Preclinical Efficacy and Molecular Mechanism of Targeting CDK7-Dependent Transcriptional Addiction in Ovarian Cancer

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Abstract

Ovarian cancer remains a significant cause of gynecologic cancer mortality, and novel therapeutic strategies are urgently needed in clinic as new treatment options. We previously showed that BET bromodomain inhibitors displayed promising efficacy for the treatment of epithelial ovarian cancer by downregulating pivot transcription factors. However, the potential antitumor activities and molecular mechanisms of other epigenetic or transcriptional therapies have not been systematically determined. Here, by performing an unbiased high-throughput drug screen to identify candidate compounds with antineoplastic effects, we identified THZ1, a recently developed covalent CDK7 inhibitor, as a new transcription-targeting compound that exerted broad cytotoxicity against ovarian tumors. Mechanistically, CDK7 represented a previously unappreciated actionable vulnerability in ovarian cancer, and CDK7 inhibition led to a pronounced dysregulation of gene transcription, with a preferential repression of E2F-regulated genes and transcripts associated with super-enhancers. Our findings revealed the molecular underpinnings of THZ1 potency and established pharmaceutically targeting transcriptional addiction as a promising therapeutic strategy in aggressive ovarian cancer.

Introduction

Epithelial ovarian cancer (EOC) represents the most life-threatening gynecologic malignancy, causing more than 140,000 deaths annually worldwide (1, 2). For the past decades, the standard treatment has been surgery and platinum-based chemotherapy. The survival improvement achieved with conventional cytotoxic agents has reached a plateau, posing formidable challenges for current translational researches to invent novel targeted therapeutic regimens (3, 4). However, ovarian cancer is characterized by low prevalence of actionable genetic alterations (5). As a result, targeted approaches engaging tumor-specific mutations are often not an attractive option. Therefore, further elucidation of disease pathogenesis is imperative to identify unique molecular vulnerabilities in ovarian cancer for developing state-of-the-art therapies (6, 7).

Recent pan-cancer analysis of tumor genomic traits has revealed a striking cancer genome hyperbola. Almost all high-grade serous ovarian carcinoma samples (HGS-OvCa), displaying large-scale copy number aberrations, are positioned in the C class and distinct from tumors of M class, which are dominated by somatic mutations (8). On the basis of these new insights into mechanisms of oncogenesis, a promising therapeutic framework emerges by exploring recurrent chromosomal gains and losses in ovarian cancer. Along this line, we and others have recently demonstrated that the bromodomain and extra terminal (BET) protein BRD4 is frequently amplified in HGS-OvCa and that pharmacologic inhibition of BRD4 using BET bromodomain inhibitors JQ1 or I-BET151 shows antiproliferative efficacy in preclinical ovarian tumor models. As in other various diseases, the anticancer activity of BET inhibitors has been attributed to transcriptional suppression of key proto-oncogenes, such as MYC or FOXM1 (9, 10). Despite these tremendous progresses on uncovering gene transcription regulation as an emerging therapeutic vulnerability against ovarian malignancy, the potential antitumor activities and molecular mechanisms of other epigenetic or transcriptional therapies remain largely elusive.

Here we used a small-molecule screen and discovered THZ1, a covalent inhibitor of cyclin-dependent kinase 7 (CDK7), as a drug candidate for the treatment of ovarian tumors. Mechanistically, THZ1 led to a profound downregulation of gene transcripts, preferentially those containing E2F-binding motifs and associated with super-enhancers (SE). These findings provided a
conceptual rationale for exploiting transcriptional addiction as a new therapeutic opportunity in ovarian cancer.

**Materials and Methods**

**Cell culture and reagents**

Tumor cell lines were originally obtained from ATCC or JCRB in 2014, where cell characterization was performed using polymorphic short tandem repeat (STR) profiling. No subsequent authentication was done by the authors. Cells were cultured in RPMI1640 (Invitrogen) supplemented with 10% FBS (Invitrogen). For gene knockout experiments using CRISPR-Cas9 system, cells were infected with lentivirus encoding sgRNAs (Supplementary Table S9, for details of the sgRNA sequences). IQ1 was purchased from Millipore (11). THZ1 was purchased from MedChem Express. Small-molecule inhibitor library was purchased from Selleck Chemicals. All inhibitors were reconstituted in DMSO (Sigma-Aldrich) at a stock concentration of 10 mmol/L.

**High throughput inhibitor screen and THZ1 IC50 measurement**

High-throughput small-molecule screen was performed as previously reported (12). Cells were seeded at optimal density and treated with the indicated inhibitors at the same concentration (500 nmol/L). Fresh medium and drugs were changed every 3 days. After 6 days of drug exposure, cells were imaged and viability was calculated using ArrayScan Infinity (Thermo Scientific). To determine the IC50 of THZ1 in ovarian cancer cell lines, seven concentrations of compounds were applied at a stepwise three-fold dilution series. Cell viability was evaluated using CellTiter-Glo reagent according to the manufacturer’s instructions (Promega). Estimates of IC50 were derived from the seven dose–response curves plotted by GraphPad Prism 6 (GraphPad Software, Inc.).

**Cell cycle and apoptosis analysis**

Cell-cycle analysis was performed 24 hours after THZ1 treatment. Cells were fixed in cold ethanol and incubated with propidium iodide (PI)/RNase Staining Solution (Cell Signaling Technology) for 15 minutes. Cell apoptosis was assayed using Dead Cell Apoptosis Kit with Annexin V–FITC and PI following manufacturer’s instructions (Life Technologies). Flow cytometric analysis was performed on a FACS AriaII cytometer (BD Biosciences). Flow cytometry data were analyzed by using FlowJo software.

**Western blot analysis**

Cells were lysed in RIPA buffer (Tris pH 7.4 50 mmol/L, NaCl 150 mmol/L, NP-40 1%, SDS 0.1%, EDTA 2 μmol/L) containing protease inhibitors (Roche) and phosphatase inhibitors (Roche), and subjected to SDS-PAGE and Western blot analysis. Antibodies against the following proteins were used: CDK4, CDK6, CDK7, CDK9, cleaved PARP, H3, actin, tubulin (Cell Signaling Technology); RNAPII S2, RNAPII S5, RNAPII S7 (Millipore).

**RNA sequencing and analysis**

Cells were treated with DMSO or THZ1 (100 nmol/L) for 6 hours, and cell numbers were measured by manually counting of Trypan blue-stained cells before lysed in buffer. ERCC Spike-In RNA Mix (Life Technologies) was added to the cell lysates in proportion to cell number as previously described (13). Total RNA (three biological replicates per condition) was extracted using RNeasy Plus Mini Kit (Qiagen) according to the manufacturer’s protocol. RNA quality was assessed using an Agilent 2100 Bioanalyzer. Sequencing libraries were generated using NEBNext Ultra RNA Library Prep Kit for Illumina (NEB). The index-coded libraries were clustered on a cBot Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumina) and sequenced on an Illumina Hiseq 2500 platform to generate 30 million 125 bp paired-end reads (Novogene). Quality control was performed on raw data in fastq format and clean reads were obtained by removing adapters, ploy-N sequences and low-quality reads. Index of the reference genome was built using Bowtie v2.2.3 (14) and clean reads were aligned to the reference genome using TopHat v2.0.12 (15). We used HTSeq v0.6.1 (16) to count the reads mapped to each gene and further normalized read counts to the control ERCC. Reads. Differential expression analysis was performed using the DESeq R package (1.18.0). P-values were adjusted using the Benjamini–Hochberg procedure for controlling the false discovery rate. Genes with an adjusted P-value<0.05 were considered differentially expressed. The sequencing data have been deposited in NCBI BioProject database (http://www.ncbi.nlm.nih.gov/bioproject/) under the accession number SRP106825.

**Chromatin precipitation, sequencing, and analysis**

Chromatin precipitation was performed as previously described. Briefly, cells were cross-linked with 1% formaldehyde and quenched with 2.5 M glycine. Cell pellets were lysed and sonicated using Sonics Vibra-Cell 505 ultrasonicator. Sonicated lysates were cleared and incubated overnight with magnetic beads bound with H3K27ac antibody (Abcam) to enrich for associated DNA fragments. Precipitated complexes were washed and cross-links were reversed overnight. RNA and protein were digested using RNase A and Proteinase K, respectively. DNA was purified with Qiaquick PCR Purification Kit. DNA libraries were generated using the Illumina TruSeq DNA Sample Preparation v2 Kit and sequenced on the Illumina HiSeq 2000 to generate 20 million 50 bp reads (Novogene). Clean reads were aligned to the reference genome using BWA v0.7.12 (17). Wiggle files for gene tracks were created and peaks were called using MACS v2.1.0 (18). Super-enhancers were identified using ROSE package as previously described (19).

**Tumor xenograft models**

For THZ1 efficacy studies, tumor cells (1 × 10^6) were mixed with Matrigel (BD Biosciences) and subcutaneously implanted in the dorsal flank of BALB/c Nude mice. When tumor sizes reached approximately 150 mm^3, mice were randomized into two groups of 10 mice each. One group of mice was treated with vehicle control (10% DMSO in D5W), and the other group was treated with THZ1 10 mg/kg twice daily. To test the effects of CDK7, control or CDK7 knockout cells were subcutaneously implanted into BALB/c Nude mice. Tumor volumes (10 animals per group) were measured with digital caliper and calculated as length × width^2 × 0.5. All animal protocols were approved by the Institutional Animal Care and Use Committee of Ren Ji Hospital.

**Histology and IHC**

Histology and IHC were performed using formalin-fixed, paraffin-embedded 5-μm-thick tumor sections. Slides were...
deparaffinized in xylene, passed through graded alcohols, and antigen retrieved with 10 mmol/L citrate buffer (pH 6.0) in a steam pressure cooker. Preprocessed tissues were either stained with hematoxylin and eosin (H&E), or treated with Peroxidase Block (Dako) to quench endogenous peroxidase activity, blocked using Protein Block (Dako), and subsequently incubated with Ki-67 or cleaved caspase-3 antibodies (Cell Signaling Technology).

Slides were then washed in 50 mmol/L Tris-Cl (pH 7.4) and incubated with horseradish peroxidase-conjugated secondary antibody. Immunoperoxidase staining was developed using a 3,3’-diaminobenzidine (DAB) chromogen (Sigma). Slides were then counterstained with hematoxylin, dehydrated in graded alcohol and xylene, and coverslipped using mounting solution.

Statistical analysis
Gene set enrichment analysis was performed using the GSEA software (20). Gene ontology and pathway analyses were performed with DAVID Bioinformatics Resources (21). In all experiments, comparisons between two groups were based on two-sided Student t-test and one-way ANOVA was used to test for differences among more groups. P values of <0.05 were considered statistically significant.

Results
Identification of THZ1 as a potent inhibitor in ovarian cancer
We reasoned that an antitumor inhibitor susceptibility profile of ovarian tumor cells might unveil additional therapeutic vulnerabilities of ovarian cancer. We therefore subjected multiple ovarian cancer cell lines, including COV 413B, OVCA420, and SKOV3, to a high-throughput small-molecule screen with a library of 181 FDA-approved drugs or other clinically relevant compounds (Supplementary Table S1). Using an

Figure 1.
Identification of THZ1 as a potent inhibitor in ovarian cancer. A, Schematic overview of high-throughput small-molecule screen. B, Top-ranked compounds suppressing ovarian cancer cell growth and their corresponding targets were presented in an inhibitory activity heatmap format. C, Representative images of cancer cells exposed to indicated inhibitors and counter stained by DAPI. D, Crystal violet staining of tumor cells treated with various concentrations of JQ1 or THZ1 for 10 days. E, Western blot analysis of RNAPII CTD phosphorylation in ovarian cancer cells that were treated with THZ1.
Imaging-based viability assay, cells were screened against each inhibitor for drug candidates with an IC_{50} of less than 0.5 μmol/L (Fig. 1A). A small subset of the tested compounds exhibited pronounced toxicity toward each cell line (Supplementary Fig. S1A) and we identified a list of top-ranked 25 compounds with IC_{50} < 0.5 μmol/L across all the three cell lines (Fig. 1B). Nine of these compounds were chemotherapeutic agents, in keeping with the generally exceptional chemosensitivity of ovarian cancer. Other major putative categories of these effective inhibitors included transcription, proteostasis, and epigenetic regulation (Supplementary Fig. S1B).

BRD4 regulates gene transcription through linking histone acetylation and core transcriptional components, and transcriptional perturbation of oncogenic drivers has been implicated in the wide range of sensitivity to BRD4 inhibition (22). As transcriptional CDKs also play essential roles in transcription initiation and elongation, we focused our further investigations on the promising therapeutic merit of CDK inhibitors, among which THZ1, a novel covalent CDK7 inhibitor (23), was particularly interesting. THZ1 has recently been demonstrated to display therapeutic activity against an emerging list of cancers, such as T-cell acute lymphoblastic leukemia (23), MYCN-amplified neuroblastoma (24), small-cell lung cancer (25), triple-negative breast cancer (TNBC; ref. 26), and esophageal squamous cell carcinoma (27). Likewise, all three ovarian cancer cell lines were highly sensitive to THZ1 in our drug screen (Fig. 1C) and we validated these findings using crystal violet staining (Fig. 1D). CDK7 regulates transcriptional initiation by directly phosphorylating serine 5 (S5) and serine 7 (S7) at the carboxyl-terminal domain (CTD) of RNA polymerase II (RNAPII). In addition, CDK7 exerts its control on transcriptional elongation via activating other CDKs to phosphorylate serine 2 (S2) of RNAPII CTD (28–30). As expected, THZ1 treatment resulted in a dose-dependent inhibition of RNAPII CTD phosphorylation at S2, S5, and S7 (Fig. 1E).

Antineoplastic effects of THZ1 across various ovarian cancer cell lines

To further validate the potency of THZ1 as a drug candidate, we extended our findings in COV 413B, OVCA420, and SKOV3 cells to a large set of distinct ovarian cancer cell lines. A comprehensive panel of 18 ovarian cancer cell lines was assembled to represent the major histologic subtypes of EOC (seven serous, five nonserous, and six unspecified). We conducted a pharmacologic screen to determine their response to THZ1. These studies confirmed that the majority of these cell lines showed a general hypersensitivity to THZ1-induced cytotoxicity (Fig. 2A and B; Supplementary Table S2). It is noteworthy that although many cell models also responded well to IQ1 (11), THZ1 was usually more potent to suppress tumor cell proliferation (Supplementary Fig. S2A), and there was a synergistic effect of growth inhibition with IQ1 and THZ1 in combination (Supplementary Fig. S2B). Furthermore, we found that multiple cell lines including A2780, COV 362, and ES-2, which were intrinsically resistant to IQ1, were efficaciously inhibited by THZ1 treatment (Fig. 2C and D).

To further characterize the antineoplastic effects of THZ1, cell-cycle progression and apoptotic index were determined using flow cytometry in three representative cell lines (A2780, COV 413B, and OVCA420). THZ1-induced cell-cycle arrest, particularly a pronounced S phase reduction, was evident in all three cases (Supplementary Fig. S3A). We also observed a concomitant increase of cell apoptosis/ necrosis in a dose-responsive and time-dependent manner as assessed by cleaved PARP Western blotting (Fig. 2E) and Annexin V-PI staining (Supplementary Fig. S3B). Finally, we explored the in vivo therapeutic potential of THZ1 in two independent xenograft transplantation models (A2780 and HEY). Once tumors reached an optimal volume of ~150 mm³, the animals were randomized into two cohorts (n = 10/group) and treated with vehicle or THZ1. Notably, mice receiving THZ1 showed a statistically significant reduction in tumor burden (Fig. 2F). Histologic analysis of THZ1-treated tumors revealed widespread necrotic tissues, decreased cell proliferation indicator Ki-67, and activation of the apoptotic marker cleaved caspase-3, whereas vehicle-treated tumors were exclusively composed of viable epithelial cells (Supplementary Fig. S4A). It was notable that although THZ1 exhibited some inhibitory effects in noncancerous cells, including HEK293T, BEAS-2B, and primary normal ovarian epithelial cells, the IC_{50} values in these cells were relatively higher than those in EOC cell lines (Supplementary Fig. S4B). In addition, THZ1 was not as efficacious in lung cancer cells as compared with ovarian cancer cells (Supplementary Fig. S4C) and there was no noticeable mice weight loss during continuous THZ1 administrations (Supplementary Fig. S4D), consistent with previous reports demonstrating the limited toxicity of THZ1 and supporting a feasible therapeutic window to use CDK7 inhibitors in clinic. We concluded that THZ1 impeded both cell-cycle and cell survival to attenuate tumor growth in different histologic subtypes of ovarian cancer.

Widespread dependence of ovarian cancer cells on CDK7

THZ1 selectively inhibits CDK7 with additional cross-reactivity against CDK12/13 at higher concentrations (23). Because CDK7 kinase activity has been implicated in the regulation of both gene transcription and cell cycle (31), the capacity of THZ1 to target ovarian cancer presumably relates to its covalent inhibition of CDK7. However, a formal investigation on the potentially critical role of CDK7 in ovarian cancer is necessary to establish CDK7 as a valid molecular target and to complement the pharmacologic studies of THZ1 in this indication. To this end, we first examined the expression levels of CDK7 protein in our ovarian cancer cell panel. CDK7, as well as RNAPII CTD phosphorylation at S2, S5, and S7, were ubiquitously expressed in essentially all cell lines (Fig. 3A). Next, we used CRISPR–Cas9 system to genetically deplete CDK7 in 11 ovarian cancer cell lines. Three independent single-guide RNA sequences targeting CDK7 (sgCDK7) led to a substantial reduction of CDK7 protein (Fig. 3B and Supplementary Fig. S5A). Strikingly, in all tested cell models, CDK7 downregulation profoundly suppressed cell viability in vitro (Fig. 3C; Supplementary Fig. S5B). Accordingly, ablation of CDK7 in HEY or ES-2 cells significantly impaired tumor xenograft formation in vivo (Fig. 3D). These data credentialed CDK7 as a functionally relevant target of THZ1 in ovarian cancer.

CDK7 belongs to a large family of ~20 serine/threonine kinases that can be roughly divided into cell-cycle CDKs and transcriptional CDKs (31). In addition to phosphorylating RNAPII CTD, CDK7 is a pivot component of CDK-activating kinase (CAK), which is involved in regulating all CDKs (32). We determined the requirement of other CDKs for ovarian tumorigenicity by

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knocking out each CDK gene using CRISPR-Cas9 technique. Interestingly, several cell-cycle CDKs (CDK1, CDK2, CDK4, and CDK6) and transcriptional CDKs (CDK7, CDK9, and CDK12) were indispensable for tumor cell growth (Supplementary Fig. S6A and S6B). Therefore, unlike TNBC (26), ovarian cancer exhibited a universal dependence on multiple CDKs, reinforcing CDK7 as a master CDK that provided a unique target for controlling ovarian cancer.

To assess the clinical relevance of CDK7 in ovarian cancer patients, we conducted a meta-analysis using the curatedOvarianData database (33). Some CDKs, including CDK7, were associated with poor prognosis in ovarian cancer (Supplementary Fig. S7; overall HR for CDK7 = 1.07, 95% confidence interval, 1.01–1.15). We also performed immunohistochemistry on a cohort of fifty EOC specimens, and found that more than 40% of these samples showed positive staining of CDK7 (Supplementary Table S3). When the patients were stratified into two groups based on CDK7 expression, the CDK7 positive group had a significant median overall survival disadvantage compared with the CDK7 negative group (Fig. 3E). Together, these analyses established a
Figure 3. Widespread dependence of ovarian cancer cells on CDK7. A, Western blot analysis of RNAPII CTD phosphorylation and CDKs in a panel of ovarian cancer cell lines. B, CDK7 was knocked out in the indicated cells using CRISPR-Cas9 system. Western blot analysis demonstrated CDK7 downregulation. C, Impaired cell viability following depletion of CDK7 in ovarian cancer cells. The left, middle, and right panels showed the bright-field images, the crystal violet staining, and the quantification of cell viability (\( P < 0.05 \), ANOVA followed by Tukey post-test), respectively. D, Images and weights of tumor xenografts derived from HEY and ES-2 cells edited with sgCDK7 or sgEGFP control (\( P < 0.05 \), ANOVA followed by Tukey post-test). E, IHC analysis of CDK7 expression in a cohort of ovarian cancer specimens. Representative images of CDK7-negative or CDK7-positive samples were shown in the top panel. The bottom panel showed Kaplan–Meier plot of overall survival in CDK7-negative versus CDK7-positive ovarian cancer patients.
therapeutic rationale for targeting CDK7 in a subset of ovarian cancer patients.

THZ1-inhibited gene transcription in ovarian cancer

Considering the prominent role of CDK7 in regulating RNAPII-mediated transcription, we next investigated the impact of THZ1 treatment on genome-wide gene expression in ovarian cancer using RNA sequencing. Treatment with 100 nmol/L THZ1 for 6 hours resulted in a dramatic decrease of global messenger RNA levels, with 41% to 87% of active transcripts showing greater than 1.5-fold reduction across the three tested cell lines (Fig. 4A). We performed gene set enrichment analysis and found that the most differentially expressed genes were associated with pathways that involve in generic transcription regulation and cell-cycle progression (Supplementary Fig. S8A). Moreover, it was of great interest to observe that gene sets containing E2F-binding motifs were significantly altered by THZ1 inhibitor in all the three cell line models (Supplementary Fig. S8B).

We focused on the 2,257 differentially expressed genes that were affected by THZ1 in all the three cell lines (Supplementary Table S4). Gene ontology analysis of these core transcripts inhibited by THZ1 revealed a significant enrichment of genes involved in cell cycle, DNA repair process and transcription regulation (Fig. 4B; Supplementary Table S5). Notably, many viral transcription factors with established roles in oncogenesis were uniformly downregulated upon THZ1 treatment, including numerous zinc finger molecules, ETVs, and most interestingly, several E2F genes. In addition, enrichment analysis of common transcription factor binding motifs showed that genes containing binding sites of transcription factors such as E2F, NRF2, and ELK1 were preferentially inhibited by THZ1 across all three cell lines. Gene ontology categories and KEGG pathways overrepresented in the core set of differentially expressed genes were associated with pathways that involve in cell cycle, DNA repair process and transcription regulation (Fig. 4H; Supplementary Table S5). Taken together, these data demonstrated that targeting CDK7 using THZ1 led to a widespread transcriptional shutdown in ovarian cancer cells, with E2F transcription factors being crucial candidate mediators of the inhibitory effects.

Preferential inhibition of super-enhancer-driven gene expression by THZ1

Previous work has established that the expression of genes associated with super-enhancers is disproportionately vulnerable to transcriptional inhibitors, which underlies the extreme sensitivity toward TZH1 in several types of cancer (24, 25). However, the identification and functional annotation of super-enhancers in ovarian cancer are elusive. We therefore sought to investigate the possible contribution of super-enhancers to the remarkable susceptibility to THZ1 in our ovarian cancer models. We performed chromatin immunoprecipitation followed by next-generation sequencing (ChIP-seq) using an antibody recognizing histone H3K27 acetylation (H3K27ac), to estimate the positions and sizes of active enhancer regions in COV 413B, OVCA420, and SKOV3 cells. Stitched enhancer domains were ranked by H3K27ac signal intensity and in each cell line (Supplementary Tables S6–S8), a subset of enhancers contained an appreciably higher H3K27ac load than regular enhancers and was identified as super-enhancers (Fig. 5A). GSEA revealed that genes with top-ranked enhancers involved in regulation of embryonic development, gene transcription, and cancer pathways (Supplementary Fig. S9A). In addition, these genes were significantly enriched for transcripts featured by E2F-binding motifs (Supplementary Fig. S9B). There was a considerable overlap of SE-associated genes

**Figure 4.**

THZ1-inhibited gene transcription in ovarian cancer. **A,** Heatmaps of global gene expression values in COV 413B, OVCA420, and SKOV3 cells that were treated with THZ1 (100 nmol/L for 6 hours) versus DMSO control. **B,** Gene ontology categories and KEGG pathways overrepresented in the core set of differentially expressed transcripts that were inhibited by THZ1 across all three cell lines.

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in three cell lines (Supplementary Fig. S9C), although each cell type displayed a distinct super-enhancer profile.

We found that many super-enhancers were adjacent to known oncogenic molecules and lineage-specific transcription factors previously implicated in ovarian cancer progression, such as PAX8, FGFR18, and SRC (refs. 34–36; Fig. 5B; Supplementary Fig. S9D). Furthermore, we identified a number of new SE-associated genes whose functions had not been described in the context of ovarian cancer (Fig. 5A). For example, Zinc Finger MIZ-Type Containing 1 (ZMIZ1), encoding a PIAS-like protein related to NOTCH and androgen receptor signaling (37, 38), was associated with super-enhancers in all the three cell lines. We also identified a common super-enhancer at the ITGB6 gene, a less studied integrin subunit with proposed functional role in breast and colon cancer (refs. 39, 40; Fig. 5B).

To determine whether THZ1 conferred preferential inhibition of SE-driven gene expression in ovarian cancer, GSEA was conducted and showed a significant enrichment of genes associated with super-enhancers in THZ1-targeted transcripts (Fig. 5C). Similar results were achieved in COV 413B, OVCA420, and SKOV3 cell lines, emphasizing the special sensitivity of SE-driven gene expression to THZ1.

Ovarian cancer genes defined by E2F-regulated and super-enhancer-associated transcripts

To further corroborate the biological significance of E2F-mediated transcription in the context of super-enhancers, we functionally inferred a cluster of genes that were defined by being both
putative E2F-regulated and SE-associated transcripts. These genes included MYC, FOSL1, FOSL2, and ELK3, some of which had been previously implicated in ovarian cancer and other malignancies (9, 41). ChIP-seq profiles for H3K27ac occupancy of these genes confirmed that they were indeed adjacent to super-enhancers (Fig. 6A). As expected, the gene expression levels of MYC, FOSL1, FOSL2, and ELK3 were uniformly sensitive to THZ1 treatment in COV 413B, OVCA420, and SKOV3 cells, based on RNA sequencing quantification (Fig. 6B). We determined the biological function of these potential ovarian cancer genes by employing CRISPR-Cas9 system to genetically knock out each of them in COV 413B, OVCA420, or SKOV3 cell lines. Two independent single-guide RNA sequences were tested for each gene and both resulted in a statistically significant reduction of cell viability, albeit to different extent (Fig. 6C and D). Therefore, E2F-regulated and super-enhancer-associated transcripts included candidate essential cancer genes to which ovarian tumors might be addicted, and as a result, targeting CDK7-dependent transcription enabled preferential and collective suppression of multiple oncogenes that were critical for ovarian tumorigenesis.

**Discussion**

The lack of actionable recurrent genetic alterations has enormously impeded the development of effective targeted therapeutics in EOC, a highly aggressive disease representing an outstanding medical challenge. In this study, through unbiased small-molecule screen approach, we found that different histotypes of ovarian cancer were exceptionally sensitive to CDK7 inhibition. Further transcriptomic and epigenetic analyses suggested that mechanism of action underlying the efficacy of CDK7 inhibitors might relate to the preferential repression of E2F-regulated gene sets and transcripts associated with super-enhancers. Our functional and mechanistic investigations...
unambiguously supported that targeting CDK7-dependent transcriptional program represented a potential therapeutic strategy in patients with EOC.

These data unraveled the transcriptional addiction of ovarian cancer, which might have important therapeutic implications in the treatment of this disease. Our previous study and other recent reports have identified BRD4 as a candidate oncogenic driver of epithelial ovarian carcinoma, whose inhibition by BET bromodomain inhibitors such as JQ1 resulted in disrupted BET-dependent transcriptional machinery and profound cell-cycle arrest of ovarian cancer (9, 10, 42). Here, we further discovered that a selective CDK7 inhibitor, THZ1, exerted potent antineoplastic activity against both JQ1-responsive and JQ1-refractory ovarian cancer cells. We further identified a widespread dependence on CDK7 and consequently an extraordinary therapeutic effect of CDK7 inhibition across different histologic subtypes of EOC. BRD4 is a crucial regulator of transcriptional elongation that recruits the positive transcription elongation factor b (P-TEFB) complex to chromatin, whereas Cd7 is a pivot subunit of RNAPII initiation factor TFIIH which phosphorylates CTD of RNAPII during transcription initiation (43, 44). It was noteworthy that certain EOC cell lines exhibited discrepancy in their sensitivity to JQ1 and THZ1, indicative of differential dependency on different transcriptional regulators. Nevertheless, because both JQ1 and THZ1 induced profound changes in the transcription landscape, this research unequivocally illustrated that inhibition of transcriptional addiction was an effective approach to target ovarian cancer that has been shown to lack obvious actionable driver genetic mutations (5).

The unique property of transcriptional addiction and conspicuous sensitivity to THZ1 presumably is mechanistically dependent on certain factors participating in and/or downstream of gene transcription that drives ovarian cancer. Our results highlighted E2F as a possible functional basis for the therapeutic effects of CDK7 inhibition. Specifically, gene expression profiling of multiple THZ1-treated EOC cell lines consistently revealed downregulation of functionally defined E2F target genes. These findings were in agreement with decreased abundance of gene sets with E2F-binding sites in the presence of BET bromodomain inhibitors, as reported by several recent studies including ours in ovarian cancer (10, 45). Emerging data indicate that E2F transcription factors, which play important roles in cell-cycle control and other tumorigenic processes, directly promote pathogenesis of ovarian cancer (46, 47). In addition to E2Fs, cell-cycle-related CDKs, for example, CDK1, CDK2, CDK4, and CDK6, were also found indispensable for ovarian tumor cell growth, implying that the intimate interaction between transcriptional machinery and cell-cycle regulation was crucial for EOC progression. We provided further insights into the preferential inhibition of E2F target genes with THZ1 by discovering that these genes tended to be adjacent to large enhancers or even super-enhancers in ovarian cancer. Therefore, at least two models may be proposed to explain the broad downregulation of THZ1-sensitive genes enriched for E2F-binding motifs. On one hand, E2Fs could function as coactivators of SE-mediated gene transcription, and on the other hand, E2F expression is likely altered by CDK7 inhibitors, as exemplified by some E2F genes uniformly downregulated across ovarian cancer cell lines upon THZ1 treatment. Of note, there are more than nine members in the mammalian E2F family (48) and these distinct gene products may have specific or redundant activities following CDK7 inhibition. Moreover, decreased E2F target gene expression should not be solely responsible for the pleiotropic efficacy of THZ1. We corroborated this point by identifying, for the first time, a set of SE-marked genes encoding both known ovarian oncoproteins and novel candidate tumor-promoting molecules. It would be interesting to further investigate the potential contribution of these previously unrecognized factors to ovarian malignancy in future work.

Super-enhancers by definition are large clustered enhancer regions, which are frequently associated with genes essential for cell lineage identity and tumor pathogenesis (19, 49). Perturbation of transcriptional components typically has a disproportionately profound effect on SE-associated genes, which underlies the extreme sensitivity toward TZH1 in other cancer types (24, 25). Nevertheless, a catalogue of super-enhancers and their biological significance in ovarian cancer had not been thoroughly characterized. By performing CHIP-seq profiling for H3K27ac occupancy, we showed the super-enhancer landscape of EOC for the first time, to our knowledge, and found that many super-enhancers were adjacent to known or novel central regulators of ovarian cancer progression and were enriched at THZ1-targeted transcripts. In accordance with previous reports (19, 49), SE-associated genes were considerably different in distinct cell lines, suggestive of oncogenic dependency on disparate SE-regulated molecules. However, we enumerated a group of ovarian cancer-driving genes that were uniformly defined by being both putative E2F-regulated and SE-associated transcripts in all three cell models. We illustrated the functionally important role of these genes including MYC, FOSL1, FOSL2, and ELK3, among which only MYC and FOSL1 had been previously implicated in ovarian tumorigenicity (9, 50). Notably, genetic depletion of each candidate ovarian cancer gene generally led to a modest decrease in cell viability, arguing that downregulation of individual genes by THZ1 was unlikely solely responsible for its antiproliferative effects. Therefore, preferential and collective suppression of multiple oncogenes conceivably contributed to rendering ovarian cancer exceptionally susceptible to THZ1 treatment.

In conclusion, we have provided extensive preclinical evidence that transcriptional inhibitors THZ1 serves as a promising therapeutic option for the control of diverse ovarian cancers. Although tumor cells may display tremendous heterogeneity and plasticity of transcriptional machinery, ovarian cancer generally remains exquisitely addicted to vibrant transcription of myriad oncogenes, especially those associated with super-enhancers. Our study pinpoints CDK7 as a new molecular target to modulate pathologic gene expression in EOC and defines a feasible treatment strategy against ovarian cancer through the pharmaceutical disruption of transcriptional program.

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

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