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Targeting super-enhancer-driven oncogenic transcription by CDK7 inhibition in anaplastic thyroid cancer

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Running title:

CDK7-dependent transcriptional addiction in ATC

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Abstract

Background: Anaplastic thyroid carcinoma (ATC) is one of the most aggressive malignancies with no effective treatment currently available. The molecular mechanisms of ATC carcinogenesis remain poorly understood. The objective of this study was to investigate the mechanisms and functions of super-enhancer-driven oncogenic transcriptional addiction in the progression of ATC and identify new drug targets for ATC treatments.

Methods: High-throughput chemical screening was performed to identify new drugs inhibiting ATC cell growth. Cell viability assay, colony formation analysis, cell cycle analysis, and animal study were used to examine the effects of drug treatments on ATC progression. ChIP sequencing was conducted to establish a super-enhancer landscape of ATC. Integrative analysis of RNA sequencing, ChIP sequencing, and CRISPR/Cas9-mediated gene editing was used to identify THZ1 target genes. Drug combination analysis was performed to assess drug synergy. Patient samples were analyzed to evaluate candidate biomarkers of prognosis in ATC.

Results: We identifed THZ1, a covalent inhibitor of cyclin-dependent kinase 7 (CDK7), as a potent anti-ATC compound by high-throughput chemical screening. ATC cells, but not papillary thyroid cancer (PTC) cells, are exceptionally sensitive to CDK7 inhibition. An integrative analysis of both gene expression profiles and super-enhancer features reveals that the super-enhancer-mediated oncogenic transcriptional amplification mediates the vulnerability of ATC cells to THZ1 treatment. Combining this integrative analysis with functional assays discovers a number of novel cancer genes of ATC, including *PPP1R15A*, *SMG9*, and *KLF2*. Inhibition of PPP1R15A with Guanabenz (GBZ) or Sephin1 greatly suppresses ATC growth. Significantly, the expression level of PPP1R15A is correlated with CDK7 expression in ATC tissue samples. Elevated expression of PPP1R15A and CDK7 are both associated with poor clinical prognosis in ATC patients. Importantly, CDK7 or PPP1R15A inhibition sensitizes ATC cells to conventional chemotherapy.

Conclusions: Taken together, these findings demonstrate transcriptional addiction in ATC pathobiology and identify CDK7 and PPP1R15A as potential biomarkers and therapeutic targets for ATC.

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Introduction

Anaplastic thyroid carcinoma (ATC) is one of the most aggressive cancers in humans, with a median survival of 6 months regardless of stage (1). Although ATC is rare and represents only 1-2% of clinically recognized thyroid cancers, it accounts for 15-39% of thyroid cancer-related deaths (1). At the time of diagnosis for most patients with ATC, the tumor has grown beyond the thyroid gland and invaded surrounding tissues of the neck, making complete resection of tumors impossible. The conventional therapeutic strategies for thyroid cancers, including radioiodine therapy, chemotherapy and radiotherapy, have failed to prevent ATC progression or mortality (2). In addition, ATC patients have received marginal survival benefits from current targeted therapies, including tyrosine kinase inhibitors, histone deacetylase inhibitors, antiangiogenic therapy, vascular disrupting agents, and peroxisomal proliferator-activated receptor-γ agonists (2-4). Therefore, understanding of the molecular mechanisms underlying ATC pathogenesis and identification of novel drug targets are urgently needed for developing effective therapeutic interventions.

Transcriptional dysregulation is a hallmark of cancer (5). The aberrant transcriptional programs cause cancer cells to become highly addicted to certain regulators of gene transcription (6). Gene transcription is regulated by a group of cyclin-dependent kinases (CDKs), termed transcriptional CDKs (CDK7, CDK8, CDK9, CDK12 and CDK13). These transcriptional CDKs, especially CDK7 and CDK9, function to facilitate transcription initiation and promote productive elongation by phosphorylating carboxy-terminal domain (CTD) of RNA polymerase II (RNAPII). Recent studies have identified a subset of aggressive cancers with exceptional sensitivity to CDK7 inhibition, including triple negative breast cancer, T-cell acute lymphoblastic leukemia, small cell lung cancer, MYCN-dependent neuroblastoma, esophageal squamous cell carcinoma, nasopharyngeal carcinoma, and aggressive ovarian cancer (7-14). Vulnerability of these malignant tumors to CDK7 inhibition has been shown to be mediated by super-enhancer (SE)-driven oncogenic transcriptional programs. SEs are defined as large clusters of enhancers that are densely loaded with master transcription factors, mediator complex, and chromatin regulators (15). In many cancers, the key oncogenic drivers, including oncogenic transcriptional

regulators, are frequently associated with SEs (15, 16). Thus, SE profiling might serve as a useful approach to identify pivotal cancer genes.

Prior studies have characterized the ATC mutational landscape via genome-wide sequencing analyses (17-19). Several transcriptional regulators, such as TP53, subunits of SWI/SNF chromatin remodeling complex and histone methyltransferases, are among the most frequently mutated genes (19). Mutations of these transcriptional regulators may produce a profound change in the transcriptional programs, thereby driving the cancer state of ATC. Therefore, we hypothesized that targeting the misregulated transcriptional programs may represent a novel therapeutic strategy for ATC.

In this study, we identifed THZ1, a covalent CDK7 inhibitor, as a highly potent anti-ATC compound. Moreover, we characterized the SE landscape in ATC cells and found that THZ1 inhibits ATC growth by suppressing the SE-linked oncogenic transcriptional addiction in ATC.

Materials and Methods

Cell culture

8505C and CAL-62 cell lines were kindly provided by Professor Haixia Guan (The First Hospital of China Medical University, China). K1, BCPAP and 8305C cell lines were purchased from Guangzhou Cellcook Biotech Co. (Guangzhou, China). C643, Hth-7, KMH-2 and KTC-1 cell lines were purchased from the Chinese Academy of Science (Shanghai, China). K1, BCPAP, KTC-1, C643, 8305C, and 8505C cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. KMH-2 and Hth-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) medium supplemented with 10% fetal bovine serum (FBS). CAL-62 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% fetal bovine serum (FBS). Cells were maintained at 37°C in a humidified atmosphere with 5% CO2. All cell lines were authenticated by short tandem repeat (STR) analysis performed by Guangzhou Cellcook Biotech Co., Shanghai Biowing Biotechnology Co. or GENEWIZ Lnc. The STR profile of 8505C cells yields an 88.88% match to the reference cell line in the Cellosaurus

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database. The STR profiles of other cell lines are 100% matched to the published data. The STR profiling reports have been included in the Supplementary data.

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Cell viability assay

Cells were seeded in 96-well plates at a density of 2000 cells/well. After 12 hours, cells were treated with drugs. After 48 hours of incubation, cells were analyzed for cell viability using the CellTiter 96®AQueous One Solution Cell Proliferation Assay and CellTiter-Glo® Luminescent Cell Viability Assay (Promega). Dose–response curves were generated using GraphPad Prism software. All assays were performed in biological triplicates.

The drug library was purchased from TargetMol. Guanabenz acetate was purchased from Selleck Chemicals. Doxorubicin hydrochloride and paclitaxel were purchased from MedChemExpress.

Drug combination

Cells were seeded in 96-well plates with a density of 2000 cells/well. After 12 hours, cells were treated with the indicated doses of drugs for 48 hours, and cell viability was measured. Calcusyn software is used for determining synergism and antagonism. A combination index (CI) plot is a Fa-CI plot in which CI < 1, = 1, > 1 indicate synergism, additive effect, and antagonism, respectively. Fa indicates the fraction that is inhibited by the drug.

Cell apoptosis assay

Cells were seeded in 6-well plate at 30% confluency. After 12 hours, cells were treated with THZ1. Cell death was assessed using a FITC Annexin V/PI Apoptosis Detection kit (BD Biosciences) according to the manufacturer's protocol. Data were generated using FlowJo software.

Cell cycle analysis

Cells were seeded in 6-well plate at 40% confluency**.** After 12 hours, cells were treated with THZ1. Cells were first fixed with 80% ethanol at -20**°**C for 15 minutes and were then resuspended in PBS supplemented with 25 mg/ml PI (Sigma-Aldrich) and 0.1 mg/ml RNase

A (TransGen Biotech). After incubation at 37°C in the dark for 30 min, cell cycle distribution was examined on FACS Calibur (Becton Dickinson).

Colony formation assay

Cells were seeded in 6-well plates at 500 cells/well. After five days, cells were treated with THZ1 for another five days, with media being replaced by fresh growth media every four days until colonies were visible. Cells were stained with crystal violet solution and colonies with more than 50 cells were manually counted. For the long term clonogenic assays, BCPAP and CAL-62 cells were treated with vehicle or THZ1 (50 nM) for 12 days. Cells were then given fresh medium, and allowed to grow for 10 days. Colonies of cells were stained with crystal violet.

Plasmids

LentiCas9-Blast (#52962) and LentiGuide-Puro (#52963) constructs were purchased from Addgene. sgRNAs were designed using sgRNA Designer (https://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design). The sgRNA sequences were chosen that match the early coding exons of targeted genes and listed in Supplementary Table S5.

Immunoblotting analysis

Cells were lysed in RIPA buffer supplemented with proteinase inhibitor cocktail (Roche) and a phosphatase inhibitor cocktail (Roche). Equal amounts of total protein were separated by SDS-PAGE, transferred to nitrocellulose membranes, and immunoblotted with the indicated primary antibodies. The sources of antibodies were: RNAPII CTD Ser2P (Millipore cat# 04-1571), RNAPII CTD Ser5P (Millipore cat# 04-1572), RNAPII CTD Ser7P (Millipore cat# 04-1570), RNAPII (Abcam cat# ab-817); CDK7 (Cell Signaling Technology cat# 2916), PARP (Cell Signaling Technology cat# 9542), and α-tubulin (Proteintech cat# 11224-1-AP).

Animal studies

Suspensions of 2×10^6 CAL-62 or 8505C cells were injected subcutaneously into the flank of 6-week female BALB/c nude mice (Charles River). Tumors were measured with a caliper.

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Tumor volume was calculated using the formula: V = 0.5 × length × width × depth. When the tumor volumes reached approximately 20 mm^3, animals were randomly divided into two groups. The animals were then treated with THZ1 (10 mg/kg) or vehicle intraperitoneally twice daily. Tumors were measured every three days. Upon harvest, tumors were fixed in formalin overnight for immunohistochemistry analysis. All the animal studies were approved by the institutional ethical committee of Tianjin Medical University (permit number: SYXK 2009-0001).

Immunohistochemistry (IHC)

The human ATC tissue microarrays were perfused with 10% formalin overnight and paraffin-embedded. Tissue specimens were blocked with 3% H_2O_2 for 15 minutes followed by 5% BSA for one hour. Tissue sections were incubated with primary antibodies against CDK7 (Cell Signaling cat# 2916), PPP1R15A (Proteintech cat# 10449-1-AP), Ki67 (Cell Signaling Technology cat# 9027), and CC3 (Cell Signaling Technology cat# 9664). Sections were incubated with anti-mouse/rabbit-HRP at room temperature for one hour followed by staining with DAB substrate. Samples were counterstained with hematoxylin for three minutes. Immunostaining was evaluated using the H-score method. The immunostaining intensity was graded as low (score 1), moderate (score 2) or strong (score 3). The H-score was calculated by multiplying the percentage of positive cells and immunostaining intensity. Thus, the range of possible scores was from 0 to 300. Scoring below 100 was defined as low expression and scoring 100 to 300 was defined as high expression. The protocols and informed consent were approved by Tianjin Medical University Cancer Institute and Hospital Ethics Committee.

RNA isolation and q-PCR

Total RNA isolation was performed using TRIzol (Invitrogen). Following isolation, total RNA was reverse transcribed using the cDNA Synthesis Kit (Roche). Gene-specific primer pairs were listed in Supplementary Table S6.

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RNA sequencing analysis

Total RNAs from cultured cells were subjected to Oligo dT selection and adaptor ligation. Sequencing was performed on BGISEQ-500 platform. Low quality reads were filtered using internal software SOAPnuke. Clean reads were assembled into unique genes. Clean reads were mapped to reference sequences using Bowtie2. DEGs (differential expressed genes) between samples were generated by DESeq2 algorithms. Sequencing data have been deposited in the Gene Expression Omnibus (GSE120177).

Chromatin immunoprecipitation sequencing (ChIP-seq) and data analysis

BCPAP and CAL-62 cells were cross-linked with 1% formaldehyde for 10 min at RT. The crosslink was stopped by adding 1/20 volume of 2.5 M glycine. Cells were washed with PBS and harvested using ChIP lysis buffer. Cells were then sonicated to obtain fragments (100-500 bp) with a Bioruptor Sonicator. Immunoprecipitation was performed with an H3K27ac antibody (Abcam cat# ab4729). After elution and reversal cross-linking, DNA was purified and sequenced on BGISEQ-500.

ChIP-seq data were mapped to the GRCh37/hg19 human reference genome by SOAPaligner/SOAP2 (Short Oligonucleotide Analysis Package). No more than two mismatches were allowed in the alignment. Reads mapped only once at a given locus were allowed for peak calling. ChIP-Seq peaks were generated using peak finding algorithm MACS (Model-based Analysis for ChIP-Seq, version MACS-1.4.2) software. Big-wig files were generated by MACS-1.4.2. ChIP-seq tracks were visualized in IGVtools (version 2.4.5). The total signal of H3K27ac ChIP-seq is expressed in units of RPM per bin. Peaks within a 12.5 kb interval were merged and stitched using Homer. Stitched enhancers were assigned to the most proximal genes and classified as SEs or TEs by ranking the H3K27ac signal. Sequencing data have been deposited in the Gene Expression Omnibus (GSE120177).

Gene Ontology (GO) analysis and gene set enrichment analysis (GSEA)

Gene Ontology (GO) analysis was performed using the DAVID web-tool (https://david.ncifcrf.gov). Significantly enriched molecular function terms were defined as

P <0.01. GSEA was performed using the GSEA standalone desktop program. Significant enrichment was defined by a false discovery rate (FDR) value < 0.25.

Statistical analysis

Statistical analyses were performed using SPSS Statistics 22.0 (IBM) or GraphPad Prism 6.02. The results were repeated in at least three independent experiments and are shown as mean ± SD (standard deviation). Unpaired two-tails Student's *t* test was used to calculate *p*-values between different treatment cohorts. Two-way ANOVA was used to compare multiple groups. Mann–Whitney *U* test was used to compare significantly downregulated transcripts in CAL-62 and BCPAP cells.

Results

High-throughput chemical screening identifies THZ1 as a potent inhibitor of ATC

To investigate the roles of transcriptional dysregulation in ATC pathogenesis and discover novel therapeutics, we performed a high-throughput screening in an ATC cell line (CAL-62). We used a library of 177 compounds with a main focus on targeting transcriptional regulators, including transcriptional CDKs, transcription factors, transcriptional cofactors, and chromatin regulators (Fig. 1A). The sensitivity of CAL-62 cells to each compound was measured by cell viability assay. A total of 27 compounds decreased cell viability by 50% or more at 1 μM comparing to vehicle control (Fig. 1B and Supplementary Table S1). Among the hit compounds, two HDAC inhibitors (Panobinostat and CUDC-907) have previously been shown to possess cytotoxic properties against ATC (20, 21), thereby validating our screening approach. To further confirm the anti-ATC properties of these compounds, we expanded the cell viability measurement in four different ATC cell lines (CAL-62, 8505C, 8305C, and C643). All four ATC cell lines showed high sensitivity to THZ1, a newly developed covalent inhibitor of CDK7 (Fig. 1C and Supplementary Table S2). We further observed that THZ1 suppressed ATC cell growth in a dose-dependent manner (Fig. 1D). Notably, although DMSO-treated ATC cells proliferated rapidly during 3 days of culture, the ATC cells ceased growing immediately upon treatment with low nanomolar concentrations of THZ1 treatment (Fig. 1D), thereby identifying THZ1 as a novel potent anti-ATC agent.

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THZ1 inhibits ATC cell growth both *in vitro* and *in vivo*

It has been reported that some aggressive cancers are highly dependent on the transcriptional function of CDK7, and therefore are exceptionally sensitive to THZ1 treatment. To test whether THZ1 exhibits selective potency in ATC, we analyzed the effect of THZ1 treatment in eight different thyroid cancer cell lines: five ATC cell lines (C643, Hth-7, KMH-2, CAL-62 and 8505C) and three papillary thyroid cancer (PTC) cell lines (KTC-1, BCPAP and K1). As shown in Fig. 2A, ATC cells were much more sensitive to low-dose THZ1, with IC_{50} values averaging five times lower than those of PTC cells. Cell cycle analysis showed that low-dose THZ1 treatment induced G2/M phase arrest in representative ATC cells, but not in PTC cells (Fig. 2B). Consistent with this, colony formation experiments demonstrated that THZ1 selectively blocked the colony formation of ATC cells (Fig. 2C). To evaluate the long term effect of THZ1, we performed clonogenic grow-out experiments. As shown in Supplementary Fig. S1A, THZ1 also selectively inhibited the colony growth of representative ATC cells comparing to PTC cells. We also observed that THZ1 induced massive apoptosis of ATC cells, as analyzed by Annexin V staining and PARP cleavage (Fig. 2D and E). The proliferation rate did not vary significantly between ATC and PTC cells, suggesting drug sensitivity does not correlate with cell proliferation rate (Supplementary Fig. S1B). Taken together, these results indicate that THZ1 induces selective cytotoxicity in ATC cells.

We next sought to examine the *in vivo* anti-cancer efficacy of THZ1. To do this, we used nude mice xenograft model implanted with CAL-62 or 8505C cells. The animals were treated with vehicle or THZ1 intravenously twice daily (10 mg/kg). As shown in Fig. 2F-K, THZ1 markedly reduced the tumor growth in mice. No systemic toxicity was observed, such as body weight loss or behavioral changes (data not shown). Xenografts isolated from THZ1-treated mice had significantly fewer proliferating cells and more apoptotic cells than those derived from vehicle-treated mice, as measured by Ki67 and cleaved caspase-3 (CC3) staining, respectively (Supplementary Fig. S1C-H). Altogether, these results demonstrate that THZ1 has potent anti-ATC activities both *in vitro* and *in vivo*.

CDK7 is essential for ATC cell growth and its elevation is associated with poor prognosis in patients with ATC

To corroborate the pharmacological studies, we investigated the impact of CDK7 depletion by CRISPR/Cas9-mediated gene editing on the biological behaviors of ATC cells. The infection with a lentivirus carrying CDK7 sgRNA markedly decreased the abundance of CDK7 protein and Serine 5 phosphorylation of Pol II CTD (Fig. 3A). Notably, depletion of CDK7 reduced cell viability, decreased colony formation, caused G2/M arrest, and induced massive cell apoptosis in ATC cells (Fig. 3B-E). These findings suggest that CDK7 is the pharmacological target of THZ1 in ATC cells.

Since ATC cells are highly dependent on CDK7, we next investigated the clinical relevance of CDK7 expression in ATC patients. We first examined the CDK7 and Ki67 protein expression in tissue samples derived from ATC (a total of 29 cases) by IHC staining. As shown in Fig. 3F CDK7 expression correlated with Ki67 staining, supporting that CDK7 may participate in the proliferation of ATC. All ATC tissues were divided into two groups: low CDK7 expression and high CDK7 expression (Fig. 3G). Both groups were subsequently assessed for associations with survival outcomes of ATC patients. A high CDK7 expression correlated significantly with a decreased survival time (Fig. 3H). We further performed CDK7 staining in 188 PTC patient samples. As shown in Fig. 3I, CDK7 expression is significantly higher in ATC samples comparing to PTC samples. The above data indicate that the elevated CDK7 expression could be a candidate biomarker of poor clinical prognosis in patients with ATC.

Preferential repression of transcription regulators by CDK7 inhibition in ATC cells

As a transcriptional kinase, CDK7 activates RNAPII-mediated transcription by phosphorylating the Serine 5 and Serine 7 (initiation-associated) and Serine 2 (elongation associated) of the RNAPII CTD (22, 23). We first examined the CDK7 expression and CTD phosphorylation in PTC and ATC cells. As shown in Supplementary Fig. S2, the expression level of CDK7 was correlated with Serine 5 phosphorylation. Importantly, we observed a higher expression of CDK7 and Serine 5 phosphorylation in ATC cells compared to PTC cells, suggesting that ATC cells possess elevated CDK7 kinase activity. Indeed, THZ1

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decreased the CTD phosphorylation at Serine 5, Serine 7 and Serine 2 in a dose-dependent manner in all four thyroid cancer cell lines (Fig. 4A). However, the phosphorylation of RNAPII CTD in ATC cells was much more sensitive to THZ1 treatment than that in PTC cells. Treatment with 100 nM THZ1 greatly reduced the CTD phosphorylation in ATC cells, but not in PTC cells (Fig. 4A).

Since THZ1 preferentially downregulates RNAPII CTD phosphorylation in ATC cells, we hypothesized that THZ1 may selectively inhibit RNAPII-mediated transcriptional programs in ATC cells. To test this hypothesis, we performed whole-transcriptome sequencing (RNA-Seq) in both ATC and PTC cells. The genome-wide gene expression analyses revealed that low-dose THZ1 had a much stronger impact on the transcriptional program of representative ATC cells than in PTC cells (Fig. 4B-C and Supplementary Table S3). In ATC cells, 1159 genes were found to be sensitive to THZ1 treatment (repressed over twofold), including some well-studied cancer genes, such as *XBP1*(24), *TSPYL5* (25), *SPC24* (26), *FOSL1* (27), *MCL1* (28, 29), *NEAT1* (30); however, THZ1 treatment only caused the suppression of 157 genes in PTC cells. Moreover, the THZ1-sensitive genes in ATC cells were repressed at a higher degree by THZ1 treatment than in PTC cells (Fig. 4D). A small group of genes (230 in ATC cells, 46 in PTC cells, respectively) were found to be upregulated by THZ1 treatment (Fig. 4C), which was due to either negative feedback or treatment-induced stress response.

Further analysis of the gene expression profiles of ATC cells showed that THZ1 repressed gene expression in a gene-selective fashion. Gene ontology (GO) analysis revealed that THZ1-sensitive transcripts were significantly enriched for genes encoding transcription factors and other nuclear proteins (DNA binding and nucleic acid binding) in ATC cells (Fig. 4E). The THZ1-sensitive genes in PTC cells were also enriched for factors with DNA and nucleic acid binding activities, but to a much lesser extent compared to those in ATC cells (Fig. 4E). These data demonstrate that THZ1 treatment preferentially targets transcription-regulating genes and the dysregulated transcriptional programs may confer the observed sensitivity of ATC cells to CDK7 inhibition.

Super-enhancers promote the transcriptional dysregulation in ATC

We then proceeded to investigate the molecular mechanisms underlying the hyper-activation of transcriptional regulators in ATC cells. Recently, it has been demonstrated that cell-identity determining genes and key oncogenic transcription regulators are frequently associated with SEs (16, 31). Thus, we wondered whether SEs promote the hyper-activation of transcriptional regulators in ATC cells. To test this, we first characterized the SE landscape of ATC and PTC cells by chromatin immunoprecipitation sequencing (ChIP-Seq) analysis of H3K27ac modification, a mark of an active enhancer. A set of enhancers, loaded with significantly higher level of H3K27ac than typical enhancers (TEs), were classified as SEs. A total of 606 SE-associated genes were identified in CAL-62 cells (Fig. 5A). Among them, PAX8 is a thyroid-specific transcription factor, which is an excellent marker for carcinomas of follicular epithelial origin, including ATC (32). Several SE-associated genes with established roles in promoting ATC progression were also identified, such as *EGFR* (33, 34) and *SPC24* (26). The GO analysis of SE-associated genes showed that they were significantly enriched for genes involved in cell-cell adhesion and transcription regulation (Fig. 5B). Notably, the list of SE-associated transcription regulators contains many transcription factors with well-known oncogenic roles in other cancers, such as *BMI1* (35), *ETS1* (36), *FOSB* (37), *MTA2* (38) and *SMAD3* (39) (Supplementary table S4).

A total of 732 SE-associated genes were identified in BCPAP cells (Fig. 5A). Unlike in ATC, the SE-associated genes in PTC were not significantly enriched for genes involved in transcriptional regulation. Interestingly, they were significantly enriched for genes involved in cell-cell adhesion, which were also seen in ATC cells, indicating that cell adhesion pathways might play key roles in maintaining the cancer state of both ATC and PTC (Fig. 5B). Many SEs were either uniquely identified in ATC cells or loaded with stronger H3K27ac signals in ATC cells than in PTC cells. Four representative samples of these ATC-specific SEs are shown in Fig. 5C. Taken together, these results suggest that SEs may play critical roles in activating the transcriptional regulators of ATC cells, thereby promoting the transcriptional dysregulation in ATC.

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Identification of SE-associated cancer genes in ATC

To further investigate the biological features of SE-associated genes, we performed gene set enrichment analysis (GSEA). We observed that only SE-associated genes in ATC were significantly enriched for THZ1-sensitive transcripts (Fig. 6A). Furthermore, these SE-associated genes were repressed to a greater extent by THZ1 than genes associated with TEs (Fig. 6B).

Several studies have demonstrated that many cancer genes are commonly associated with SEs (31, 40). The SE-associated oncogenes often exhibit high expression and are highly sensitive to THZ1 treatment (7-10). To identify the critical cancer genes in ATC, we performed an integrative analysis of the transcriptome data and the SE profiling data obtained from ATC cells. We selected the candidate cancer genes by the following criteria: (i) associated with SEs, (ii) highly sensitive to low-dose THZ1, (iii) and expression levels within the top 20% of all active transcripts. As a result, 19 candidate genes were identified (Fig. 6C). Among them, there were several known oncogenes, such as *PAX8* (41, 42), *EGFR* (43), and *NEAT1* (30). Further analysis of RNA-seq data revealed that THZ1 selectively repressed these genes in ATC cells, but not in PTC cells (Fig. 6D). The reduction of four representative genes was confirmed by quantitative PCR analysis (Fig. 6E). Among these candidate cancer genes, *EGFR* has been reported to be important for ATC initiation and development (34, 44).This supports the effectiveness of our approach to predict critical cancer genes.

PPP1R15A functions as a novel druggable target in ATC

To test the biological functions of these candidate genes in ATC cells, we silenced all these 19 genes using CRISPR/Cas9-mediated gene editing and assessed ATC cell viability after gene depletion. We have validated all the mutations by DNA sequencing (Supplementary Fig. S3) and further confirmed the knockdown efficiency by western blotting for EGFR, PAX8, MAT2A, FOXD1, PPP1R15A (Supplementary Fig. S4A). Depletion of 12 out of 19 candidates significantly impaired the colony formation ability of ATC cells (Fig. 7A). Silencing of *PPP1R15A* caused the greatest reduction of colony formation in ATC cells. PPP1R15A binds to protein phosphatase 1 (PP1), and together with PP1 forms the holo-phosphatase of eIF2α. Guanabenz (GBZ), an FDA-approved drug for hypertension,

selectively inhibits PPP1R15A by disrupting the interaction between PPP1R15A and PP1, and thereby prolongs the phosphorylation of eIF2α (45). We then test the anti-ATC effect of GBZ. As shown in Fig. 7B and Supplementary Fig. S4B, GBZ inhibited cell growth in two ATC cell lines. The effect of PPP1R15A inhibition on ATC cell growth was further confirmed by Sephin1 (45), a newly developed PPP1R15A inhibitor (Fig. 7B and Supplementary Fig. S4B).

To investigate the clinical relevance of PPP1R15A in ATC, we analyzed the protein expression of PPP1R15A in ATC tissue samples. We observed that the expression level of PPP1R15A correlated significantly with CDK7 expression (Fig. 7C-D). Similar to CDK7, a high PPP1R15A expression was significantly associated with decreased survival time of patients with ATC (Fig. 7E). Taken together, these results indicate that *PPP1R15A* is a critical cancer gene involved in ATC pathogenesis, and may serve as a candidate drug target for ATC treatment.

CDK7 or PPP1R15A inhibition sensitizes the ATC cells to conventional chemotherapy

Doxorubicin (DOX) is the only cytotoxic agent that is approved by the FDA as single-drug chemotherapy for ATC treatment (46). Unfortunately, the response rate is below 22% and high dose of DOX can cause severe side effects (47). Thus, searching for better therapeutic combinations to improve DOX effects has been an urgent need. Since THZ1, GBZ and Sephin1 effectively inhibited ATC cell growth, we wondered if these chemicals could improve the poor outcomes of conventional chemotherapies for ATC. To test this, we evaluated the efficacy of combination treatment of DOX with these drugs. THZ1 significantly enhanced the anti-ATC effect of DOX (Fig. 7F). Similarly, combining DOX with GBZ or Sephin1 showed synergistic anti-ATC effects (Fig. 7F and Supplementary Fig. S4C and D). Furthermore, we evaluated the efficacy of a combination treatment of paclitaxel (PTX) with THZ1 or GBZ. THZ1 or GBZ significantly enhanced the anti-ATC effects of PTX (Fig. 7G), suggesting the synergistic effects are not limited to genotoxic agents. Altogether, these results indicate that inhibition of CDK7 or PPP1R15A strongly enhances the potency of conventional chemotherapy in ATC cells.

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Discussion

ATC is the most aggressive subtype of thyroid cancer and lacks effective treatments. Recent large-scale genome analysis have established the mutational landscape of ATC, which is characterized by a greater mutational burden and intertumoral heterogeneity compared to other types of thyroid cancer (17-19). The high genetic complexity and scarce actionable driver mutations have greatly limited the development of effective targeted therapies. In the present study, we address this high therapeutic need. We demonstrate that targeting the transcriptional dysregulation, instead of specific genomic mutations, might be an effective alternative approach against ATC. We find that ATC cells are exceptionally sensitive to THZ1. Functional studies validate the high dependency of ATC cells on CDK7. Mechanistic analyses reveal that SE-driven hyper-activation of transcriptional regulators confer the exceptional sensitivity of ATC cells to CDK7 inhibition. The underlying mechanisms may include: (i) the SE-driven hyper-activation of transcriptional regulators promotes continuously active transcription in ATC cells, thereby supporting the high expression of cancer genes; (ii) the adaptation of ATC cells to this highly active transcription makes them more vulnerable to transcriptional perturbation elicited by CDK7 inhibition than other types of thyroid cancer.

Prior studies have demonstrated that some transcription-targeting drugs exhibited anti-ATC activity by inhibiting transcriptional cofactors, such as HDACs and BRD4 (20, 21, 48-51). However, these drugs showed much lower potency than THZ1 in our chemical screen. By inhibition of CDK7, THZ1 directly targets RNAPII transcription machinery. Thus, THZ1 treatment represses the cancer genes addicted to continuously active transcription more effectively and confer higher sensitivity than other agents.

The knowledge about critical cancer genes involved in ATC pathogenesis is limited. In this study, we identified 14 candidate cancer genes by integrative analysis of transcriptome data, SE profiling data and functional assays. One of the candidates, EGFR, had been reported to play critical roles in ATC progression (33, 34). Unfortunately, an oral EGFR kinase inhibitor, Gefitinib, has shown poor efficacy in an ATC clinical trial (52). The reason for this discrepancy may be explained by the existence of kinase-independent roles of EGFR in ATC pathogenesis. Furthermore, our integrative analysis also identified another actionable candidate cancer gene, *PPP1R15A*.

Depletion of PPP1R15A shows the strongest reduction in ATC cell viability among all the candidate cancer genes. Inhibition of PPP1R15A by GBZ causes a profound decrease in ATC cell proliferation. Further investigations of THZ1 and GBZ in clinical studies are needed. At present, despite the poor response rate, DOX-mediated chemotherapy is still the first-class treatment for patients with ATC (1, 47, 53). Our data suggest potential roles for THZ1 and GBZ in modulating the sensitivity of ATC cells to DOX treatment. This may provide new therapeutic strategies for the fraction of ATC patients with poor response to conventional chemotherapies.

Due to the complex and heterogeneous genetic changes, limited success has been achieved regarding biomarker identification in ATC. In this study, we demonstrate that the high expression of CDK7 and PPP1R15A correlate with poor prognosis of patients with ATC. Thus, our work not only identifies CDK7 and PPP1R15A as promising therapeutic targets, but also highlights their value as potential prognostic biomarkers in ATC.

In summary, the current study demonstrates that targeting transcriptional addiction by CDK7 inhibition is a promising therapeutic strategy for ATC. Moreover, by characterizing the transcriptional profile and SE landscape of ATC, our work provides significant insights into the molecular pathogenesis of ATC.

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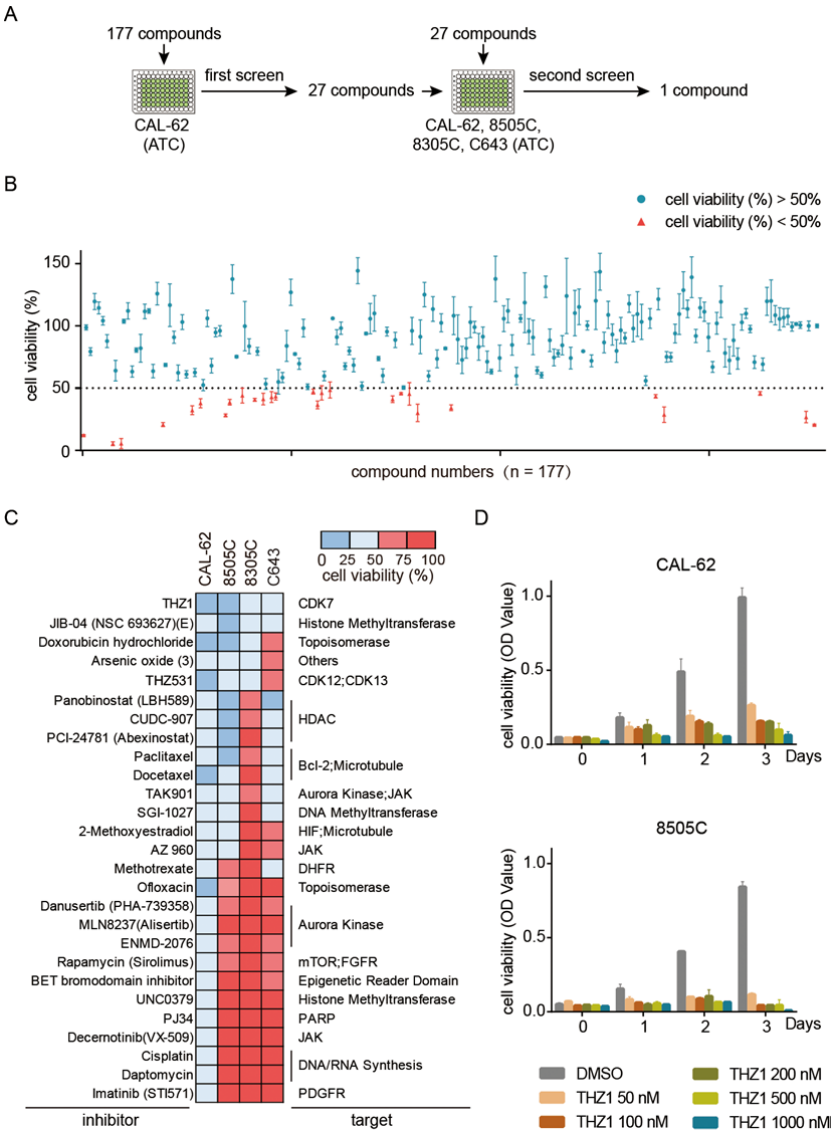

Figure 1. High-throughput small-molecule screen identified THZ1 as a potent inhibitor of ATC cells. A, Schematic of high-throughput small-molecule screen. **B,** Relative cell viability of CAL-62 cells in a high-throughput screen with 177 compounds. A total of 27 compounds reduced cell viability by more than 50%. **C,** The heatmap displays the sensitivity of four ATC cell lines to the 27 compounds selected from (**B**). **D,** Cell viability analysis of THZ1 treatment in two ATC cell lines at the indicated concentrations for the indicated times. Data represent mean ± SD of three replicates.

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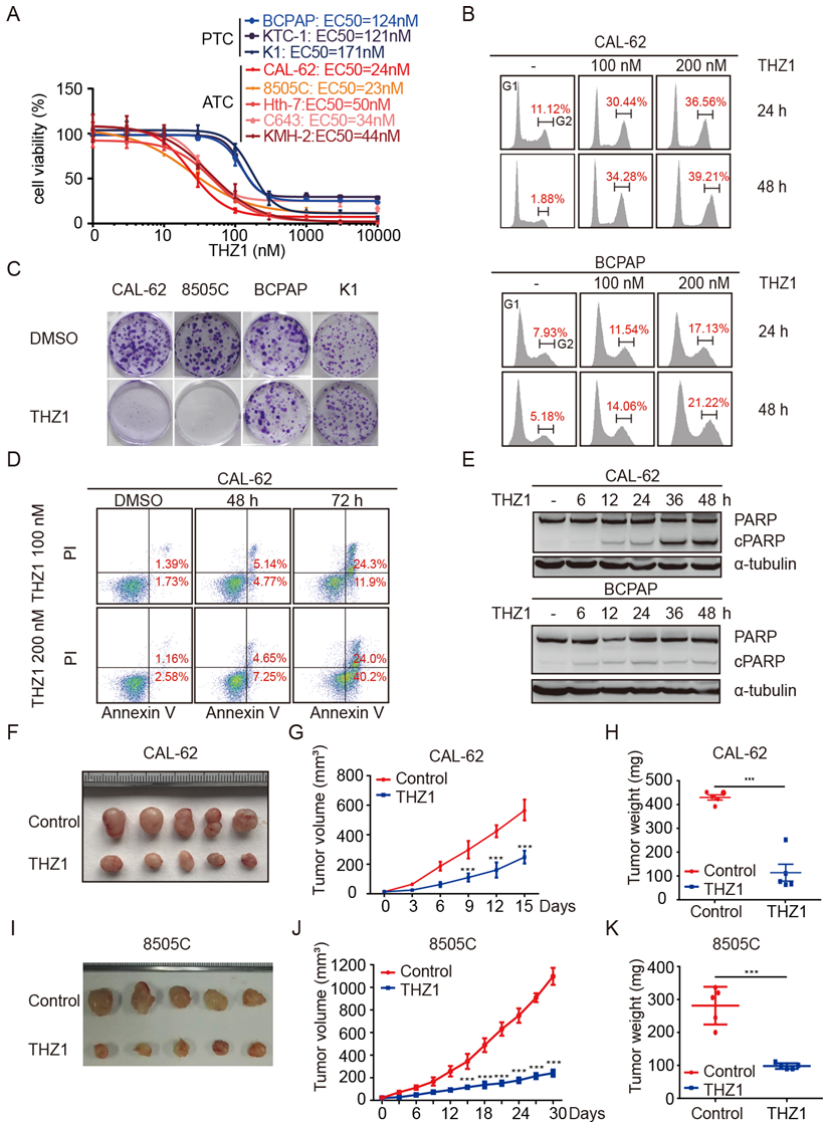

Figure 2. THZ1 exhibits high potency against ATC both *in vitro* and *in vivo*. A, Dose-response curves of seven thyroid cancer cell lines to THZ1 treatment. All cells were treated with increasing doses of THZ1 for 48 hours, and IC50 values were determined by cell viability assay. Data represent mean ± SD of three replicates. **B,** Cell-cycle analysis of cells exposed to THZ1 treatment at the indicated concentrations and durations. The cell phase distribution was measured by flow cytometry with propidium iodide staining. **C,** Colony formation assays of cells treated with vehicle or THZ1 (50 nM) for 5 days. **D,** Cell apoptosis analysis of CAL-62 cells treated with either THZ1 or DMSO. Apoptosis was determined by Annexin V–FITC/ propidium iodide staining. **E,** Immunoblotting analysis quantifying the expression of PARP and cleaved PARP upon THZ1 treatment (200 nM). α-tubulin was used

as a loading control. **F-H,** THZ1 suppressed the growth of ATC xenografts in nude mice. **F-H**, Representative tumor photographs, tumor growth curves, and end-point tumor weights in mice bearing CAL-62 xenografts treated with vehicle (n = 5) or THZ1 (n = 5) (10 mg/kg intravenously [i.v.] twice daily) for 15 days. **I-K**, Representative tumor photographs, tumor growth curves, and end-point tumor weights in mice bearing 8505C xenografts treated with vehicle (n = 5) or THZ1 (n = 5) (10 mg/kg intravenously [i.v.] twice daily) for 30 days. Scale bars represent 100 μm. Mean ± SD values are presented. ***, $p < 0.001$.

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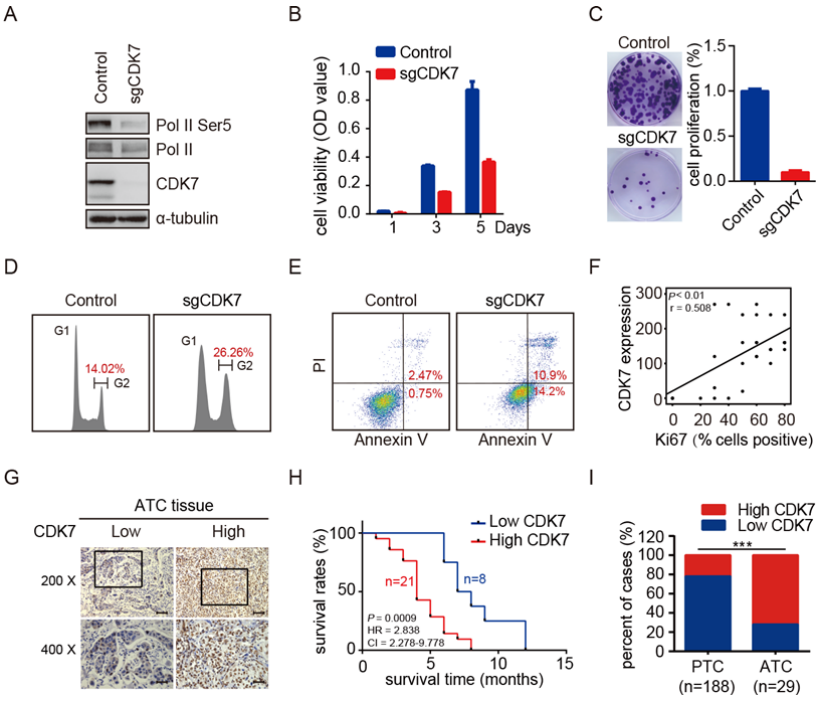

Figure 3. Depletion of CDK7 inhibits ATC cell growth and elevation of CDK7 expression is correlated with poor prognosis. A, Immunoblotting analysis of lysates from CAL-62 cells infected with lentivirus carrying sgRNA against CDK7 or control sgRNA. **B-E,** Cell viability analysis (**B**), colony formation analysis (**C**), cell-cycle distribution (**D**), and apoptosis analysis (**E**) of CAL-62 cells infected with lentivirus carrying sgRNA against CDK7 or control sgRNA. Data represent mean ± SD of three replicates. **F,** Spearman correlation of CDK7 and Ki67 protein expression in ATC samples. **G,** Representative immunohistochemistry analysis of CDK7 protein expression in ATC patient samples. Scale bars represent 100 μm and 50 μm, respectively. **H,** Kaplan-Meier survival curves of patients with ATC categorized by CDK7 expression status. **I,** The expression of CDK7 in 188 PTC and 29 ATC patients. ***, P < 0.001.

Thyroid
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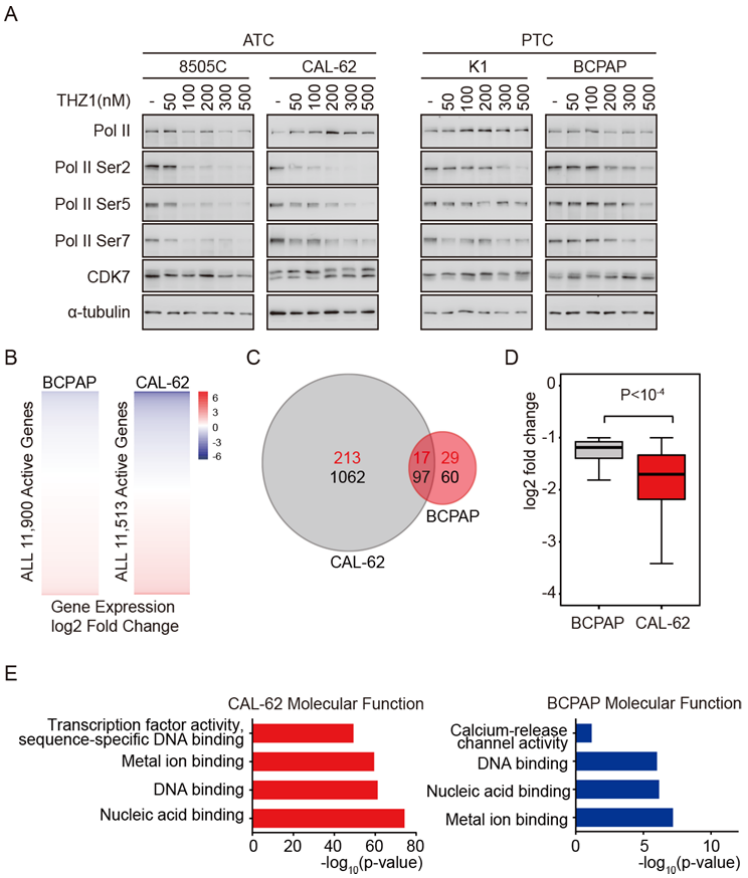

Figure 4. THZ1 preferentially downregulates transcription-regulating genes in ATC cells. **A,** Immunoblotting analysis of RNAPII C-terminal domain (CTD) phosphorylation in CAL-62 and BCPAP cells treated with vehicle or indicated concentrations of THZ1 for 6 hours. **B,** Heatmap of gene expression changes (log_2 fold change) in BCPAP and CAL-62 cells treated with THZ1 (100 nM for 6 hours) versus vehicle control. **C,** Venn diagram illustrating the number of differentially expressed transcripts (THZ1 versus DMSO, log_2 fold change ≥1.0) in CAL-62 and BCPAP cells. The upregulated transcripts (red) and downregulated transcripts (black) are indicated. **D,** Box plots of log_2 fold changes for significantly downregulated transcripts in CAL-62 and BCPAP cells after THZ1 treatment for 6 hours. Whiskers extend to 1.5 times the interquartile range (Mann-Whitney *U* test: $p < 10^{-4}$). **E,** Enriched gene ontology (GO) for the top "Molecular Function" categories of the THZ1-down-regulated genes in CAL-62 and BCPAP cells.

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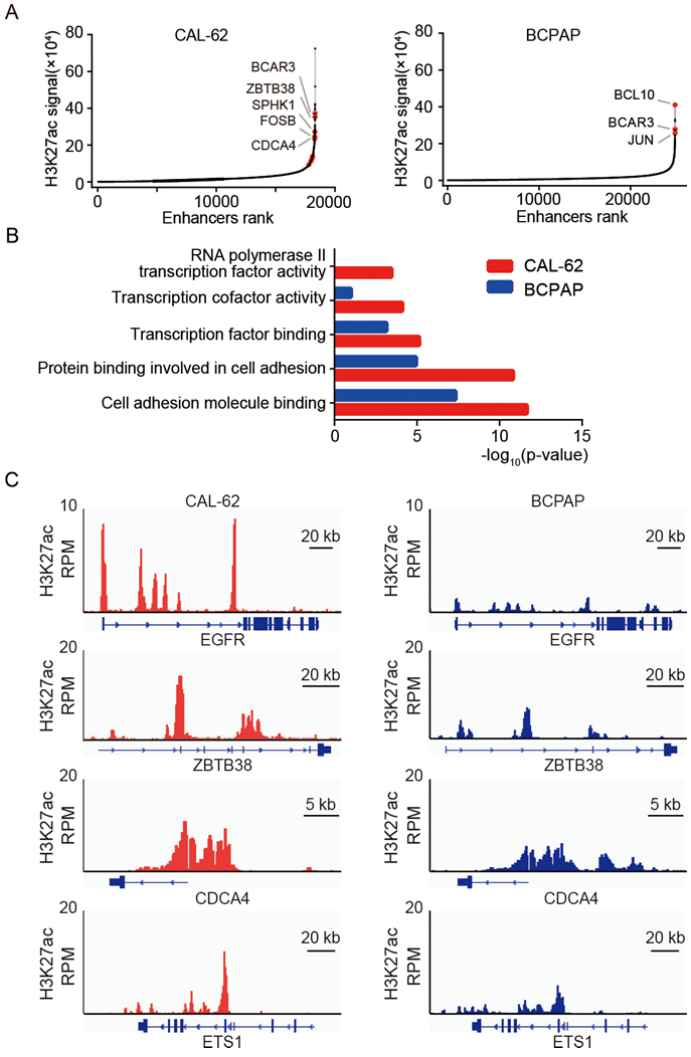

Figure 5. Characterization of super enhancer landscapes in CAL-62 and BCPAP cells. A, Distribution of normalized and rank-ordered H3K27Ac signals (length×density) at enhancers. **B,** Enriched gene ontology (GO) for the top "Molecular Function" categories of SE-associated genes in CAL-62 and BCPAP cells. **C,** ChIP-seq tracks of H3K27Ac at representative SE-associated gene loci in CAL-62 and BCPAP cells.

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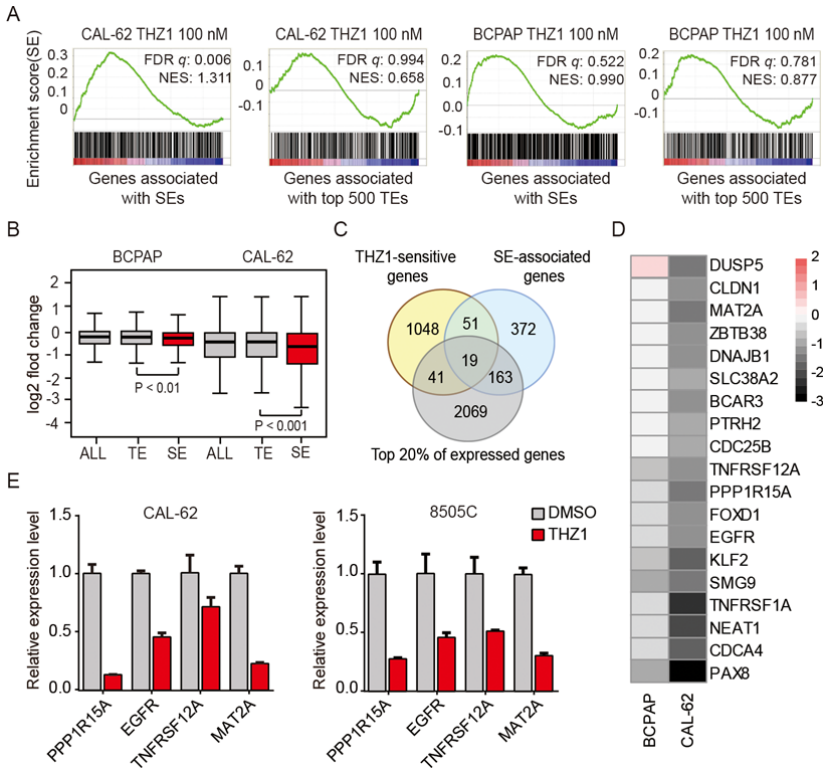

Figure 6. Discovery of super-enhancer (SE)-associated cancer genes in ATC. A, Gene set enrichment analysis (GSEA) depicting the enrichment between genes regulated by THZ1 and SE-associated-genes. **B,** Box plots illustrating $\log_2$ fold change for transcripts (Fragments Per Kilobase Million≥1) correlated with different types of enhancer in BCPAP and CAL-62 cells upon THZ1 treatment for 6 hours. Whiskers extend to 1.5 times the interquartile range. *p*-value was calculated using a two-sided Mann-Whitney U test. **C** and **D,** Identification of the candidate novel oncogenes. Venn diagram showing overlap of THZ1-sensitive genes, SE-associated genes and top expressed genes (**C**). Heatmap demonstrating $\log_2$ fold change of candidates upon THZ1 treatment (**D**). **E,** q-PCR analysis of representative candidate gene expression upon THZ1 treatment. Data represent mean ± SD of three replicates.

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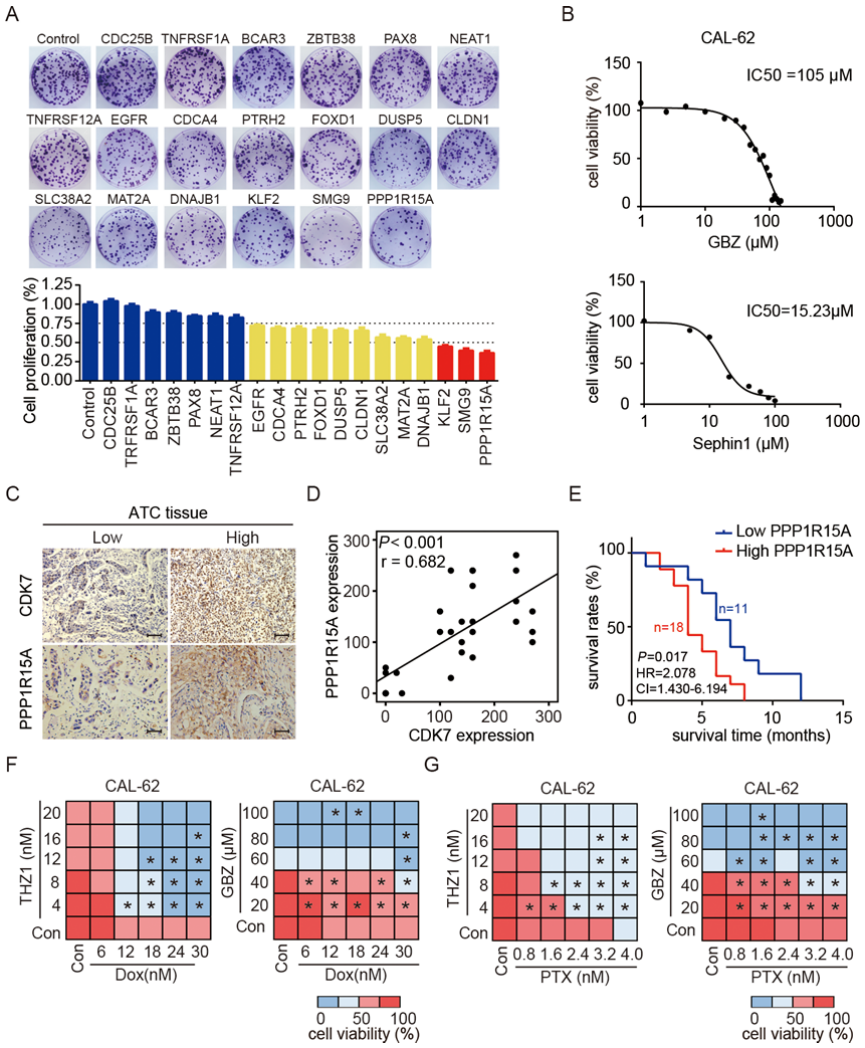

Figure 7. Identification of PPP1R15A as a novel druggable target and rational drug combination treatments for ATC. A, Colony formation assays of cells depleted with indicated genes in CAL-62 cells. Data represent mean ± SD of three replicates. **B,** Cell viability assay following treatment for 48 hours with indicated concentrations of GBZ in CAL-62 cells. **C,** Representative immunohistochemistry analysis of CDK7 and PPP1R15A protein expression in ATC patient tissue samples. Scale bars represent 100 μm. **D,** Spearman correlation curve between PPP1R15A and CDK7 protein expression (H score) in ATC samples. **E,** Kaplan-Meier survival curves of patients with ATC categorized by PPP1R15A expression status. **F,** Combination analysis of THZ1/GBZ and doxorubicin in CAL-62 cells. G, Combination analysis of THZ1/GBZ and PTX in CAL-62 cells. *, combination index (CI) <0.8.

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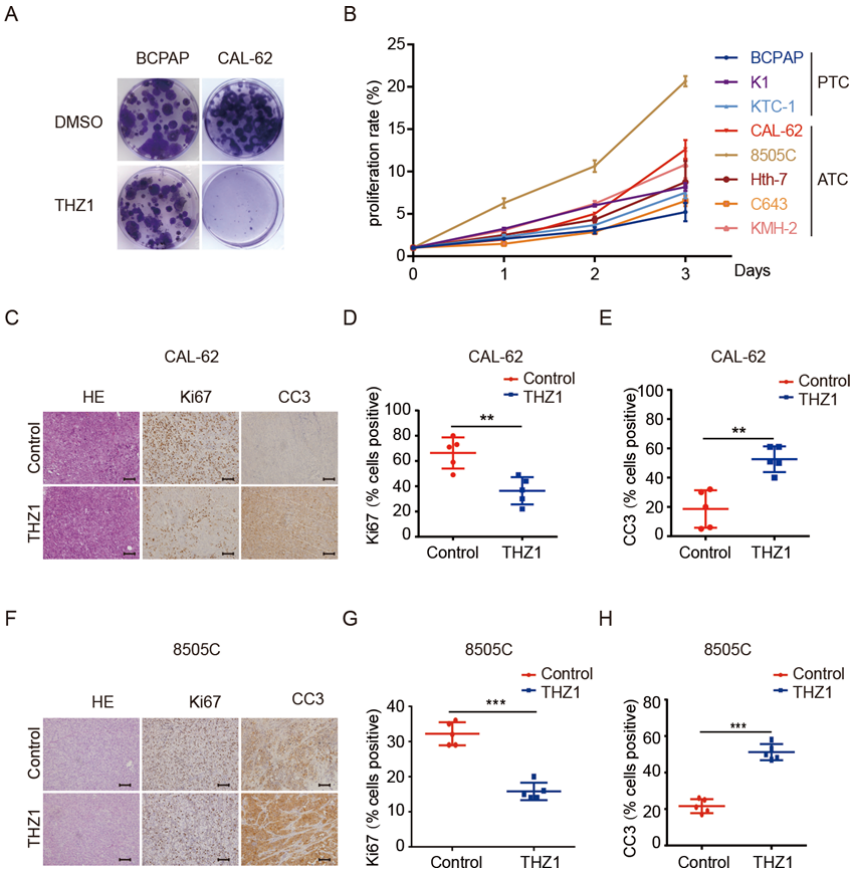
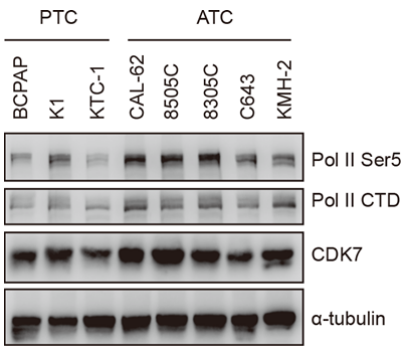


Supplementary Figure S1.

A, Colony formation assays of BCPAP and CAL-62 cells. **B,** MTS assays analyzing the proliferation rates of indicated PTC and ATC cells. **C,** Representative images of hematoxylin and eosin [H&E], Ki67 and cleaved caspase 3 [CC3] staining of tumors in mice bearing CAL-62 xenografts treated with vehicle or THZ1. **D-E,** Quantification of Ki67-positive cells (**D**) and CC3-positive cells (**E**) in tumor sections from mice bearing CAL-62 xenografts treated with vehicle or THZ1. **F,** Representative images of hematoxylin and eosin [H&E], Ki67 and cleaved caspase 3 [CC3] staining of tumors in mice bearing 8505C xenografts treated with vehicle or THZ1. **G-H,** Quantification of Ki67-positive cells (**G**) and CC3-positive cells (**H**) in tumor sections from mice bearing CAL-62 xenografts treated with vehicle or THZ1. Scale bars represent 100 μm (**C** and **F**). Mean ± SD values are presented. **, $p < 0.01$, ***, $p < 0.001$.



Supplementary Figure S2.

Immunoblotting analysis showing the expression of CDK7 and the level of RNAPII C-terminal domain (CTD) phosphorylation in PTC and ATC cells.

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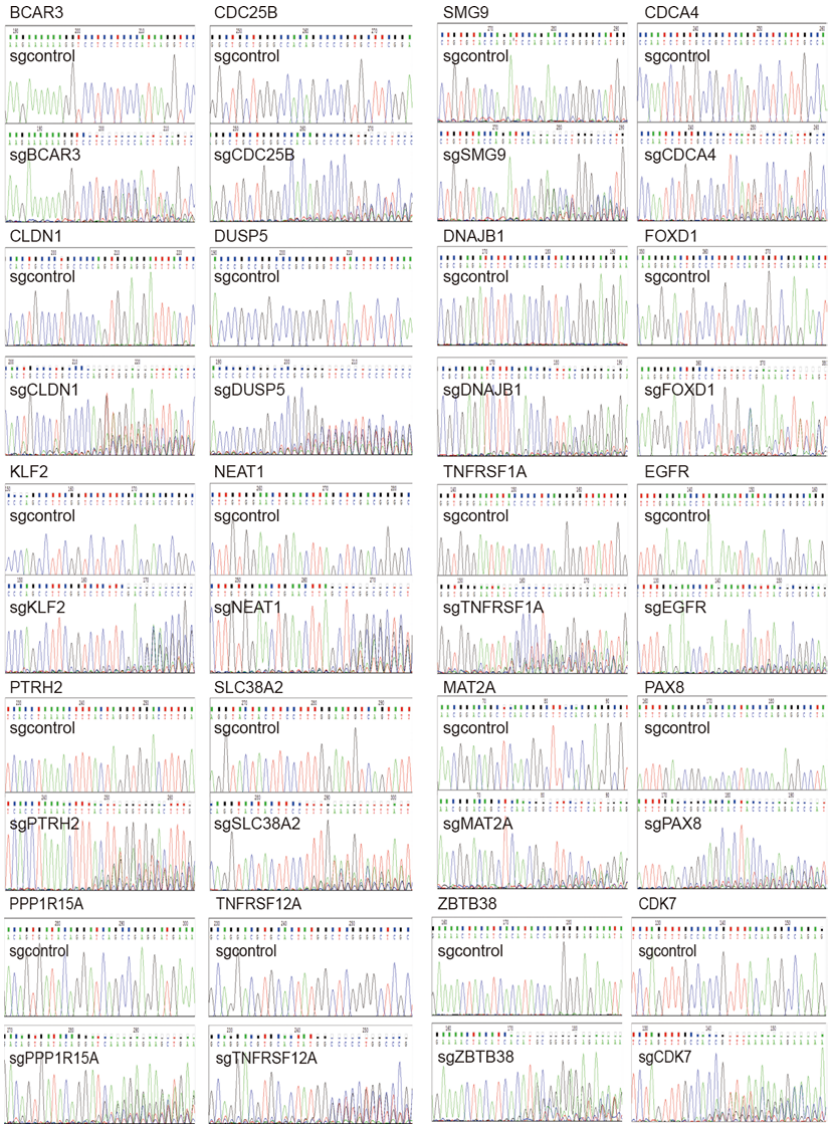

Supplementary Figure S3.

Sequence verification of CRISPR targeting regions in the indicated genes.

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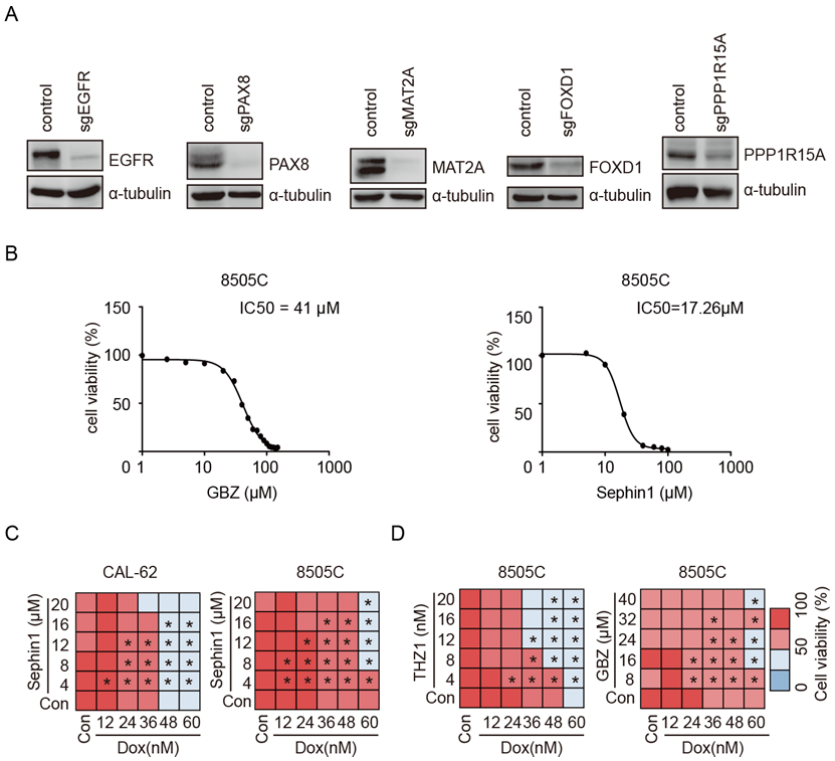

Supplementary Figure S4.

A, Verification of sgRNA-mediated knockdown of indicated genes by immunoblotting analysis. **B,** Cell viability analysis of 8505C cells treated with GBZ/Sephin1 for 48 hours. **C,** Combination analysis of Sephin1 and DOX in CAL-62 and 8505C cells. **D,** Combination analysis of THZ1/GBZ and DOX in 8505C cells. *, CI < 0.8.

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Cell Line Authentication Service

STR Profiling Report

Sample From: TianJin Medical University

Sample Type: Cell Line
Testing Method: STR Genotyping
Report Time: 12/14/2018

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E-mail:market@biowing.com.cn

Thyroid
Targeting super-enhancer-driven oncogenic transcription by CDK7 inhibition in anaplastic thyroid cancer (DOI: 10.1089/thy.2018.0550)

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Cell Line Authentication – STR Profiling Report

Sample code

Table 1. Sample Code

Customer's code	Company Code
KTC-1	20181010-02

Sample Number: 1

Sample Type: Cell line

Testing Type: STR

Testing Method:

DNA was extracted by a commercial kit from CORNING (AP-EMN-BL-GDNA-250G). The twenty STRs including Amelogenin locus were amplified by six multiplex PCR and separated on ABI 3730XL Genetic Analyzer. The signals were then analyzed by the software GeneMapper.

Data Interpretation:

Cell lines were authenticated using Short Tandem Repeat (STR) analysis asdescribed in 2012 in ANSI Standard (ASN-0002) by the ATCC Standards Development Organization (SDO) and in Capes-Davis et al., Match criteria for human cell line authentication: Where do we draw the line? Int J Cancer.2013;132(11):2510-9.

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Test Results:

1. STR profile

Table 2. STR and Amelogenin Genotyping Results of Cell line 20181010-02.

Loci	Sample information			Cell Bank information		
	Sample name : KTC-1			Cell line name : KTC-1		
	Allele1	Allele2	Allele3	Allele1	Allele2	Allele3
D5S818	11	12		11	12	
D13S317	11	11		11	11	
D7S820	11	11		11	11	
D16S539	12	12		12	12	
VWA	14	17		14	17	
TH01	9	9		9	9	
AMEL	X	Y		X	Y	
TPOX	11	11		11	11	
CSF1PO	10	12		10	12	
D12S391	18	23				
FGA	23	26				
D2S1338	22	23				
D21S11	29	29				
D18S51	12	13				
D8S1179	11	14				
D3S1358	14	15				
D6S1043	14	18				
PENTAE	13	20				
D19S433	13	14				
PENTAD	13	14				
D1S1656	14	14				

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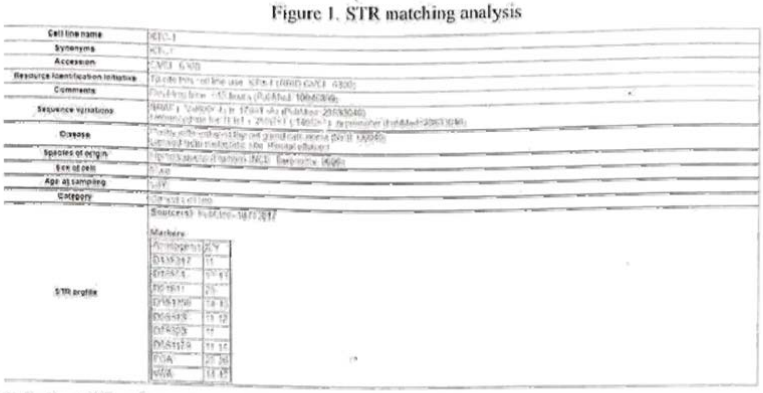
2. database annotation

Figure 1. STR matching analysis



Note: The STR online match analysis of the test cell against DSMZ database, showing cell number (Cell No.) and cell name.

3. Authentication

1. The STR results showed that the sample do not have any multiple alleles were found in this cell line, and no cross contamination of human cells was found in the cell line.
2. The search result in EXPASY and DAMZ databases.
3. The submitted profile is an exact match for the following human cell line(s) in the DSMZ STR database (8 core loci plus Amelogenin): KTC-1.

- **Note:**

1. EV=N*2/M

N:number of the matching peaks ; M: number of all peaks

2. A cell line can be considered to be authenticated when 80% (exact match) of the alleles in its STR profile match profiles from tissue or other cell line samples from that donor or from database. Cell lines with between a 55% to 80% (similar) match require further profiling for investigation of relatedness.

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Appendix:

1. Genotyping Strategy and Site Distribution

Table S1. Experimental Strategy and Sites

	Strategy 1	Strategy 2	Strategy 3	Strategy 4
1	D3S1358	D8S1179	D19S433	AMEL
2	VWA	D21S11	TH01	D1S1656
3	D7S820	D16S539	D13S317	D5S818
4	CSF1PO	D2S1338	TPOX	D12S391
5	PENTAE	PENTAD	D18S51	FGA
6			D6S1043	

The allele match algorithm compares the 8 core loci plus amelogenin only, even though alleles from all lociwill be reported when available.

2. DSMZ tools was used to carry on the cell line comparison, which contains 2455 cell lines STR data from ATCC, DSMZ, JCRB ,ECACC, GNE and RIKEN databases. If the cell is not included in the above cell library, users need to compared with other databases.

Technician: Jianan Zhang
Checked by: Ning Qian
Issued by: Yang Bai
Issue date: 12/14/2018

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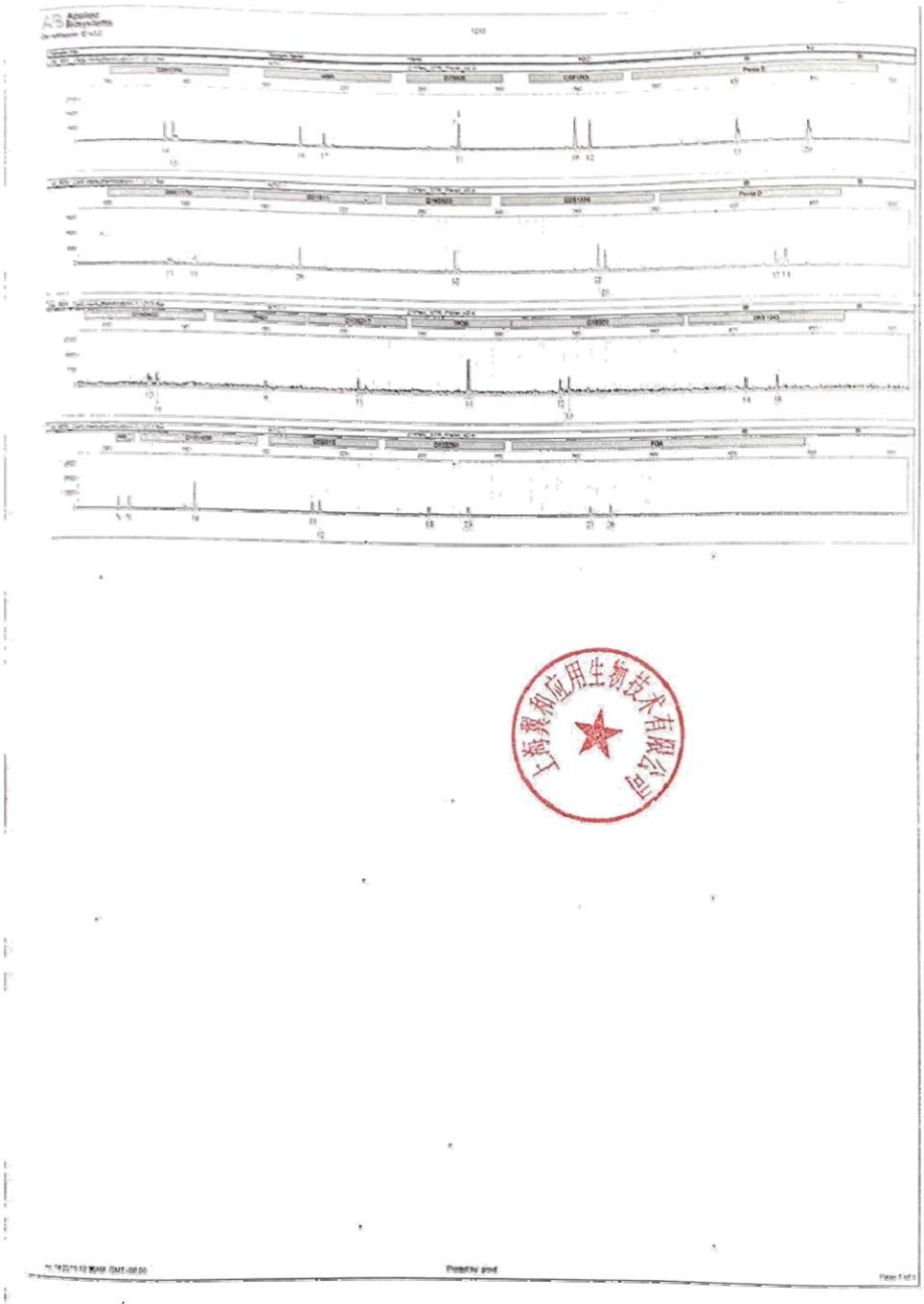

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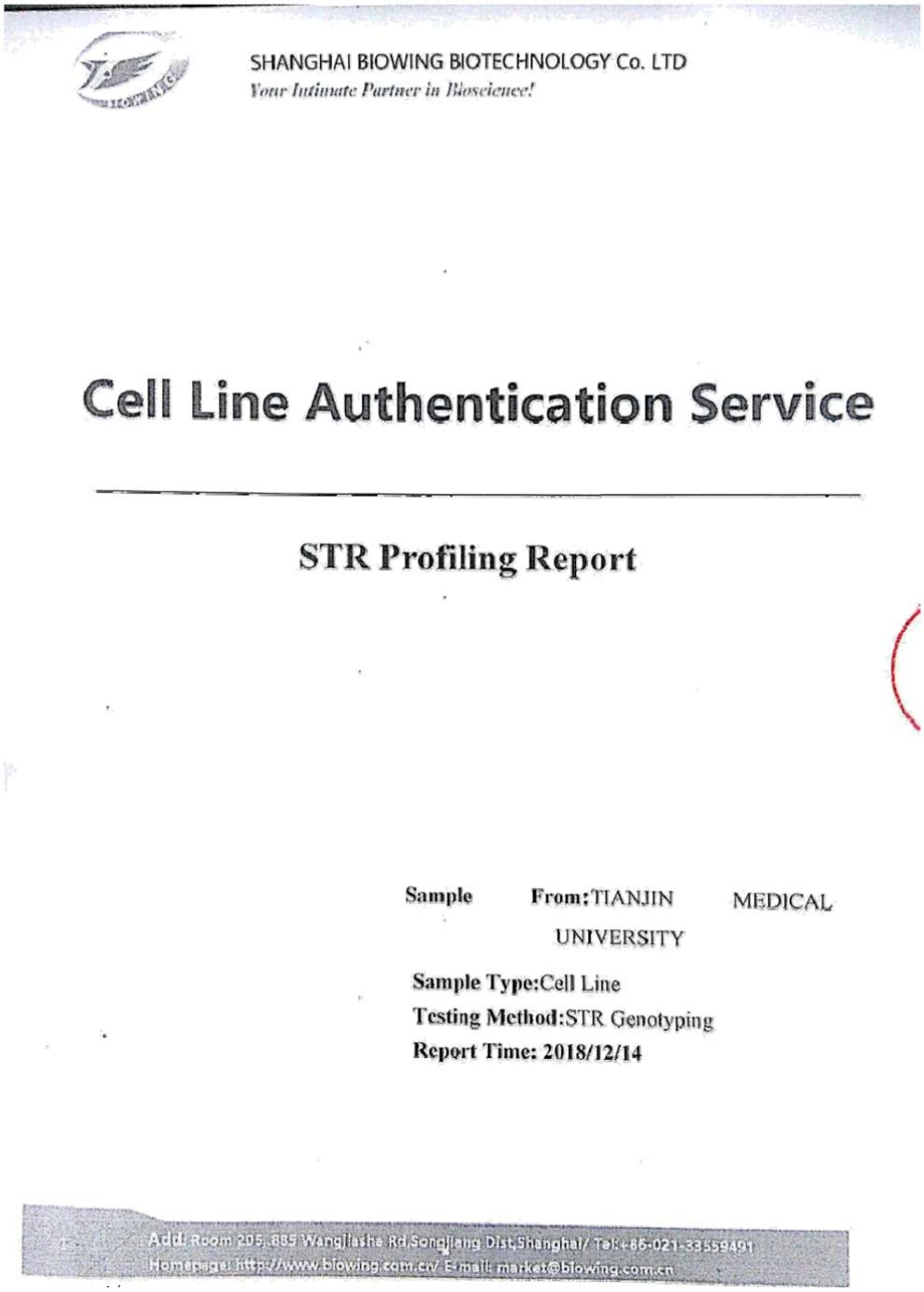

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Cell Line Authentication Service

STR Profiling Report

Sample From:TIANJIN MEDICAL UNIVERSITY

Sample Type: Cell Line

Testing Method:STR Genotyping

Report Time: 2018/12/14

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Cell Line Authentication – STR Profiling Report

Sample code

Table 1. Sample Code

Customer's code	Company Code
KMH-2	20181207-01

Sample Number:1

Sample Type: Cell line

Testing Type: STR

Testing Method:

DNA was extracted by a commercial kit from CORNING (AP-EMN-BL-GDNA-250G). The twenty STRs including Amelogenin locus were amplified by six multiplex PCR and separated on ABI 3730XL Genetic Analyzer. The signals were then analyzed by the software GeneMapper.

Data Interpretation:

Cell lines were authenticated using Short Tandem Repeat (STR) analysis asdescribed in 2012 in ANSI Standard (ASN-0002) by the ATCC StandardsDevelopment Organization (SDO) and in Capes-Davis et al., Match criteria forhuman cell line authentication: Where do we draw the line? Int J Cancer.2013;132(11):2510-9.

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Test Results

1. STR profile

Table 2. STR and Amelogenin Genotyping Results of Cell line20181207-01.

Loci	Sampleinformation			Cell Bank information		
	Sample name: KMH-2			Cell line name: KMH-2		
	Allele1	Allele2	Allele3	Allele1	Allele2	Allele3
D5S818	12	13		12	13	
D13S317	9	9		9	9	
D7S820	11	11		11	11	
D16S539	9	12		9	12	
VWA	14	15		14	15	
TH01	9	9		9	9	
AMEL	X	Y		X	Y	
TPOX	8	11		8	11	
CSF1PO	10	11		10	11	
D12S391	21	22				
FGA	20	22				
D2S1338	18	18				
D21S11	29	30				
D18S51	14.2	17				
D8S1179	13	13				
D3S1358	15	16.2				
D6S1043	11	11				
PENTAE	15	15				
D19S433	15	15.2				
PENTAD	9	10				
D1S1656	15	16				

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2. database annotation

Figure 1. STR matching analysis

EV	Cell No.	Cell name	Locus names								
			D5S818	D13S317	D7S820	D16S539	VWA	TH01	AM	TPOX	CSF1PO
	Query (Your Cell)		*12,13*	*9,9*	*11,11*	*9,12*	*14,15*	*9,9*	*X,Y*	*8,11*	*10,11*
1.00(36/36)	JCRB1066	KMH-2	12,13	9,9	11,11	9,12	14,15	9,9	X,Y	8,11	10,11

Note: The STR online match analysis of the test cell against DSMZ database, showing cell number (Cell No.) and cell name.

3. Authentication

☐ The submitted sample profile is human, but not a match for any profile in the DSMZ STR database.

☑ The submitted profile is an exact match for the following human cell line(s) in the DSMZ STR database (8 core loci plus Amelogenin):**KMH-2**.

☐ The submitted profile is similar to the following DSMZ human cell line: /.

- **Note:** A cell line can be considered to be authenticated when 80% (exact match) of the alleles in its STR profile match profiles from tissue or other cell line samples from that donor or from database. Cell lines with between a 55% to 80% (similar) match require further profiling for investigation of relatedness.

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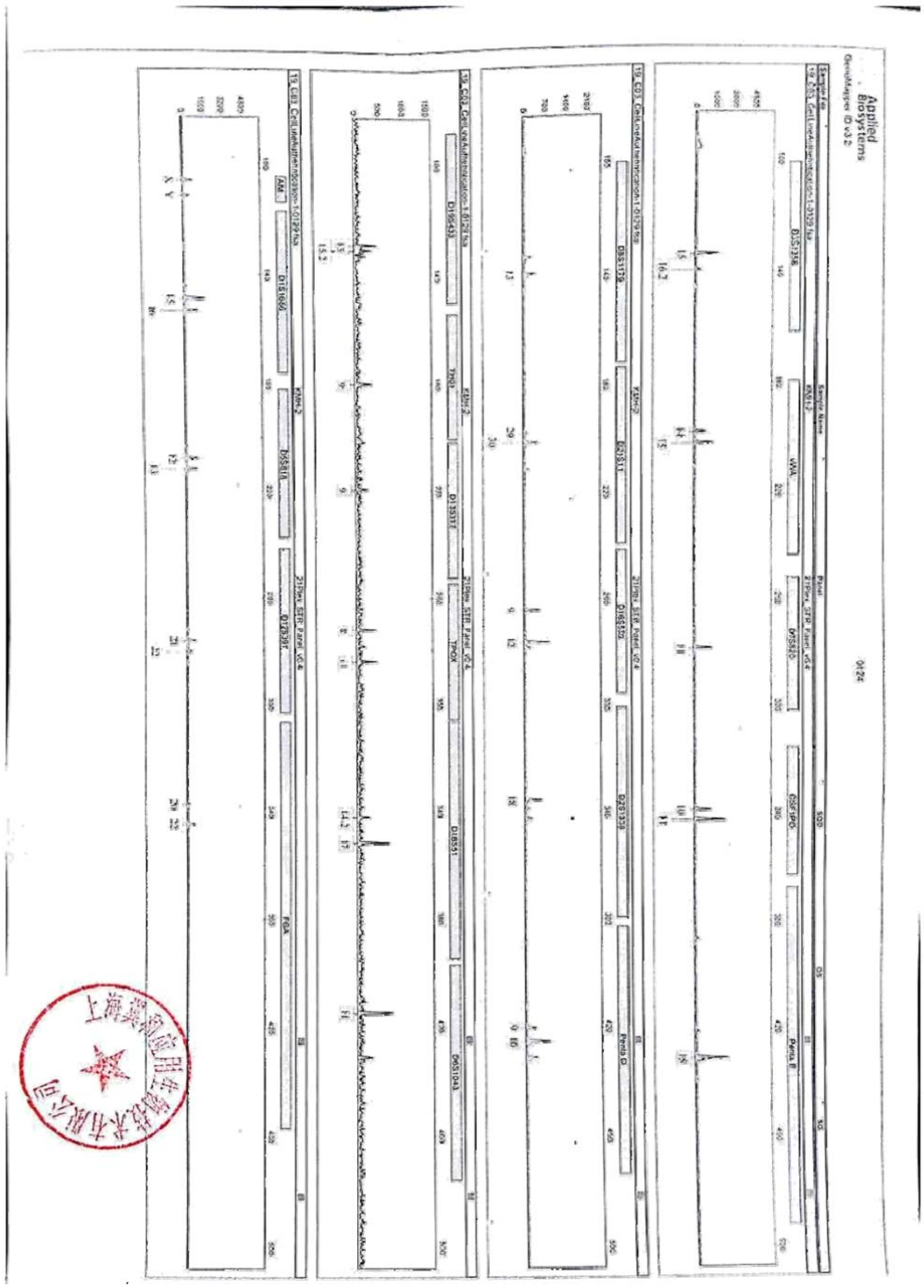

Thyroid
Targeting super-enhancer-driven oncogenic transcription by CDK7 inhibition in anaplastic thyroid cancer (DOI: 10.1089/thy.2018.0550)

SHANGHAI BIOWING BIOTECHNOLOGY Co. LTD

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Cell Line Authentication Service

STR Profiling Report

Sample From: TIANJIN MEDICAL UNIVERSITY

Sample Type: Cell Line

Testing Method: STR Genotyping

Report Time: 2018/12/14

Add: Room 205, 885 Wangjiashe Rd,Songjiang Dist,Shanghai/ Tel:+86-021-33559491
Homepage: http://www.biowing.com.cn/ E-mail: market@biowing.com.cn

Thyroid
Targeting super-enhancer-driven oncogenic transcription by CDK7 inhibition in anaplastic thyroid cancer (DOI: 10.1089/thy.2018.0550)

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Testing Company: Shanghai Biowing Applied Biotechnology Co. Ltd
Address: Room 205, NO.885 Wangjiashe Road, Songjiang District, Shanghai
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Contact: Wenyao Zhang
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Cell Line Authentication – STR Profiling Report

Sample code

Table 1. Sample Code

Customer's code	Company Code
Hth-7	20181207-02

Sample Number:1

Sample Type: Cell line

Testing Type: STR

Testing Method:

DNA was extracted by a commercial kit from CORNING (AP-EMN-BL-GDNA-250G). The twenty STRs including Amelogenin locus were amplified by six multiplex PCR and separated on ABI 3730XL Genetic Analyzer. The signals were then analyzed by the software GeneMapper.

Data Interpretation:

Cell lines were authenticated using Short Tandem Repeat (STR) analysis asdescribed in 2012 in ANSI Standard (ASN-0002) by the ATCC StandardsDevelopment Organization (SDO) and in Capes-Davis et al., Match criteria forhuman cell line authentication: Where do we draw the line? Int J Cancer.2013;132(11):2510-9.

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Test Results

1. STR profile

Table 2. STR and Amelogenin Genotyping Results of Cell line 20181207-02.

Loci	Sampleinformation			Cell Bank information		
	Sample name: Hth-7			Cell line name: HTh7		
	Allele1	Allele2	Allele3	Allele1	Allele2	Allele3
D5S818	11	11		11	11	
D13S317	11	14		11	14	
D7S820	8	11		8	11	
D16S539	9	13		9	13	
VWA	14	18		14	18	
TH01	9	9.3		9	9.3	
AMEL	X	X		X	X	
TPOX	11	11		11	11	
CSF1PO	12	12		12	12	
D12S391	23	23				
FGA	20	23				
D2S1338	19	21				
D21S11	29	30				
D18S51	16	16				
D8S1179	12	13				
D3S1358	14	15				
D6S1043	14	20				
PENTAE	12	12				
D19S433	12	12				
PENTAD	10	11				
D1S1656	11	16				

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2. database annotation

Figure 1. STR matching analysis

Amelogenin	X
CSF1PO	12
D3S1358	14,15
D5S818	11
D7S820	8,11
D8S1179	12,13
D13S317	11,14
D16S539	9,13
D18S51	16
D21S11	29,30
FGA	20,23
TH01	9.9.3
TPOX	11
vWA	14 (PubMe 14,18 (Put

Note: The STR online match analysis of the test cell against DSMZ database, showing cell number (Cell No.) and cell name.

3. Authentication

☐ The submitted sample profile is human, but not a match for any profile in the DSMZ STR database.

☑ The submitted profile is an exact match for the following human cell line(s) in the DSMZ STR database (8 core loci plus Amelogenin): **HTh7**.

☐ The submitted profile is similar to the following DSMZ human cell line: /.

- **Note:** A cell line can be considered to be authenticated when 80% (exact match) of the alleles in its STR profile match profiles from tissue or other cell line samples from that donor or from database. Cell lines with between a 55% to 80% (similar) match require further profiling for investigation of relatedness.

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Appendix:

1. Genotyping Strategy and Site Distribution

Table S1. Experimental Strategy and Sites

	Strategy 1	Strategy 2	Strategy 3	Strategy 4
1	D3S1358	D8S1179	D19S433	AMEL
2	VWA	D21S11	TH01	D1S1656
3	D7S820	D16S539	D13S317	D5S818
4	CSF1PO	D2S1338	TPOX	D12S391
5	PENTAE	PENTAD	D18S51	FGA
6			D6S1043	

The allele match algorithm compares the 8 core loci plus amelogenin only, even though alleles from all lociwill be reported when available.

2. DSMZ tools was used to carry on the cell line comparison, which contains 2455 cell lines STR data from ATCC, DSMZ, JCRB ,ECACC, GNE and RIKEN databases. If the cell is not included in the above cell library, users need to compared with other databases.

Technician: Jianan Zhang

Checked by: Ning Qian

Issued by: Yang Bai

Issue date: 2018/12/14

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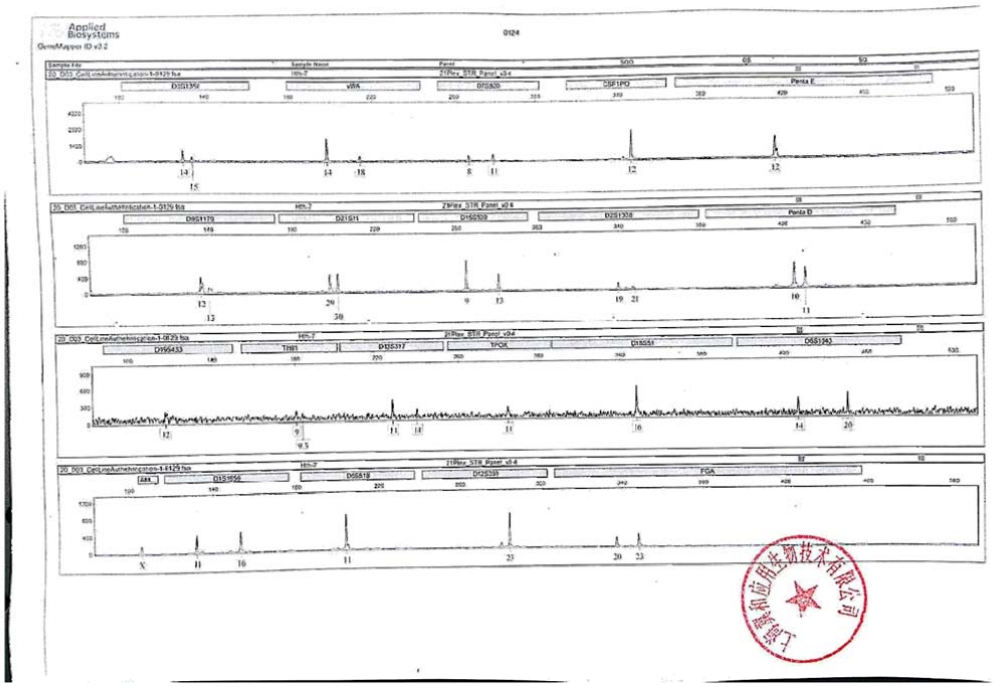

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Report of Human Cell Line Authentication

(Notice:This authentication report is restricted to the cell sold from Guangzhou Cellcook Biotech Co., Ltd, and the date with seal is the date of delivery.)

I. Sample

Sample Name: labeled as 'B-CPAP'

II. Method and Procedure

1. PCR is amplified with STR Multi-amplification Kit (PowerPlex™16HS System);
2. PCR products are assayed with 3100 DNA Analyzer (Applied Biosystems®).
3. Amplification of gene COX1 and electrophoresis are employed to survey the species of the sample.

III. Results

1. The STR profiles of the cell line sample are in the attached table and figure.
2. The search result in ATCC and DSMZ databases.
3. The electrophoresis figure of gene COX1.

B-CPAP: ①No loci has tri-alleles or tetra-alleles. Contamination of other human cell line is not found (Figure 1 & Table 1). ②100% matched cell lines are not found in ATCC data banks. 100% matched cell lines are found in DSMZ data banks. And the cell line named as "B-CPAP" *et al.* (Figure 2 & Figure 3). ③The sample is a human cell line. Contamination of other species cells are not found in the sample (Figure 4).

Operator: Xiaohua Mo

Auditor: Xuanyi Liang

GUANGZHOU CELLCOOK BIOTECH CO.,LTD 23 NOV 2018 CELL STR PROFILING PASS

Guangzhou Cellcook Biotech Co., Ltd

Thyroid
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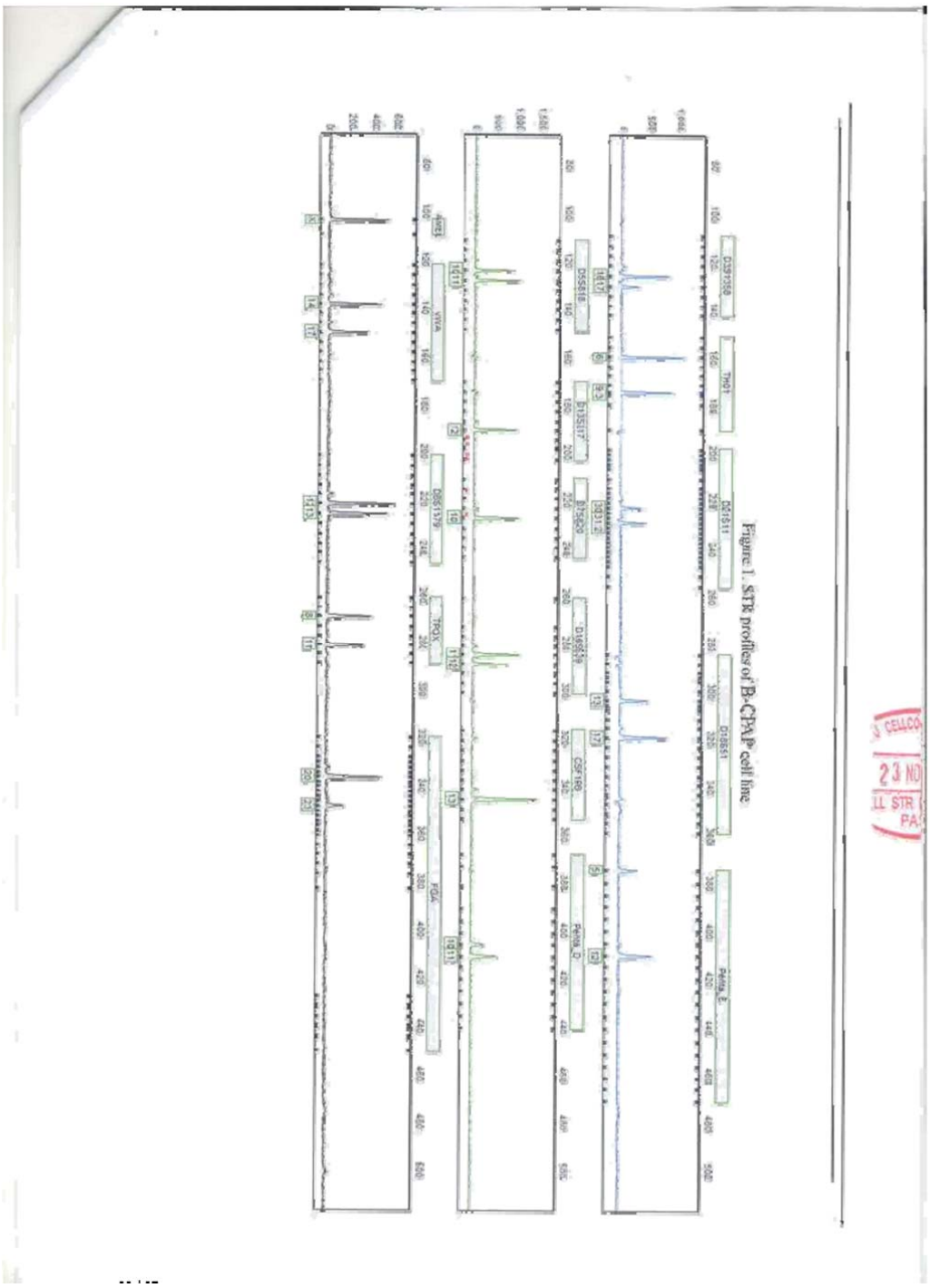

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Table 1. STR profiles of B-CPAP cell line

	Allele1	Allele2
D3S1358	16	17
TH01	6	9.3
D21S11	30	31.2
D18S51	13	17
Penta_E	5	12
D5S818	10	11
D13S317	12	
D7S820	10	
D16S539	11	12
CSF1PO	13	
Penta_D	10	11
AMEL	X	
vWA	14	17
D8S1179	12	13
TPOX	8	11
FGA	20	23

Figure 2. Search result in ATCC database

As part of our continuing efforts to characterize and authenticate the cell lines in the Cell Biology collection, ATCC has developed a comprehensive database of short tandem repeat (STR) DNA profiles for all of our human cell lines. View our brief tutorial before starting.

1. STR Profiling Analysis
2. Matching Algorithm
3. Interrogating the Database

There are no results.

Figure 3. Search result in DSMZ database

Result of STR matching analysis by your data.

- DSMZ Profile Database -

A graphical presentation is shown at the bottom of this page.

EV	Cell No.	Cell name	Locus names									Figures
			D5S818	D13S317	D7S820	D16S539	TWA	TH01	AM	TPOX	CSF1PO	
		Query (Your Cell)	*10,11*	*12,12*	*10,10*	*11,12*	*14,17*	*6,9.3*	*x,x*	*8,11*	*11,13*	
1.00(36/36)	279	B-CPAP	10,11	12,12	10,10	11,12	14,17	6,9.3	X,X	8,11	11,13	-
0.67(24/36)	105	CPC-36	10,12	12,12	9,10	11,12	14,17	6,9.3	X,X	8,8	10,13	-
0.67(24/36)	248	KTSE-349	10,20	12,12	10,10	20,12	14,16	7,9	X,X	8,8	11,13	-
0.67(24/36)	446	CAL-27	11,12	10,13	10,10	11,12	14,17	6,9.3	X,X	8,8	10,12	-
0.67(24/36)	571	SU-DHL-6	12,12	12,16	10,10	11,12	14,17	6,9.3	X,X	11,12	10,10	-
0.67(24/36)	687	MDS0	11,12	12,12	10,11	11,12	14,17	9.3,9.3	X,Y	8,11	8,11	-
0.67(24/36)	756	PGA-1	11,12	8,12	10,11	12,12	17,17	6,9.3	X,Y	8,11	11,13	-
0.67(24/36)	779	PG-ESO	11,12	8,12	10,11	9,12	17,17	6,9.3	X,Y	8,11	12,13	-
0.67(24/36)	CVCL_1179	Detroit 510	10,12	10,11	9,10	11,12	14,16	6,9.3	X,X	8,11	12,12	-
0.67(24/36)	CRL-2486	HEPM	11,11	8,12	9,10	11,12	17,18	6,9.3	X,X	8,11	10,13	-
0.67(24/36)	CRL-2095	CAL-27	11,12	10,12	10,10	11,12	14,17	6,9.3	X,X	8,8	10,12	-
0.67(24/36)	CRL-2958	SU-DHL-6	12,12	12,14	10,10	11,12	14,17	6,9.3	X,X	11,12	10,10	-

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Figure 4. Authentication of the species of the sample

M: Marker. As the size of 700, 600, 500, 400, 300, 200 and 100bp from up to down.

Nine species are checked, as follow: *Homo sapiens* 391bp, *Cricetulus griseus*315bp, *Macaca mulatta*287bp, *Cercopithecus aethiops*222bp, *Rattus norvegicus*196bp, *Canis familiaris*172bp, *Mus musculus*150bp, *Bos Taurus*102bp, IC 70bp

The sample: The band size is 391bp which matches the size of human.

Thyroid
Targeting super-enhancer-driven oncogenic transcription by CDK7 inhibition in anaplastic thyroid cancer (DOI: 10.1089/thy.2018.0550)

Report of Human Cell Line Authentication

(Notice:This authentication report is restricted to the cell sold from Guangzhou Cellcook Biotech Co., Ltd. and the date with seal is the date of delivery.)

I. Sample

Sample Name: labeled as "8305C"

II. Method and Procedure

1. PCR is amplified with STR Multi-amplification Kit (PowerPlex™16HS System);
2. PCR products are assayed with 3100 DNA Analyzer (Applied Biosystems®).
3. Amplification of gene COX1 and electrophoresis are employed to survey the species of the sample.

III. Results

1. The STR profiles of the cell line sample are in the attached table and figure.
2. The search result in ATCC and DSMZ databases.
3. The electrophoresis figure of gene COX1.

8305C: ①No loci has tri-alleles or tetra-alleles. Contamination of other human cell line is not found (Figure 1 & Table 1). ②100% matched cell lines are not found in ATCC data banks. 100% matched cell lines are found in DSMZ data banks. And the cell line named as "8305C" *et al*. (Figure 2 & Figure 3).③The sample is a human cell line. Contamination of other species cells are not found in the sample (Figure 4).

GUANGZHOU CELLCOOK BIOTECH CO.,LTD 23 NOV 2018 PASS

Operator: Xiaohua Mo Auditor: Xuanyi Liang

Guangzhou Cellcook Biotech Co., Ltd

Targeting super-enhancer-driven oncogenic transcription by CDK7 inhibition in anaplastic thyroid cancer (DOI: 10.1089/thy.2018.0550)

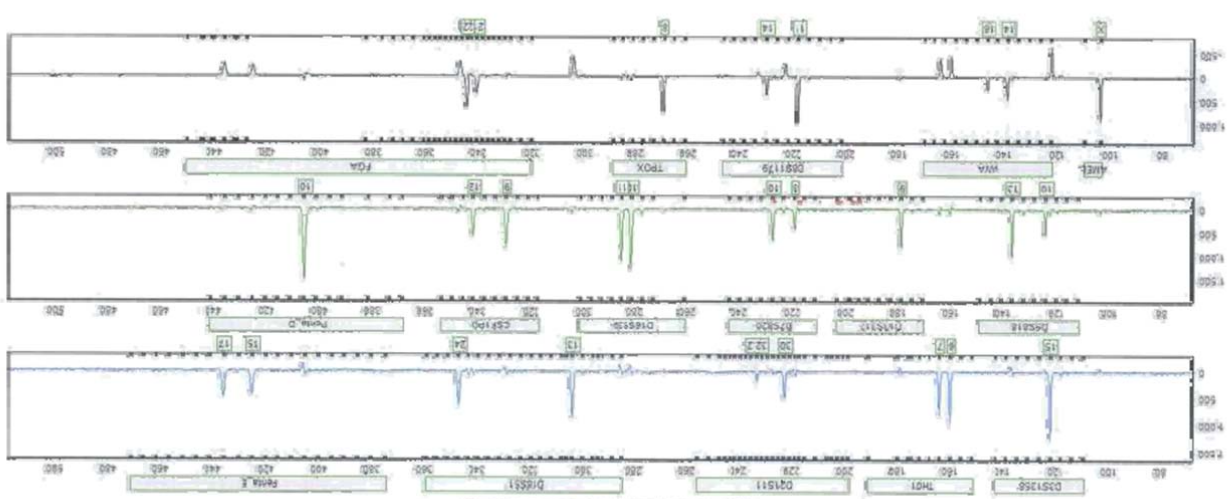

Figure 1. S1K profiles of 8305C cell line

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Table 1. STR profiles of 8305C cell line

	Allele1	Allele2
D3S1358	15	
TH01	6	7
D21S11	30	32.2
D18S51	13	24
Penta_E	15	17
D5S818	10	13
D13S317	9	
D7S820	8	10
D16S539	10	11
CSF1PO	9	12
Penta_D	10	
AMEL	x	
vWA	14	16
D8S1179	11	14
TPOX	8	
FGA	21	22

Figure 2. Search result in ATCC database

As part of our continuing efforts to characterize and authenticate the cell lines in the Cell Biology collection, ATCC has developed a comprehensive database of short tandem repeat (STR) DNA profiles for all of our human cell lines. View our brief tutorial before starting.

1. STR Profiling Analysis
2. Matching Algorithm
3. Interrogating the Database

There are no results.

Figure 3. Search result in DSMZ database

Result of STR matching analysis by your data.

- DSMZ Profile Database -

A graphical presentation is shown at the bottom of this page.

TV	Cell No.	Cell name	Locus name									Figures
			D5S818	D13S317	D7S820	D16S539	vWA	TH01	AML	TPOX	CSF1PO	
	Query (Your Cell)		10,13	9,9	8,10	10,11	14,16	6,7	x,x	8,8	9,12	
1.000(6/6)	ACC 388	8305C	10,13	9,9	8,10	10,11	14,16	6,7	X,X	8,8	9,12	-
1.000(6/6)	ECACC 94090	8305C	10,13	9,9	8,10	10,11	14,16	6,7	X,X	8,8	9,12	-
0.940(34/36)	[illegible]	[illegible]	10,13	9,9	8,8	10,11	14,16	6,7	X,X	8,8	9,12	-
0.782(28/36)	ACC 675	ES-2	12,12	9,12	8,10	10,11	14,16	6,8	X,X	8,8	12,12	-
0.671(24/36)	ACC 517	JEKO-1	10,13	9,9	10,11	12,12	14,16	7,9	X,X	8,8	9,12	-
0.671(24/36)	CRL-3006	[illegible]	10,12	9,9	10,11	12,12	14,16	7,9	X,X	8,8	9,12	-
0.671(24/36)	CRL-7373	Hs 246.Ts	12,13	9,9	8,10	10,11	15,16	6,6	X,X	8,8	12,12	-
0.671(24/36)	CRL-7360	Hs 606.St	11,13	9,12	8,10	11,12	14,16	7,9	X,X	8,8	11,12	-
0.671(24/36)	CRL-7360	Hs 606.T	11,13	9,12	8,10	11,12	14,16	7,9	X,X	8,8	11,12	-
0.671(24/36)	ACC 525	KP-N-RT-BM-1	10,13	9,12	10,11	10,12	17,20	6,7	X,X	8,8	11,12	-
0.671(24/36)	ACC 675	HDC	11,13	9,12	12,12	8,11	14,16	6,7	X,X	8,8	9,12	-
0.671(24/36)	ACC 675	SNU-...	11,13	9,9	10,11	11,12	14,16	6,7	X,X	8,8	11,12	-

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Nine species are checked, as follow: *Homo sapiens* 391bp, *Cricetulus griseus*315bp, *Macaca mulatta*287bp, *Cercopithecus aethiops*222bp, *Rattus norvegicus*196bp, *Canis familiaris*172bp, *Mus musculus*150bp, *Bos Taurus*102bp, IC 70bp

The sample: The band size is 391bp which matches the size of human.

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电话:0512-68731001
传真:0512-62629520
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Cell Line Authentication Report

Customer: Lin.Dang

Institution: TIANJIN MEDICAL UNIVERSITY

Quotation Number: 80-227636616

Completion Date: 11/13/2018

1. **Sample ID: C643**
2. **Original Material: Cell pellets**
3. **Methods:**

   1).Genomic DNA was extracted from the cell pellets provided by the customer.

   2).Samples, together with positive and negative control were amplified using GenePrint 10 System (Promega).

   3). Amplified products were processed using the ABI3730xl Genetic Analyzer.

   4).Data were analyzed using GeneMapper4.0 software and then compared with the ATCC, DSMZ or JCRB and RIKEN .etc databases for reference matching.

4. **Results:**

   1) **10 Loci STR Profile:**

Genetic Site	Cell Bank information		Customer sample	
(Locus)	C643		C643	
Amelogenin	X	Y	X	Y
CSF1PO	10	11	10	11
D13S317	8	10	8	10
D16S539	9	13	9	13
D5S818	11	12	11	12
D7S820	9	12	9	12
THO1	9.3	10	9.3	10
TPOX	11	12	11	12
vWA	15	17	15	17
D21S11			28	28
Percent match between the sample and the database profile: 100%				

Summary:

Your cell line is considered to be "identical" to the reference cell line in the ATCC STR database, as the STR profile yields a 100% match.

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中国苏州工业园区
星湖街218号纳米科技园B3楼 215123
电话:0512-68731001
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Notes:

1. P=100% x (2xM)/N; M=18, N=36P=100% x (2x18)/36=100%

M: number of the matching peaks; N: number of all peaks

2. Based on the ANSI Standard, cell lines with ⩾80% match are considered to be related; i.e., derived from a common ancestry. Cell lines with between a 55% to 80% match require further profiling for authentication of relatedness.

3. The *short tandem repeat (STR) profile generated* by GENEWIZ Inc. is indicative only of the sample sent to GENEWIZ Inc. at the time it was sent. This data and analysis are for research use only.

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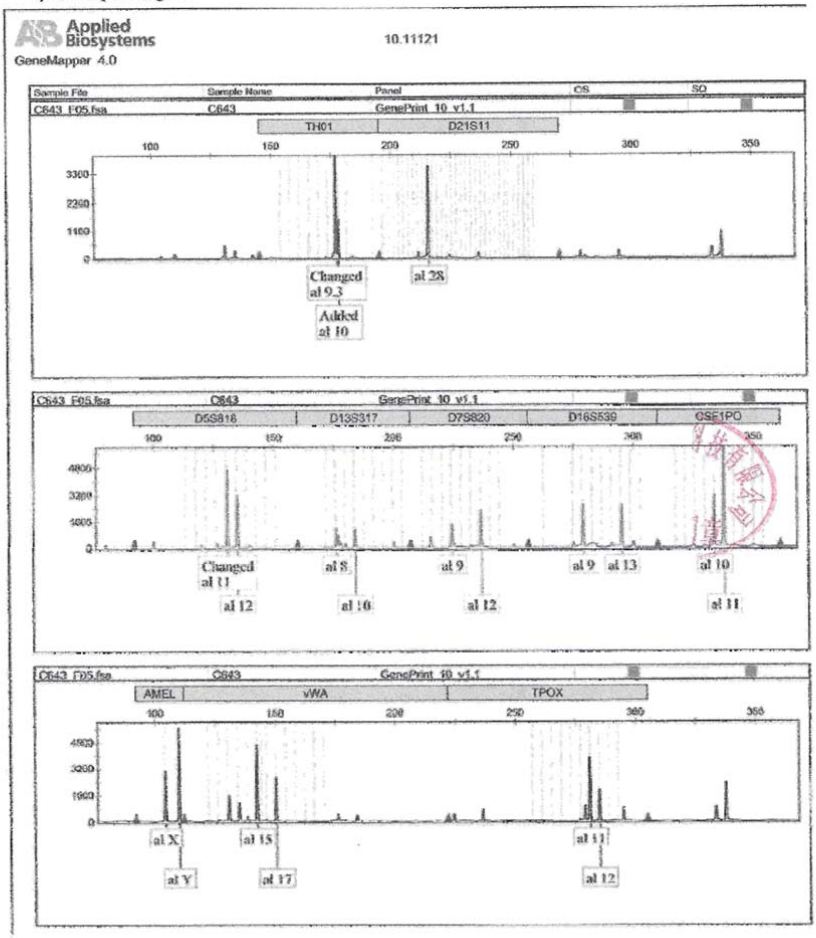
2) Electrophoretogram



Note: Raw data in appendix

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Cell Line Authentication Report

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Thyroid
Targeting super-enhancer-driven oncogenic transcription by CDK7 inhibition in anaplastic thyroid cancer (DOI: 10.1089/thy.2018.0550)

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苏州金唯智生物科技有限公司
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Cell Line Authentication Report

Customer: Lin.Dang

Institution: TIANJIN MEDICAL UNIVERSITY

Quotation Number: 80-227636616

Completion Date: 11/13/2018

1. **Sample ID: CAL-62**
2. **Original Material: Cell pellets**
3. **Methods:**

1).Genomic DNA was extracted from the cell pellets provided by the customer.

2).Samples, together with positive and negative control were amplified using GenePrint 10 System (Promega).

3). Amplified products were processed using the ABI3730xl Genetic Analyzer.

4).Data were analyzed using GeneMapper4.0 software and then compared with the ATCC, DSMZ or ICRB and RIKEN .etc databases for reference matching.

4. **Results:**

1) **10 Loci STR Profile:**

Genetic Site	Cell Bank information		Customer sample	
(Locus)	CAL-62		CAL-62	
Amelogenin	X	X	X	X
CSF1PO	9	12	9	12
D13S317	12	12	12	12
D16S539	12	13	12	13
D5S818	9	12	9	12
D7S820	10	10	10	10
THO1	7	9	7	9
TPOX	8	9	8	9
vWA	16	16	16	16
D21S11	32.2	32.2	32.2	32.2
Percent match between the sample and the database profile: 100%				

Summary:

Your cell line is considered to be "identical" to the reference cell line in the ATCC STR database, as the STR profile yields a 100% match.

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传真:0512-62629530
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Notes:

1. P=100% x (2xM)/N; M=18, N=36P=100% x (2x18)/36=100%

M: number of the matching peaks; N: number of all peaks

2. Based on the ANSI Standard, cell lines with ≥80% match are considered to be related; i.e., derived from a common ancestry. Cell lines with between a 55% to 80% match require further profiling for authentication of relatedness.

3. The short tandem repeat (STR) profile generated by GENEWIZ Inc. is indicative only of the sample sent to GENEWIZ Inc. at the time it was sent. This data and analysis are for research use only.

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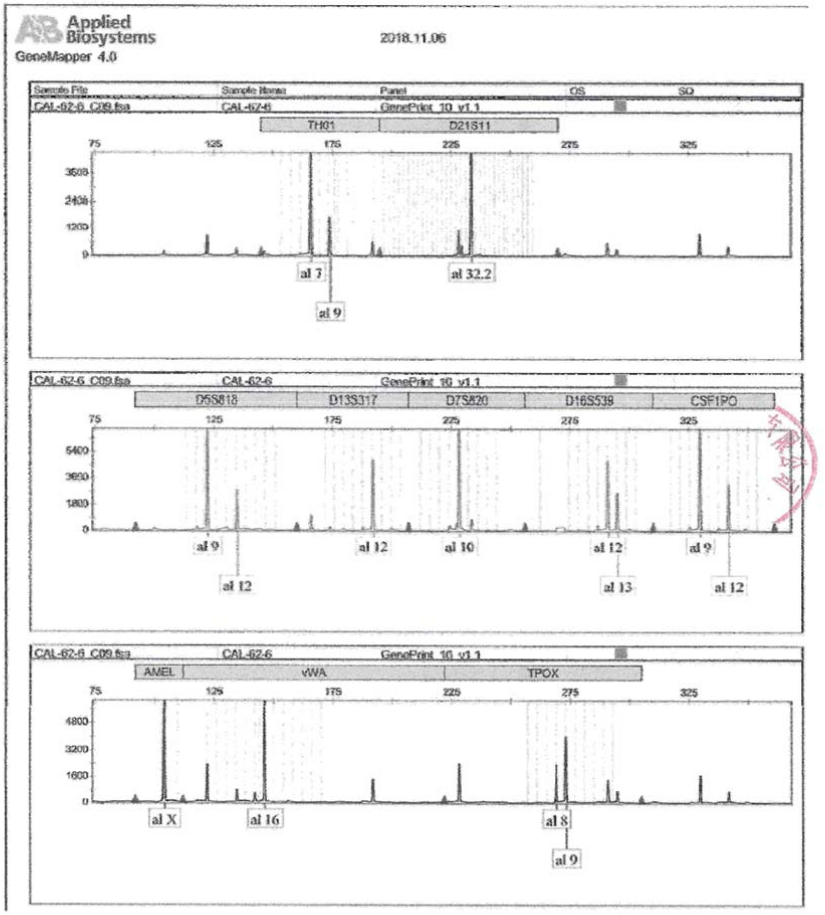
2) Electrophoretogram



Note: Raw data in appendix

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Cell Line Authentication Report

Customer: Lin.Dang

Institution: TIANJIN MEDICAL UNIVERSITY

Quotation Number: 80-227636616

Completion Date: 1/29/2019

1. **Sample ID: 8505C**
2. **Original Material: Cell pellets**
3. **Methods:**

   1).Genomic DNA was extracted from the cell pellets provided by the customer.

   2).Samples, together with positive and negative control were amplified using GenePrint 10 System (Promega).

   3). Amplified products were processed using the ABI3730xl Genetic Analyzer.

   4). Data were analyzed using GeneMapper4.0 software and then compared with the ATCC, DSMZ, JCRB and RIKEN .etc databases for reference matching.

4. **Results:**

   1) **10 Loci STR Profile:**

Genetic Site	Cell Bank information		Customer sample	
(Locus)	8505C		8505C	
Amelogenin	X	X	X	X
CSF1PO	12	13	12	13
D13S317	13	13	13	13
D16S539	12	12	12	12
D5S818	10	11	10	11
D7S820	10	10	10	10
THO1	6	9	6.3	9
TPOX	11	11	11	11
vWA	17	19	17	19.3
D21S11			28	32.2
Percent match between the sample and the database profile: 88.88%				

Summary:

Your cell line is considered "related" to the reference cell line in the Cell Bank STR database, as the STR profile yields matches that are ≥ 80% but less than 100%.

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Notes:

1. P=100% x (2xM)/N; M=16, N=36P=100% x (2x16)/36=88.88%

M: number of the matching peaks; N: number of all peaks

2. Based on the ANSI Standard, cell lines with ≥80% match are considered to be related; i.e., derived from a common ancestry. Cell lines with between a 55% to 80% match require further profiling for authentication of relatedness.

3. The short tandem repeat (STR) profile generated by GENEWIZ Inc. is indicative only of the sample sent to GENEWIZ Inc. at the time it was sent. This data and analysis are for research use only.

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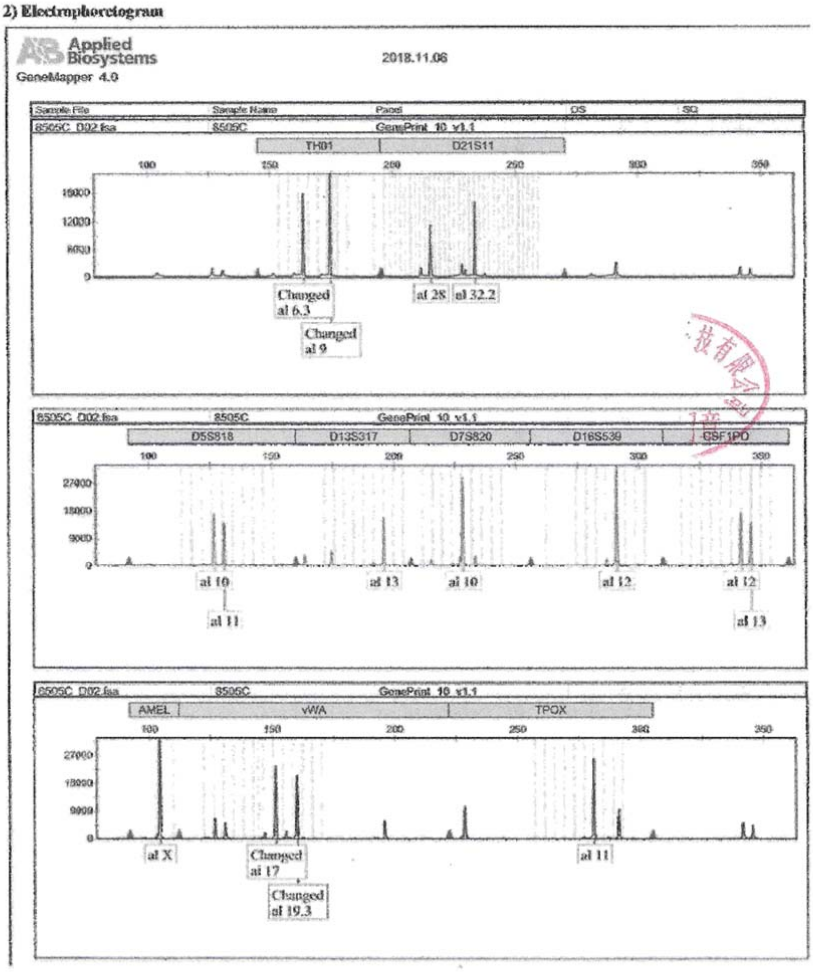

Note：Raw data in appendix

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Report of Human Cell Line Authentication

(Notice:This authentication report is restricted to the cell sold from Guangzhou Cellcook Biotech Co., Ltd, and the date with seal is the date of delivery.)

Ⅰ. Sample

Sample Name: labeled as 'K1'

Ⅱ. Method and Procedure

1. PCR is amplified with STR Multi-amplification Kit (PowerPlex™16HS System);
2. PCR products are assayed with 3100 DNA Analyzer (Applied Biosystems®).
3. Amplification of gene COX1 and electrophoresis are employed to survey the species of the sample.

III. Results

1. The STR profiles of the cell line sample are in the attached table and figure.
2. The search result in ATCC and DSMZ databases.
3. The electrophoresis figure of gene COX1.

K1: ①No loci has tri-alleles or tetra-alleles. Contamination of other human cell line is not found (Figure 1 & Table 1). ②100% matched cell lines are found in ATCC and DSMZ data banks And the cell line named as "K1" et *al*. (Figure 2 & Figure 3).③The sample is a human cell line. Contamination of other species cells are not found in the sample (Figure 4).

Operator: Xiaohua Mo

Auditor: Xuanyi Liang

Guangzhou Cellcook Biotech Co., Ltd

GUANGZHOU CELLCOOK BIOTECH CO.,LTD
23 MAY 2018
CELL STR PROFILING
PASS

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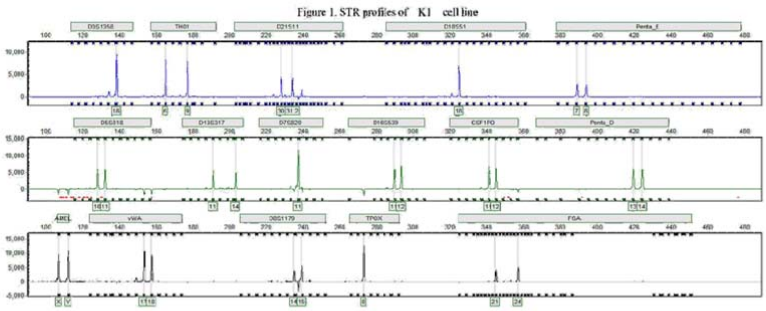

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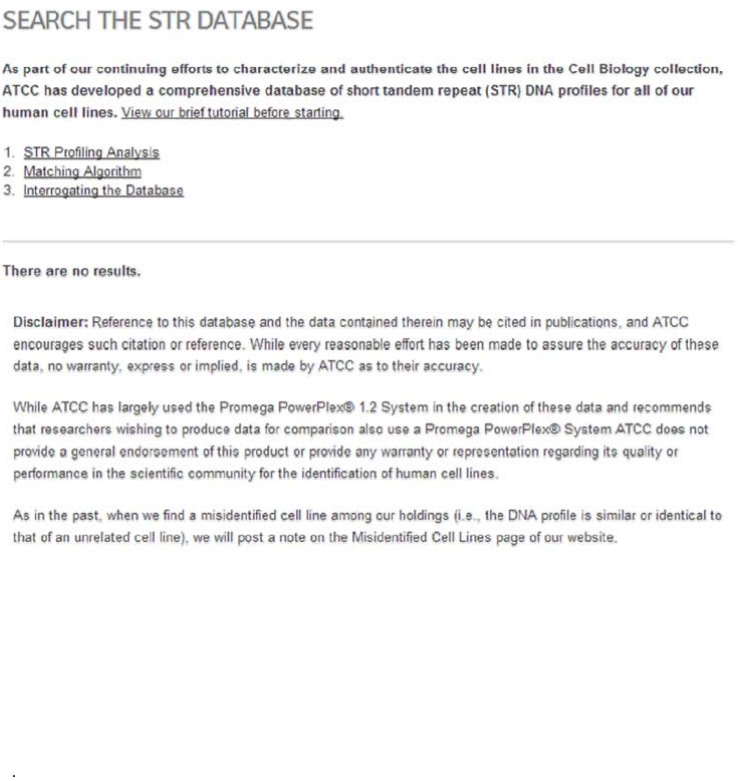
Table 1. STR profiles of K1 cell line

	Allele1	Allele2
D3S1358	18	
TH01	6	9
D21S11	30	31.2
D18S51	18	
Penta_E	7	8
D5S818	10	11
D13S317	11	14
D7S820	11	
D16S539	11	12
CSF1PO	11	12
Penta_D	13	14
AMEL	x	y
vWA	17	18
D8S1179	14	15
TPOX	8	
FGA	21	24

Figure 2. Search result in ATCC database



SEARCH THE STR DATABASE

As part of our continuing efforts to characterize and authenticate the cell lines in the Cell Biology collection, ATCC has developed a comprehensive database of short tandem repeat (STR) DNA profiles for all of our human cell lines. View our brief tutorial before starting.

1. STR Profiling Analysis
2. Matching Algorithm
3. Interrogating the Database

There are no results.

Disclaimer: Reference to this database and the data contained therein may be cited in publications, and ATCC encourages such citation or reference. While every reasonable effort has been made to assure the accuracy of these data, no warranty, express or implied, is made by ATCC as to their accuracy.

While ATCC has largely used the Promega PowerPlex® 1.2 System in the creation of these data and recommends that researchers wishing to produce data for comparison also use a Promega PowerPlex® System ATCC does not provide a general endorsement of this product or provide any warranty or representation regarding its quality or performance in the scientific community for the identification of human cell lines.

As in the past, when we find a misidentified cell line among our holdings (i.e., the DNA profile is similar or identical to that of an unrelated cell line), we will post a note on the Misidentified Cell Lines page of our website.