China (2019YFA0110300 and 2017YFA0505600-04 to QL); the National Natural Science Foundation of China (81820108024 and 81630005 to QL, 81773166 to ZW); and the Science and Technology Planning Project of Guangzhou (201804020044 to QL).

COMPETING INTERESTS
The authors declare no competing interests.

ETHICS APPROVAL
Our study involved a commercially available human PCa TMA (HProA150CS01, Outdo Biotech, Shanghai, China).

ADDITIONAL INFORMATION
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41419-021-04027-6.

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