Am J Cancer Res 2023;13(11):5656-5666
www.ajcr.us /ISSN:2156-6976/ajcr0150313

Original Article

The transcription factor TBP promotes hepatocellular carcinoma progression by activating AKT3

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Received March 23, 2023; Accepted July 20, 2023; Epub November 15, 2023; Published November 30, 2023

Abstract: The present work was performed to clarify the role of TATA-binding protein (TBP) in hepatocellular carcinoma (HCC). TBP expression in adjacent liver tissues and HCC tissue sample was detected by immunohistochemistry and qRT-PCR. With CCK-8, BrdU, flow cytometry, and transwell assays, the malignancy of cancer cell lines were evaluated. The binding sites of TBP and AKT serine/threonine kinase 3 (Akt3) promoter region were predicted by PROMO database, and the binding relationship between TBP and AKT3 promoter was verified with dual luciferase reporter gene assay and ChIP-qPCR assay. The effect of TBP on AKT3 expression was examined by immunoblotting. The signaling pathways associated with AKT3 were predicted by gene set enrichment analysis (GSEA) with LinkedOmics database. It was revealed that, TBP expression in HCC tissues and cell lines was up-regulated, which was associated with the short survival time of patients. Up-regulation of TBP promoted the viability and aggressiveness of HCC cells, while knockdown of TBP had opposite effects. TBP could bind with AKT3 promoter region, and TBP overexpression promoted the expression of AKT3, while its knockdown worked oppositely. Additionally, TBP/AKT3 axis modulated mTOR expression in HCC cells. In conclusion, TBP promotes the transcription of AKT3, thus accelerating the malignant progression of HCC.

Keywords: TBP, AKT3, HCC, proliferation, apoptosis, migration, invasion

Introduction

Primary liver cancer is one of the most deadly human malignancies globally. There are about 841,000 new cases and 782,000 deaths annually [1, 2]. Hepatocellular carcinoma (HCC) accounts for about 85%-90% of the total cases of liver cancer [3]. The etiology and pathogenesis of HCC are associated with gene polymorphism, environmental factors, life style (e.g. alcohol consumption and fat intake) and hepatitis virus infection [4]. The prognosis of HCC is still far from satisfactory, due to its high recurrence rate after surgery, chemoresistance and radioresistance [5]. Therefore, to improve the clinical outcome of HCC patients, further exploration on the molecular mechanisms of HCC tumorigenesis is necessary.

Transcription factor (TF) is a class of proteins which bind with specific DNA regulatory sequences and activate or repress transcription at trans-activating or trans-repressive domains [6]. Some TFs are reported to be involved in HCC progression by regulating gene expression [7-10]. For example, scutellarein up-regulates PTEN and inactivates PI3K/Akt/NF-κB signaling, and thus blocks HCC progression [11]; activating transcription factor 2 (ATF2) directly binds with LINC00882 promoter region and activate its transcription, and promotes the malignant biological behaviors of HCC cells [12]; E2F transcription factor 4 (E2F4) binds to CDCA3 promoter region, and facilitates its transcription, and thus the malignant biological behaviors of HCC cells are promoted [13]. TATA-binding protein (TBP) is the main component of TFIID, which positions RNA polymerase and serves as the scaffold for other transcription factors. It has a long string of glutamines in N-terminus, which modulates DNA binding activity of the C-terminus; some studies suggest that TBP plays a crucial role in cancer biology [14-17]. For example, TBP promotes the

expression of vascular endothelial growth factor (VEGF) in colon cancer [14]. TBP expression is up-regulated by Ras oncogene, and TBP contributes to malignant transformation of cells [15]. A recent study reports that TIF1γ suppresses lung adenocarcinoma metastasis by interacting with the TAF15/TBP complex [16]. Some studies also show that TBP can bind with MYC oncoprotein to regulate transcription [17]. Interestingly, some previous studies suggest that the abnormal expression of TBP is associated with the tumorigenesis of HCC. Specifically, alcohol induces the up-regulation of TBP, and hepatitis C virus infection exacerbates this phenomenon [18]; another study reports that the X protein of hepatitis B virus up-regulates TBP expression, thus leading to the abnormal transcription during the course of hepatocarcinogenesis [19]. However, the specific function and underlying mechanism of TBP in HCC are still unclear.

Serine/threonine protein kinase 3 (Aktserine/threonine kinase 3, Akt3), reportedly, can promote the progression of HCC [20, 21]. In the present work, bioinformatics suggested that TBP could bind to AKT3 promoter region. Herein, we report that, TBP is highly expressed in HCC tissues, and its aberrant up-regulation promotes the growth, migration and invasion of HCC cells, and represses apoptosis, via modulating AKT3 at transcriptional level.

Materials and methods

Clinical samples

Thirty patients with HCC who were admitted to Puren Hospital of Wuhan University of Science and Technology, from February 2017 to July 2019, were enrolled in the present study. Resected cancer tissue samples and the corresponding adjacent tissue samples were collected immediately after removal and stored in liquid nitrogen. All patients were not treated with anti-cancer treatments before the surgery. Ethics approval of this study was obtained from the ethics committee of the hospital.

Cell lines and cell culture

HCC cell lines (Bel-7402, HepG2, SK-Hep1, MHCC-97H) and immortalized liver cell line (L02) were purchased from American Type Culture Collection. All cells were grown in RPMI-1640 medium (Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum, 100 U/mL penicillin, and 0.1 mg/mL streptomycin (all from Gibco) in 5% CO_2 at 37°C.

Cell transfection

Empty plasmid (negative control, NC), TBP overexpression plasmid (TBP), AKT3 overexpression plasmid (AKT3), small interfering RNA (siRNA) targeting TBP (si-TBP), siRNA targeting AKT3 (si-AKT3), and negative control siRNA (si-NC) (RiboBio, Shanghai, China) were transfected into Bel-7402 and HepG2 cells with Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA), with the efficiency measured by quantitative real-time PCR (qPCR) 12 h after transfection.

Immunohistochemistry (IHC)

All tissue samples were fixed in 10% formaldehyde and embedded in paraffin, and then consecutively sectioned at 4-μm intervals and then mounted on polylysine-coated glass slides. Then the slides were incubated for 2 h at 62°C, deparaffinized, and rehydrated. Heat-mediated antigen retrieval was performed in 10 mM Tris-citrate buffer (pH 7.0) in a pressure cooker. After incubated with 3% H_2O_2 to remove the peroxidase, the tissue samples were subsequently incubated with 3% bovine serum albumin (BSA), and then incubated with anti-TBP antibody (1:500, ab220788, Abcam, Cambridge, UK) overnight at 4°C in a wet box. After washing, each section was incubated with the appropriate horseradish peroxidase-labeled secondary antibody and then the color was developed with 3, 3'-Diaminobenzidine solution (Genetech, San Diego, CA, USA) before counterstaining with hematoxylin. Ultimately, the samples were observed and photographed under an optical microscope, and the staining intensity was evaluated by two independent pathologists.

qRT-PCR

The RNA in tissues and cells was extracted by a TRIzol kit (Invitrogen) and reversely transcribed to cDNA with a Miscript Reverse Transcription Kit (Qiagen, Hilden, Germany). With cDNA as the template, qRT-PCR was performed with a SYBR Premix-EX-TAQ kit (Takara, Dalian, China) on ABI7300 system (ThermoFisher Scientific),

with GAPDH or U6 as the internal references. The relative expression levels of TBP and AKT3 were calculated by $2^{-\Delta\Delta Ct}$ method. The primer sequences: TBP forward: 5'-GAGGACGTTCGGTTTAG-3', reverse: 5'-AGCAGCCACAGTACGAGCAA-3'; U6 forward: 5'-CTCGCTTCGGCAGCACA-3', reverse: 5'-AACGCTTCACGAATTTGCGT-3'; AKT3 forward: 5'-CACCATGAAGACTTTCTGTGGTACA-3', reverse: 5'-CAAGAGGCCTGAAAGCAATGAT-3'; GAPDH forward: 5'-CGACCACTTTGTCAAGCTCA-3', reverse: 5'-AGGGGTCTACATGGCAACTG-3'.

Cell counting kit-8 (CCK-8) assay

The transfected Bel-7402 and HepG2 cells were collected and inoculated onto 96-well plates at the density of 1×10^4 cells/well. After 24 h, 48 h and 72 h, the medium was mixed with 10 μL of CCK-8 reagent (Invitrogen) for 2 h. The optical density was detected by a microplate reader at 450 nm wavelength.

EdU assay

Bel-7402 and HepG2 cells were inoculated into 24-well plates until cells grow normally. Cells were inoculated into a 96-well plate at 2×10^4/well, and mixed with 200 μL 5 μmol/L EdU solution (Beyotime, Shanghai, China) for 2 h, followed by immersing in phosphate buffer saline (PBS) and fixation with 4% paraformaldehyde. The cells were then incubated with Apollo® fluorescence staining solution (Beyotime) in darkness for 30 min, and stained with DAPI reaction solution (Beyotime) for 30 min. The cells were then observed after PBS washing and photographed under fluorescence microscope.

Flow cytometry

The transfected HCC cells were harvested and washed with PBS twice. The cells were mixed with 500 μL of pre-cooled 1× binding buffer and 5 μl of Annexin V-FITC (Yeasen Biotech Co., Ltd., Shanghai, China) and incubated in darkness for 15 min at ambient temperature. Then, 2.5 μL of propidium iodide (Yeasen Biotech Co., Ltd.) was added for staining the cells for 5 min, and after the cells were washed with PBS, finally the apoptosis of the cells was detected by flow cytometry.

Transwell assay

Cell migration and invasion abilities were evaluated with transwell inserts. Transwell chambers (8 μM pore size, Costar, Cambridge, MA, USA) with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) were utilized for cell invasion assays, and the chambers without Matrigel were used for cell migration assay. Approximately 5×10^4 cells were suspended in 200 μL of serum-free medium and loaded to the upper chamber. In the lower chamber, 600 μL of medium containing 10% FBS was added, and then the cells were cultured for 24 h. After taking out the transwell chamber, the cells that did not migrate/invade were gently wiped off with a cotton swab, and the cells on the below surface of the filter were fixed in methanol for 10 min, and stained with 0.1% crystal violet for 40 min. After the fixed cells were washed with the tap water, five fields (×200) of the filter were randomly selected under the microscope, and the cells were photographed and counted.

Western blot

The transfected cells were collected and lysed in RIPA lysis buffer (Pierce, Rockford, IL, USA) in an ice bath, and the supernatant was collected after centrifugation. After protein extraction, protein concentrations were measured with a BCA Protein Detection Kit (Pierce). Subsequently, protein samples were mixed with loading buffer, and heated in boiling water for 10 min for protein denaturation, and then the loading buffer was added, and sodium dodecyl sulfate-polyarylamide gel electrophoresis was performed, and the protein on the gel was then transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA), which were then blocked with 5% skimmed milk for 1 h at room temperature. Next, the PVDF membranes were incubated with primary antibodies including anti-AKT3 (ab179463, 1:500, Abcam, Shanghai, China), anti-mammalian target of rapamycin (mTOR) (ab134903, 1:500, Abcam), anti-p-mTOR (phospho S2448, ab109268, 1:200, Abcam, Shanghai, China), anti-LC3 (ab63817, 1:500, Abcam, Shanghai, China), anti-PTEN (ab267787, 1:1000, Abcam, Shanghai, China) overnight at 4°C. Next, the membrane was washed with tris buffered saline tween (TBST) three times, 5 min each time, and next the membranes were incubated with the secondary antibody goat anti-Rabbit IgG H&L (ab182016, 1:1000, Abcam Inc., Cambridge, UK) at room temperature for 2 h. Bio-Rad Geldolez Imager was utilized for imaging the protein bands and the gray

levels of the protein bands were analyzed by Image J software. GAPDH was used as the internal reference.

Chromatin immunoprecipitation (ChIP)

ChIP assay was performed with an EZ-ChIP™ kit (Millipore). After Bel-7402 and HepG2 cells were cultured for 36 h, they were fixed with formaldehyde, and then the fixation was terminated by glycine after incubation. Next, the cell precipitation was isolated, and then the cell lysis buffer containing PMSF was added, and then the nuclear precipitate was obtained. DNA was truncated ultrasonically in an ice bath, and 10% (of the volume) supernatant in the lysates was used as the control, and the remaining 90% of the lysates were incubated with anti-TBP antibody with magnetic bead with oscillation, and then centrifuged. Next, elution buffer was used to elute the DNA sequence bound to TBP, and then a DNA purification kit was adopted to purify the DNA, and finally qRT-PCR was performed to detect the sequence of AKT3 promoter region.

Dual-luciferase reporter gene assay

The binding site on the promoter region of AKT3 and TBP was predicted by PROMO database, and three potential binding sites were predicted, and the wild type (WT) sequence (...TTAAAAA...TTTTATA...TTTTTTA...) and mutant type (MUT) type sequence (...TTGGGGG...TTTTGTG...TTTTTTG...) were cloned into luciferase reporter vector pGL3 (Promega, Madison, WI, USA) to construct pGL3-AKT3-wild type (AKT3-WT) and pGL3-AKT3-mutant type (AKT3-MUT) reporter vectors. The reporter vectors and TBP overexpression plasmid (or si-TBP) were then co-transfected into Bel-7402 and HepG2 cells respectively, using Lipofectamine™ 2000 (Invitrogen) according to the manufacturer's instructions. Forty-eight hours after transfection, the luciferase activity was detected by a dual luciferase activity reporting system (Promega).

Statistical analysis

Statistical analysis was performed with SPSS 22.0 software, and the experimental data were plotted by GraphPad Prism 9. The comparisons between two groups or among multiple groups were performed with student's *t*-test or one-way ANOVA. Chi-square test was used for data comparison in a contingency table, and $P < 0.05$ indicated that the differences were statistically significant.

Results

TBP is highly expressed in HCC samples and cell lines

First of all, the data in UALCAN database showed a significant increase in TBP expression in HCC tissue samples, and an association between up-regulation of TBP and higher tumor grade and lymph node metastasis of the patients with HCC (**Figure 1A-C**). IHC suggested that TBP expression was remarkably higher in HCC tissues (chi-square = 6.696, $P = 0.01$, **Figure 1D**). The results of qPCR also indicated, TBP was up-regulated in four HCC cell lines (Bel-7402, HepG2, SK-Hep1, MHCC-97H) compared with that of the immortalized liver cell line L02 (**Figure 1E**). In addition, UALCAN database and the StarBase database implied a strong association between high expression of TBP and a short overall survival of the patients (**Figure 1F**, **1G**).

Regulatory effects of TBP on the malignant biological behaviors of HCC cells

Next, we transfected TBP overexpression plasmid into Bel-7402 cells and transfected si-TBP into HepG2 cells, to construct the gain-of-function model and loss-of-function model, and qRT-PCR showed that the transfection is successful (**Figure 2A**). CCK-8, EdU, Transwell assays and flow cytometry indicated that compared with control group, exogenous TBP promoted HCC cell growth, migration, and invasion and reduced the percentage of apoptotic cells, while knockdown of TBP worked oppositely (**Figure 2B-F**).

TBP interacts with AKT3 promoter regain

Next, PROMO databases was searched and it was revealed that TBP could probably bind to AKT3 promoter region, and there were three potential binding sites (**Figure 3A**). Interestingly, with the data from StarBase database, a positive correlation between TBP expression and AKT3 expression was observed in HCC tissue samples (**Figure 3B**). We next focused on verifying whether TBP promoted the transcription of AKT3. Western blot showed that TBP overex-

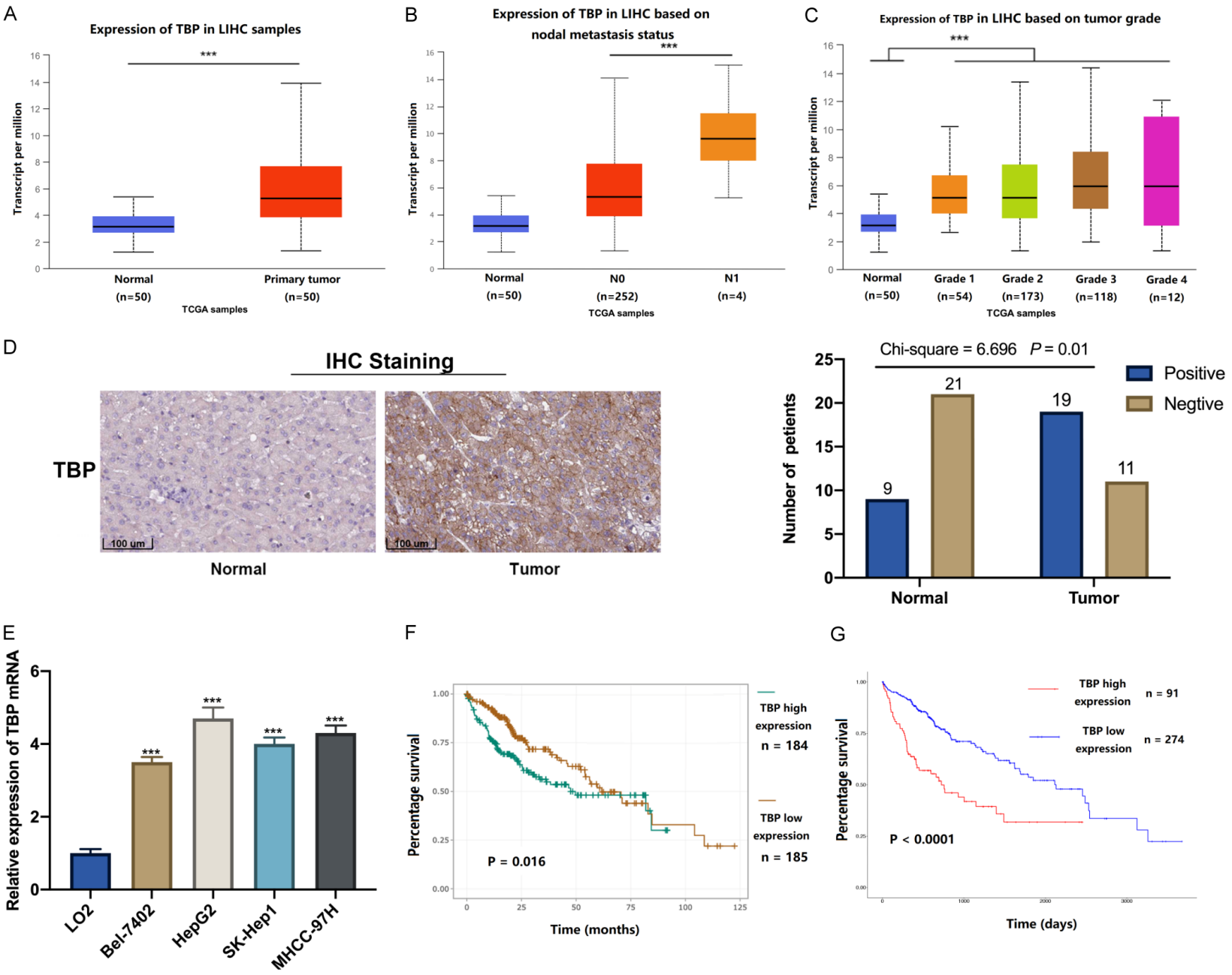

A
Expression of TBP in LIHC samples
Transcript per million
***
Normal (n=50)
Primary tumor (n=50)
TCGA samples
B
Expression of TBP in LIHC based on nodal metastasis status
Transcript per million
***
Normal (n=50)
N0 (n=252)
N1 (n=4)
TCGA samples
C
Expression of TBP in LIHC based on tumor grade
Transcript per million
***
Normal (n=50)
Grade 1 (n=54)
Grade 2 (n=173)
Grade 3 (n=118)
Grade 4 (n=12)
TCGA samples
D
IHC Staining
TBP
100 um
Normal
Tumor
Chi-square = 6.696 P = 0.01
Positive
Negtive
Number of petients
21
19
9
11
Normal
Tumor
E
Relative expression of TBP mRNA
***
LO2
Bel-7402
HepG2
SK-Hep1
MHCC-97H
F
Percentage survival
TBP high expression n = 184
TBP low expression n = 185
P = 0.016
Time (months)
G
Percentage survival
TBP high expression n = 91
TBP low expression n = 274
P < 0.0001
Time (days)

Figure 1. TPB is highly expressed in HCC. A. UALCAN database was used to analyze the difference of TBP expression in HCC tissue samples and adjacent liver tissues. B, C. UALCAN database was used to analyze the relationship between TBP expression and tumor grade and lymph node metastasis in patients with HCC. D. The expressions of TBP in 30 HCC tissues and normal tissues were detected by immunohistochemistry. Scale bar = 100 μm. E. qRT-PCR was used to detect the expression of TBP mRNA in normal hepatocytes and HCC cell lines. F, G. UALCAN database and StarBase database were used to analyze the relationship between the expression of TBP and the overall survival of patients. ***P < 0.001.

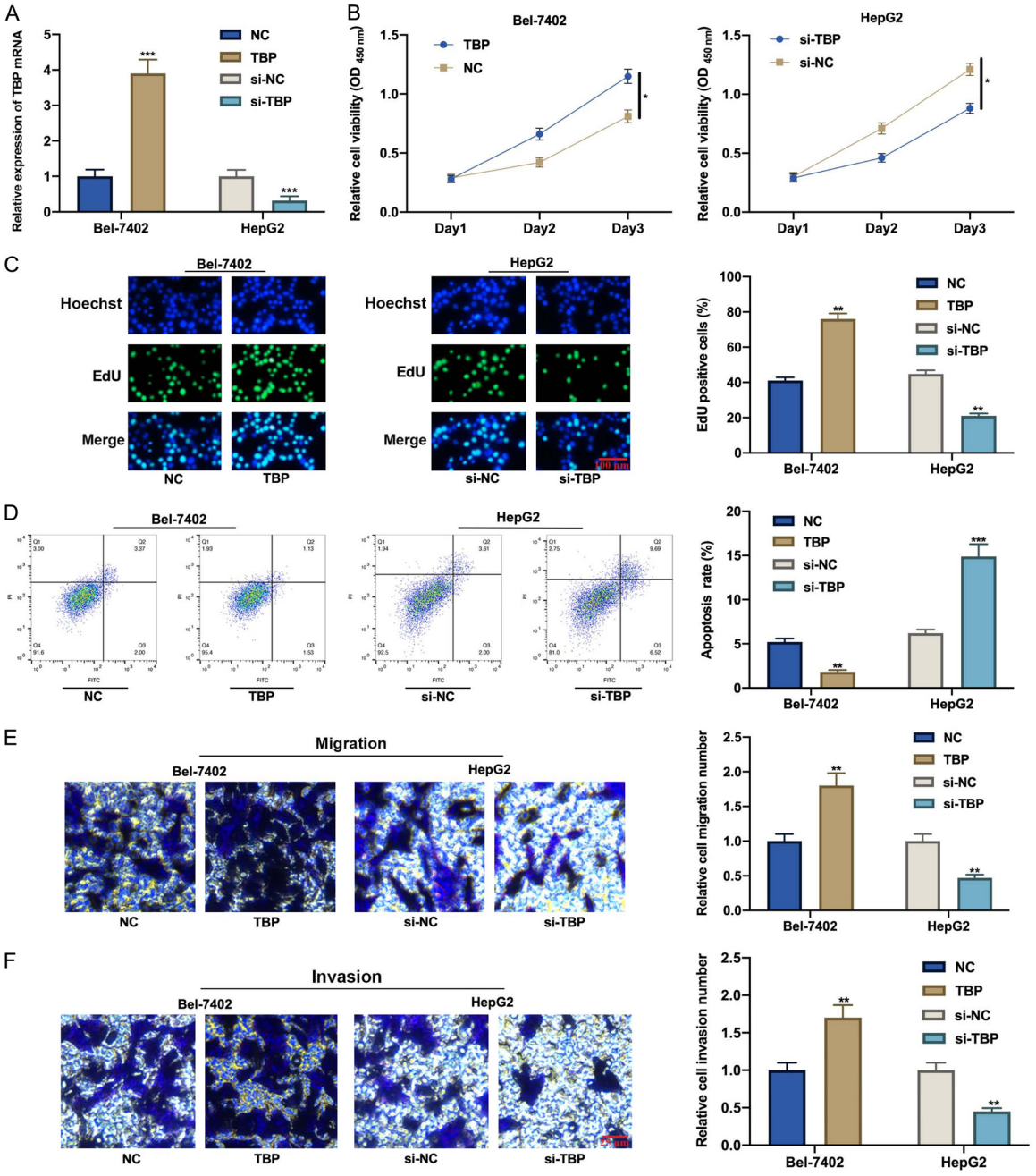
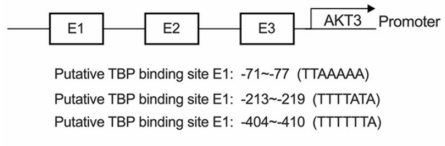
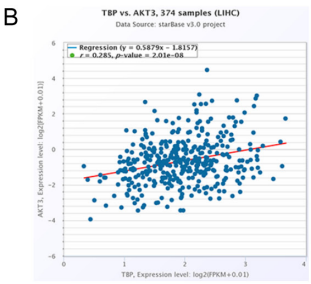


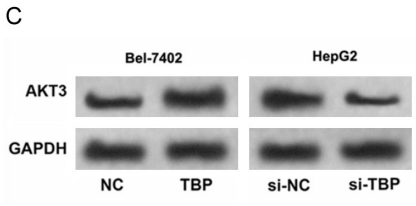
Figure 2. Effects of TBP on the proliferation, migration, invasion and apoptosis of HCC cells. A. The expression of TBP mRNA after transfection of Bel-7402 or HepG2 cells with TBP overexpression plasmid or si-TBP was detected by qRT-PCR. B, C. After transfection, the proliferation of HCC cells was assessed by CCK-8 and EdU experiments. Scale bar = 100 μm. D. After transfection, the apoptosis of HCC cells was assessed by flow cytometry. E, F. After transfection, migration and invasion abilities of HCC cells were assessed by transwell assay. Scale bar = 25 μm. *P < 0.05, **P < 0.01, and ***P < 0.001.

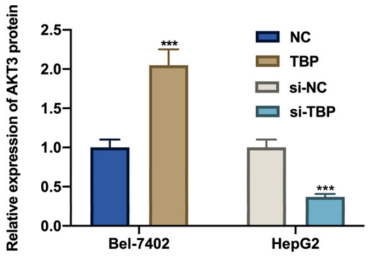
A

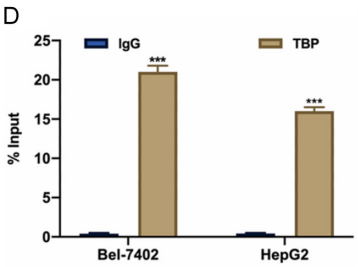
Name	Dissimilarity	RE equally	RE query	Start	End	Suquence
TBP	0.00%	1.15356	0.59124	71	77	TTAAAAA
TBP	0.00%	1.15356	0.59124	213	219	TTTTATA
TBP	0.00%	1.15356	0.59124	404	410	TTTTTTA

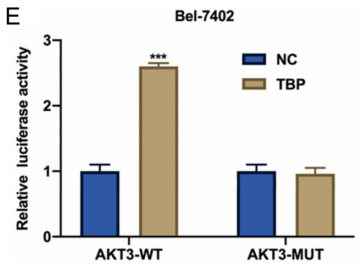












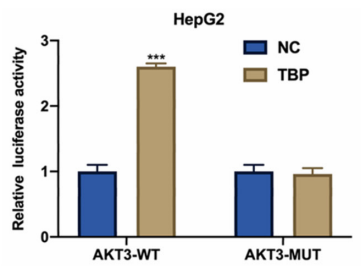


Figure 3. TBP interacts with AKT3 promoter in HCC cells. A. The site at which TBP binds to the AKT3 promoter region was predicted by PROMO databases. B. The correlation between TBP expression and AKT3 expression in HCC tissue samples was analyzed by the data of StarBase database. C. Western blot was used to detect the effect of overexpression or knockdown of TBP on the expression of AKT3 in HCC cells. D. The binding affinity of TBP and AKT3 promoter region was detected by a ChIP-qPCR assay. E. The effect of TBP overexpression on AKT3-WT and AKT3-MUT luciferase activities was validated by a dual luciferase reporter gene assay. ***$P < 0.001$.

pression promoted the expression of AKT3; its knockdown functioned oppositely (**Figure 3C**). ChIP-qPCR assay showed that TBP was significantly enriched in the promoter region of AKT3 (v.s. IgG control group) (**Figure 3D**). Additionally, the dual-luciferase reporter gene assay showed, overexpression of TBP markedly increased the luciferase activity of the AKT3 WT reporter, but not that of the MUT reporter, in both Bel-7402 and HepG2 cells (**Figure 3E**). Collectively, these data suggested that the transcription of AKT3 was modulated by TBP.

TBP exerts its biological function in HCC cells via modulating AKT3

To further confirm whether TBP promoted the progression of HCC by regulating AKT3, we co-transfected TBP overexpression plasmid with si-AKT3 into Bel-7402 cells; si-TBP and AKT3 overexpression plasmids were transfected into HepG2 cells (**Figure 4A**). *In vitro* assays showed that TBP overexpression facilitated the malignant biological behaviors of HCC cells, while knocking down AKT3 weakened this effect; the malignancy of HCC cells was repressed after TBP knockdown, which was partly reversed by restoration of AKT3 (**Figure 4B-F**).

TBP/AKT3 axis may modulate the activation of mTOR pathway in HCC cells

Next, to explore the downstream mechanism of AKT3 in HCC progression, LinkedOmics database was applied for gene set enrichment analysis (GSEA), and it was revealed that high AKT3 expression level was positively associated with the activation of ECM-receptor interaction, focal adhesion, and P13K/AKT signaling pathways (**Figure 5A**). Western blot showed that, overexpression of TBP promoted of the expression of mTOR, p-mTOR, and reduced the activation of LC3 and PTEN, while knockdown of AKT3 weakened this effect. Down-regulation of TBP caused a decrease in mTOR, p-mTOR expression, and increase in LC3 activation and PTEN expression, while up-regulation of AKT3 functioned oppositely (**Figure 5B**).

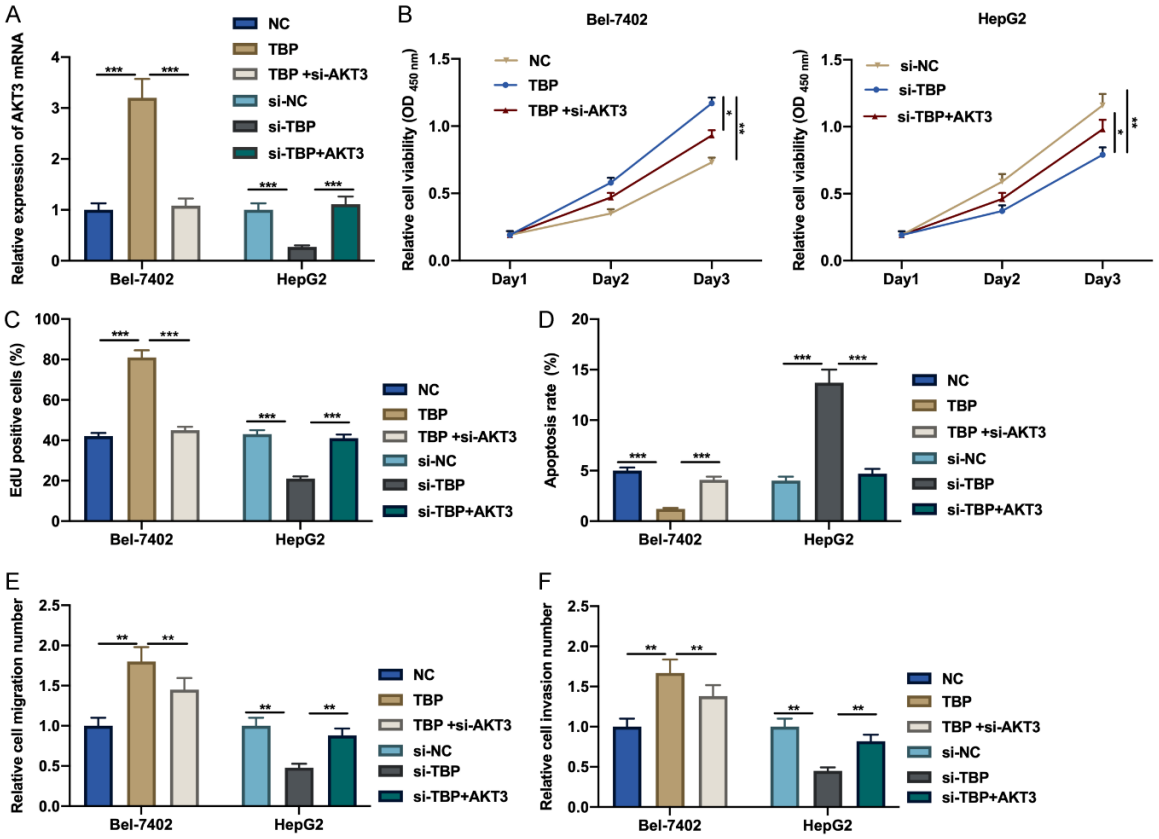


Figure 4. TBP participates in regulating HCC cell proliferation, migration, invasion and apoptosis by modulating AKT3. A. TBP overexpression plasmid and si-AKT3 were co-transfected into Bel-7402 cells, and si-TBP and AKT3 overexpression plasmid were co-transfected into HepG2 cells, and subsequently AKT3 mRNA expression was detected by qRT-PCR. B, C. After transfection, HCC cell proliferation was assessed by CCK-8 assay and EdU assay. D. After transfection, the apoptosis was assessed by flow cytometry. E, F. After transfection, cell migration and invasion abilities were evaluated by transwell experiments. $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

Discussion

At present, the incidence of HCC worldwide is gradually increasing [22]. Unfortunately, most patients with HCC have unsatisfactory clinical outcomes, due to the high risk of recurrence, and the natural properties of treatment resistance of HCC cells [23]. Increasing number of TFs are reported to be involved in HCC progression, and they modulate disease progression via regulating gene expression, and indirectly modulating protein degradation, protein/protein interaction, et al, and have shed new light on the exploration of new cancer targets [6]. Here we found that TBP, a TF, was highly expressed in HCC, and its abnormal expression was associated with some adverse clinical features and shorter survival time of the patients.

TBP is recruited to gene promoters via directly binding to DNA or protein-protein interactions, and thus the active transcription initiation complexes are formed [24-26]. Previous studies report that, TBP dysregulation is associated with the pathogenesis of a variety of neurodegenerative diseases and certain cancers. It regulates tumor microenvironment via modulating hormone pathway and NF-κB signaling [27, 28]. In Huntington disease cell model, the expression of miR-146a is inhibited, and miR-146a is associated with the pathogenesis of HD by targeting TBP [29, 30]; as a signal mediator or receptor from the enhancer-binding transcriptional activator protein, TBP can directly bind to hepatitis B X-interacting protein (HBxIP) and activate the transcription of oncogenic lncRNA Lin28B in breast cancer cells [31]; increased TBP expression leads to colorectal cancer progression via promoting the transcription of VEGF [14]. However, in HCC, the role of TBP deserves further investigation. Here we found that TBP expression in HCC tissues and cells was significantly increased, and its abnormal expression was significantly correlated

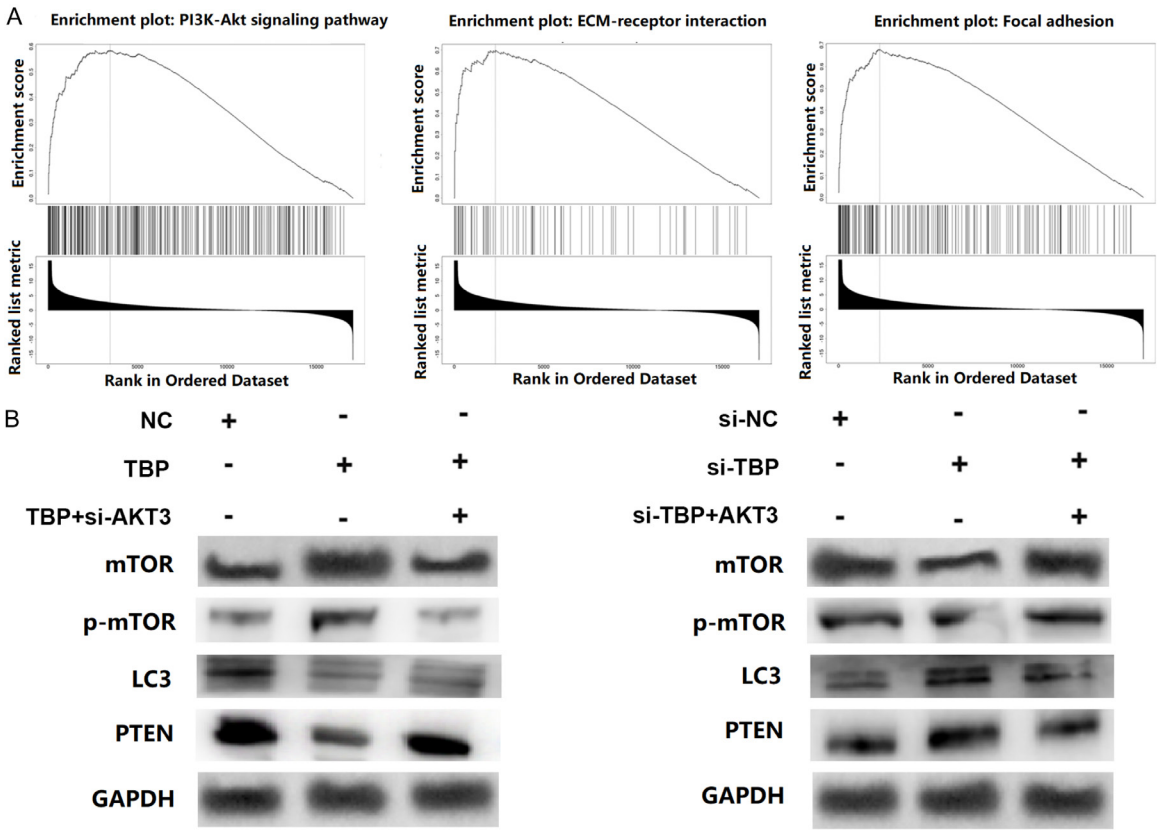


Figure 5. AKT3 activates ECM-receptor interaction, focal adhesion, and AKT/mTOR pathways. A. GSEA was used for predicting the pathway enriched by AKT3. B. TBP overexpression plasmid and si-AKT3 were co-transfected into Bel-7402 cells, and si-TBP and AKT3 overexpression plasmid were co-transfected into HepG2 cells. Subsequently, Western blot was used to detect the expression of mTOR, p-mTOR, LC3 and PTEN.

with the poor prognosis of HCC patients, and knocking down TBP inhibited the malignancy of HCC cells. Based on these findings, we conclude that TBP may play a cancer-promoting role in HCC.

Akt protein kinase (also known as protein kinase B) plays a crucial role in multiple physiological and pathological processes of human cells such as apoptosis, proliferation, differentiation, metabolism, aging and tumorigenesis [32]. AKT3, as one of its subtypes, participates in the pathogenesis of various diseases [33-36]. For instance, AKT3 is up-regulated in non-small cell lung cancer (NSCLC), and circWHSC1 can regulate miR-296-3p/AKT3 axis [36]. AKT3 is highly expressed in endometrial carcinoma, and LINC01224 promotes the expression of AKT3 via decoying miR-485-5, thereby driving the progression of endometrial carcinoma [34]. Importantly, the role of AKT3 in HCC has also been emphasized. For example, LINC00680 enhances the stemness of HCC cells and increases chemoresistance of HCC cells via decoying miR-568 to up-regulate AKT3 [14]; another work reports that miR-424 targets the Akt3/E2F3 axis and inactivates pRb-E2F signaling pathway, and thus the growth of HCC cells is suppressed [37]. Here we found that the transcription of AKT3 could be activated after TBP's binding to the AKT3 promoter region. In addition, AKT3 depletion counteracted the biological effects of TBP in HCC cells, which suggests that TBP is an oncogenic protein in HCC, and its role is at least partially mediated by AKT3.

Interestingly, our data indicated that TBP/AKT3 axis could probably modulate PI3K/Akt/mTOR pathway, ECM-receptor interaction and focal adhesion. These preliminary data imply that, besides the malignant biological behaviors investigated in the present work, TBP/AKT3 may also contributes to HCC progression via modulating autophagy (factually we observed that TBP overexpression contributes to inacti-

vation of LC3), extracellular remodeling, epithelial-mesenchymal transition, et al. These topics are interesting, and require further investigation. Additionally, TBP's role as a diagnostic biomarker remains to be further evaluated with a larger cohort of patients with complete follow-up data. Last but not least, animal models are helpful for further verifying the property of TBP as an oncoprotein in HCC.

In conclusion, TBP is highly expressed in HCC tissues and cell lines, and shows promising value as a biomarker. Functionally and mechanistically, TBP activates the transcription of AKT3, thereby promoting malignant progression of HCC cells. The present work may provide insights for HCC diagnosis and treatment.

Disclosure of conflict of interest

None.

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