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RESEARCH

General Transcription Factor IIB Overexpression and a Potential Link to Proliferation in Human Hepatocellular Carcinoma

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Abstract The general transcription factor IIB (TFIIB) plays a central role in preinitiation complex (PIC) assembly, providing a bridge between promoter-bound TFIID and RNA Polymerase II (RNA POLII). TFIIB functionally counteracts the transcriptional activation of hepatitis B virus X protein (HBx), which has been shown to play a role in the development of human hepatocellular carcinoma (HCC). However, the function of TFIIB in HCC remains unclear. In this article, we demonstrate that TFIIB plays an important role in HCC pathogenesis. TFIIB expression was immunohistochemically examined in a series of 100 HCC tissue specimens. The expression level of TFIIB showed significant correlation with the histological grade (P=0.030), the level of AFP (P=0.011) and the proliferation marker Ki-67 (P=0.0002). High TFIIB expression level correlated with poor survival. Western blot analysis also confirmed that the TFIIB protein was overexpressed in HCC tissue compared to benign normal tissue. Additionally, Western blot and qRT-PCR analyses showed a high expression level of TFIIB protein in the HCC cell lines SMMC7721, HepG2, BEL7404, and Huh7 and the immortalized normal line BEL7702 but a lower expression in the normal Chang hepatocyte cell line. Following the release of Huh7 cells from serum starvation, the expression of TFIIB was upregulated. A cell growth assay suggested that TFIIB was involved in the proliferation and growth of HCC cells. In conclusion, our results demonstrate that TFIIB overexpression may play essential roles in the pathogenesis of hepatocellular carcinoma by affecting the proliferation of HCC cells.

Keywords Human hepatocellular carcinoma (HCC) · General transcription factor IIB (TFIIB) · Cell proliferation · Pathogenesis

Introduction

Hepatocellular carcinoma (HCC) is one of the five most common cancers worldwide and mainly occurs in patients with chronic liver disease, such as those with hepatitis B and C infections [1]. Its lethal nature currently accounts for approximately 610,000 deaths a year worldwide [2]. Despite advances in therapy for HCC and modern surgical innovations, overall, patient outcome has not substantially improved [3]. A number of studies have suggested the molecular mechanisms involved in hepatocarcinogenesis [4–7], and understanding the molecular mechanism of HCC development and progression is imperative in establishing novel, effective, and targeted therapies for this highly aggressive cancer.

TFIIB is involved in RNA polymerase II (RNAPII) recruitment and transcription initiation in eukaryotes [8]. TFIIB stabilizes the binding of TATA-binding protein (TBP) to the TATA-box and provides additional sequence

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specificity through contacts with the B-recognition elements (BREs) flanking the TATA box on either side [9]. The complex then recruits RNAPII and other general transcription factors (GTFs). Once the preinitiation complex (PIC) is assembled, DNA at the promoter is melted, an open complex is formed and transcription is initiated [10]. Recent research has revealed that the phosphorylation of TFIIB is a critical event in transcription that links the gene promoter and terminator, and triggers initiation by RNA pol II [11]. The C-terminal of TFIIB serves as a TBP-binding and promoter-recognition domain; the N-terminal domain, which contains a Zn-ribbon accompanied with a linker domain, is required for binding to RNAPII [12, 13].

Although the structure and transcription initiation function of TFIIB has been studied widely and extensively, no studies have linked TFIIB to oncogenesis. However, several discoveries suggest a potential role for TFIIB in this process. In vivo, TFIIB is a target of HBx; a C-terminal domain mutant of TFIIB inhibits transcriptional coactivation by HBx [14]. Additionally, HBx and RNAPII subunit RPB5-mediating protein (RMP) are competitively associated with TFIIB [15]. Recent research has also revealed that RMP may play essential roles in HCC growth by affecting the proliferation and apoptosis of HCC cells [16]. All of these studies suggest that TFIIB may also play a role in the pathogenesis of HCC. Moreover, in rats with traumatic brain injury (TBI), TFIIB expression gradually increased as the proliferative capacity increased [17]. We therefore performed a detailed experimental analysis to investigate whether TFIIB has a novel role in hepatocarcinogenesis.

Materials and Methods

Patients and Tissue Samples

One hundred human HCC tissue specimens were collected from patients who underwent surgical treatment without postoperative systemic chemotherapy at the Surgery Department, Nantong Tumor Hospital, China, between 2003 and 2006. The diagnosis was histologically confirmed in all of the cases, based mainly on the examination of sections stained with H&E. The patient ages ranged from 21 to 75 years, with an average age of 48.6 years. The male: female ratio was 4:1. A total of 79 patients were positive for the HBV surface antigen, and 81 patients were positive for cirrhosis. The histological grades were classified as well-differentiated tumors (grade I; n=51), moderately differentiated cancers (grade II; n=43), and poorly differentiated tumors (grade III; n=6). The follow-up time for the 100 patients ranged from 1 to 192 weeks from the date of surgery (mean=43.18 weeks). The main clinicopathological variables of the patients are shown in Table 1. Informed consent was obtained from all of the patients.

The tissue specimens were immediately processed after surgical removal. For histological examination, all tumorous and surrounding non-tumorous tissue samples were processed as 10 % buffered formalin-fixed, paraffin-embedded blocks. Protein was analyzed in 11 snap-frozen tumorous and adjacent non-tumorous tissue samples that were stored at −80 °C.

Immunohistochemistry

Human HCC tissue microarrays were obtained from Nantong Tumor Hospital of China. The sections were deparaffinized using a graded ethanol series, and endogenous peroxidase activity was blocked by soaking in 0.3 % hydrogen peroxide. Then, the sections were processed in 10 mmol/L citrate buffer (pH 6.0) and heated to 121 °C in an autoclave for 20 min to

Table 1 TFIIB and Ki-67 expression and clinicopathological parameters in 100 HCC specimens

Parameters	Total	TFIIB		P^{α}	Ki67		P^{α}
		Low ≤0.55	High >0.55		Low ≤0.71	High >0.71	
Age (year)							
≤48	48	25	23	0.118	15	33	0.358
>48	52	19	33		12	40	
Gender							
Male	80	32	48	0.107	19	61	0.143
Female	20	12	8		8	12	
Histological stage							
Stage I	51	29	22	0.03	17	34	0.038
Stage II	43	13	30		9	34	
Stage III	6	2	4		1	5	
Metastasis							
Positive	16	7	9	0.982	2	14	0.154
Negative	84	37	47		25	59	
Tumor size (cm)							
≤4.8	59	24	35	0.422	14	45	0.377
>4.8	41	20	21		13	28	
HBsAg							
Positive	79	35	44	0.906	22	57	0.711
Negative	21	9	12		5	16	
Cirrhosis							
Positive	81	35	46	0.742	18	63	0.026
Negative	19	9	10		9	10	
AFP (ng/ml)							
≤50	39	11	28	0.011	6	33	0.036
>50	61	33	28		21	40	

Statistical analyses were performed by the Pearson χ^2 test. P<0.05 was considered significant

retrieve the antigen. After rinsing in PBS (pH 7.2), PBS containing 10 % goat serum was added for 1 h at room temperature to block any nonspecific reactions. The sections were then incubated overnight at 4 °C with anti-TFIIB rabbit polyclonal antibody (diluted 1:50; Novus Biologicals) and anti-Ki-67 mouse monoclonal antibody (diluted 1:100; clone 7B11; Zymed Laboratories, San Francisco, California, USA). Negative-control slides were also processed in parallel using a nonspecific immunoglobulin IgG (Sigma Chemical Company, St. Louis, Missouri, USA) at the same concentration as the primary antibody. All of the slides were processed using the peroxidase/anti-peroxidase method (Dako, Hamburg, Germany). After rinsing in PBS, the peroxidase reaction was visualized by incubating the sections with diaminobenzidine tetrahydrochloride in 0.05 mol/L Tris buffer (pH 7.6) containing 0.03 % H_2O_2. After rinsing in water, the sections were counterstained with hematoxylin, dehydrated, and coverslipped.

Immunohistochemical Evaluation

Two observers (A.X.Z. and J.C.) independently evaluated the immunostaining results, and similar results were obtained for all of these samples. For the assessment of TFIIB and Ki-67, five high-power fields were randomly selected for each specimen, and nuclear and cytoplasmic staining was examined under high-power magnification. More than 500 cells were counted to determine the labeling index, which represented the percentage of immunostained cells relative to the total number of cells.

Cell Culture and Cell Cycle Analysis

All of the cell lines were obtained from Fudan University, China. The SMMC7221, HepG2, BEL7404 and Huh7 cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin in 5 % CO_2 at 37 °C. BEL7702 and Chang cells were cultured in RPMI-1640 medium supplemented with 10 % FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin in 5 % CO_2 at 37 °C. For cell cycle analysis, the cells were fixed with 70 % ethanol for 1 h at 4 °C and then incubated with 1 mg/mL RNase A for 200 min at 37 °C. Subsequently, the cells were stained with propidium iodide (50 μg/mL PI) (Becton Dickinson, San Jose, CA) in PBS containing 0.5 % Tween-20 and analyzed using a Becton Dickinson flow cytometer BD FACScan (San Jose, CA) and Cell Quest acquisition and analysis programs.

Western Blot Analysis

Tissue and cell proteins were promptly homogenized in a homogenization buffer containing 1 M Tris–HCl, pH 7.5, 1 % Triton X-100, 1 % NP-40 (nonidet p-40), 10 % sodium dodecyl sulfate (SDS), 0.5 % sodium deoxycholate, 0.5 M EDTA, 10 μg/mL leupeptin, 10 μg/mL aprotinin, and 1 mM PMSF and then centrifuged at 10,000 g for 30 min to collect the supernatant. The protein concentrations were determined with a Bio-Rad protein assay (Bio-Rad, Hercules, CA, USA). The supernatant was diluted in 2× SDS loading buffer and boiled. The proteins were separated with SDS polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidine difluoride filter (PVDF) membranes (Millipore, Bedford, MA). The membranes were blocked with 5 % dried skim milk in TBST (20 mM Tris, 150 mM NaCl, and 0.05%Tween-20). After 2 h at room temperature, the membranes were incubated overnight with polyclonal primary antibodies. The antibodies used were as follows: anti-TFIIB (1:1,000; Novus Biologicals), anti-PCNA (1:1,000; Santa Cruz), and anti-β-actin (1:2,000; Sigma). Then, horseradish peroxidase-conjugated IgG was used as the secondary antibody. The immunoreactive bands were visualized through chemiluminescence (NEN Life Science Products, Boston, MA). After the chemiluminescence was exposed to X-ray films, the films were scanned using a Molecular Dynamics densitometer (Imaging Technology, Ontario, Canada). The values are representative of at least three independent experiments.

Statistical Analysis

Statistical analysis was performed using the Stata View 7.0 software package. The association between Ki-67 and TFIIB expression and clinicopathological features was analyzed using the χ^2 test. Ki-67 and TFIIB expression in human hepatocellular carcinoma (HCC) was analyzed using the Spearman rank correlation test because the data were not normally distributed. The survival curves were calculated using the Kaplan–Meier method, and the log-rank test was used for analysis. $P<0.05$ was considered statistically significant.

Transient Transfection Experiments

A total of 2×10^5 Chang cells were seeded into each well of a 24-well plate. After incubation for 24 h, these cells were transfected with Lipofectamaine™2000 (Invitrogen, Shanghai, China) according to the manufacturer's protocol. The cells were transfected with the plasmid pEGFP-N3-TFIIB for TFIIB overexpression. The vector alone (pEGFP-N3) was used as the negative control.

PCR

Total RNA was extracted from the cells using Trizol reagent as described by the manufacturer (Invitrogen). Briefly,

500 ng of total RNA was reverse-transcribed into cDNA using the ThermoScript RT system (Fermentas, Ontario, Canada). The following primers were used: TFIIB: forward, 5′-AAGAATTCCAATGGCGTCTACCAGCCG-3′, and reverse, 5′-AAGGATCCTAGCTGTGGTAGTTTGTCCAC-3′; and GAPDH: forward, 5′-TGATGACATCAAGAAGGTGGTGAAG-3′, and reverse, 5′-TCCTTGGAGGCCATGTGGGCCAT-3′. PCR amplification was performed with an initial denaturing step at 94 °C for 5 min; 30 cycles at 94 °C for 45 s, 58 °C for 45 s and 72 °C for 45 s; and a further extension at 72 °C for 10 min. The PCR products were electrophoresed through a 1 % agarose gel and visualized with ethidium bromide staining. The relative differences in the expression between groups were normalized with GAPDH

Cell Growth Assay

Chang cells were plated in 96-well plates and maintained in DMEM before being transiently transfected with various recombinant plasmids. At five time points after transfection, the cell viability was determined with a commercial Cell Counting Kit 8 (CCK-8, Dojindo, Japan). A total of 10 μl of CCK-8 reagent was added to each well and incubated at 37 °C for 2 h until the media turned yellow. The absorbance was measured at 450 nm in a spectrophotometer. Each experiment was performed in triplicate and repeated at least three times.

Immunofluorescence

The cells were plated onto coverslips the day before synchronization. After synchronization, they were fixed with 3 % paraformaldehyde and permeabilized with 0.1 % Triton X-100 for 1 h. Nonspecific binding was blocked with 1 % BSA for 30 min. After overnight incubation with primary antibodies against rabbit anti-TFIIB, the coverslips were rinsed 3 times in PBS and incubated for 1 h with goat anti-rabbit IgG-fluorescein isothiocyanate (Santa Cruz, CA, USA) in the dark. The nuclei were counterstained with DAPI (Santa Cruz, CA, USA). Images were acquired using a Leica confocal microscope. Digital images of the fluorescent antibody-stained cells were acquired with software provided by Leica.

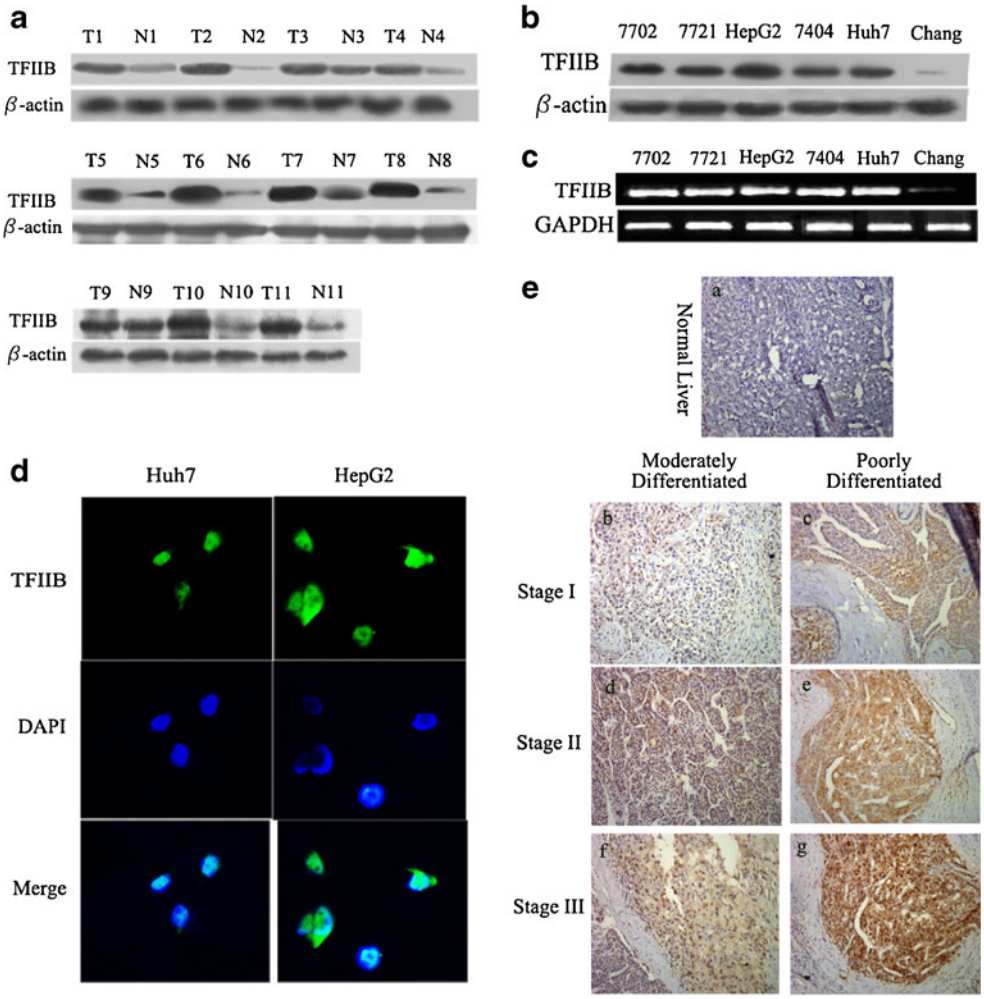

Fig. 1 TFIIB is overexpressed in HCC. **a** Western blot analysis of whole-cell protein extracts prepared from eleven paired HCC (T) and non-tumor liver (N) specimens. β-actin was used as a control for protein load and integrity. **b** TFIIB expression was detected with Western blot in the indicated cells. **c** The relative TFIIB transcript level was detected in these cells with RT-PCR. GAPDH was used as an internal control. **d** Immunofluorescence staining of TFIIB was performed in Huh7 and HepG2 cells. The nuclei were counterstained with DAPI. Original magnification, ×400. **e** The analysis of TFIIB expression in a tissue microarray with immunohistochemistry. Original magnification, ×200

Results

Elevated TFIIB Expression in HCC Tissue and Cell Lines

The expression of TFIIB was studied with immunoblotting in eleven paired normal human liver and HCC biopsy samples. TFIIB was dramatically increased in tumors as compared with the adjacent normal tissues (Fig. 1a). We next screened baseline TFIIB expression levels in the human HCC cells SMMC7221, HepG2, BEL7404, and Huh7, the immortalized normal line BEL7702 and normal Chang hepatocytes. In the HCC cell lines and BEL7702, both the mRNA (>1.5 fold) and protein expression levels of TFIIB were increased compared to the normal Change liver cell line (Fig. 1b and c). In agreement with previous report [17], TFIIB expression was predominantly detected in the nucleus of the HCC cells Huh7 and HepG2 (Fig. 1d).

To characterize the TFIIB expression patterns, an immunohistological analysis was performed in tissue microarrays containing 100 primary HCC and 9 normal adjacent liver samples that were immunostained using the anti-TFIIB antibody. Little to no TFIIB immunostaining was detected in the 9 normal liver samples, whereas significant TFIIB staining was observed in HCC samples (Fig. 1e). The expression of TFIIB gradually increased with the tumor stages, from I to III, and the grades of differentiation, from well-differentiated to poorly differentiated (e.g., compare Fig. 1e *b*, *e*, and *c* with *f*, *d* and *g*). To investigate the correlation of the frequency of TFIIB-positive cells in tumor tissue with clinicopathological features, the mean number of TFIIB-positive cells in the tumor tissue in 100 HCC cases was calculated. Based on whether the percentage of TFIIB-positive cells was above the mean level (55 %) in

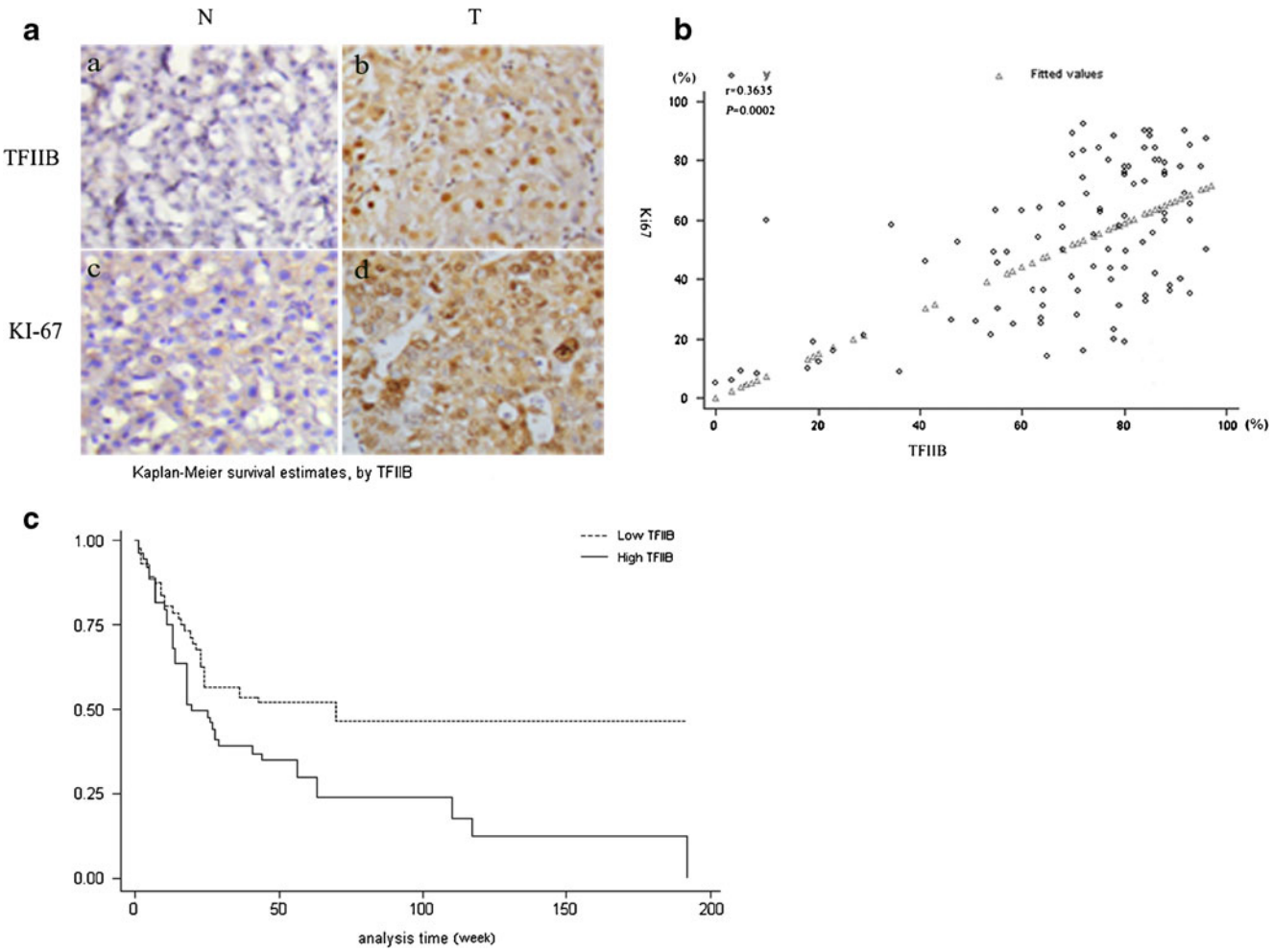

Fig. 2 TFIIB overexpression correlates with a proliferation marker and the poor prognosis of HCC patients. **a** Immunohistochemical staining of TFIIB (*a*, *b*) and Ki-67 (*c*, *d*) in adjacent normal tissues (N) and HCC tissues (T). Original magnification, ×400. **b** The relationship between the Ki-67 proliferation index and TFIIB expression in HCC. **c** Kaplan-Meier survival curves for low TFIIB expression versus high TFIIB expression in 100 patients of HCC (P=0.010, log-rank test)

tumor tissue, the HCC cases were divided into two groups: the TFIIB-high group (above the mean level, n=56) and the TFIIB-low group (below the mean level, n=44). The frequency of TFIIB-positive cells in tumor tissues was not significantly associated with cirrhosis, age, gender, metastasis or tumor size, but it was significantly correlated with the histological grade (P=0.030) and the level of AFP (P=0.011) (Table 1). In univariate analysis, the Kaplan-Meier survival curves showed that high TFIIB expression was significantly related to poor survival (Fig. 2c). Collectively, these data suggest that TFIIB plays a potential role in hepatocarcinogenesis.

The Expression of TFIIB is Associated with the Proliferation Marker Ki-67

TFIIB has been reported to be involved in proliferation in the central nervous system during TBI [17]. Therefore, we performed immunohistochemical staining of the same cohort of HCC tissues and adjacent normal tissues to evaluate the expression of TFIIB and the proliferation marker Ki-67. Representative examples of reactivity for TFIIB and Ki-67 are shown in Fig. 2a. There was no or low TFIIB and Ki-67 expression in adjacent normal tissue (Figs. 2a *a*, *c*). Therefore, we focused on the TFIIB expression in HCC. TFIIB expression in HCC was scored as positive when staining was strong in the nuclei (Fig. 2a *b*). The LI of TFIIB ranged from 0.5 % to 94.83 %. The mean percentage of positive cells was 55.81 %. Ki-67 expression in HCC was scored as positive when staining was strong in the nuclei (Fig. 2a *d*). The LI of Ki-67 ranged from 0.15 % to 97.02 %. The mean percentage of positive cells was 71.79 %. In most specimens, the proportion of TFIIB -positive tumor cells was similar to the proportion of Ki-67-positive tumor cells. A positive correlation was found between TFIIB expression and Ki-67-based proliferative activity (P=0.0002) (Fig. 2b).

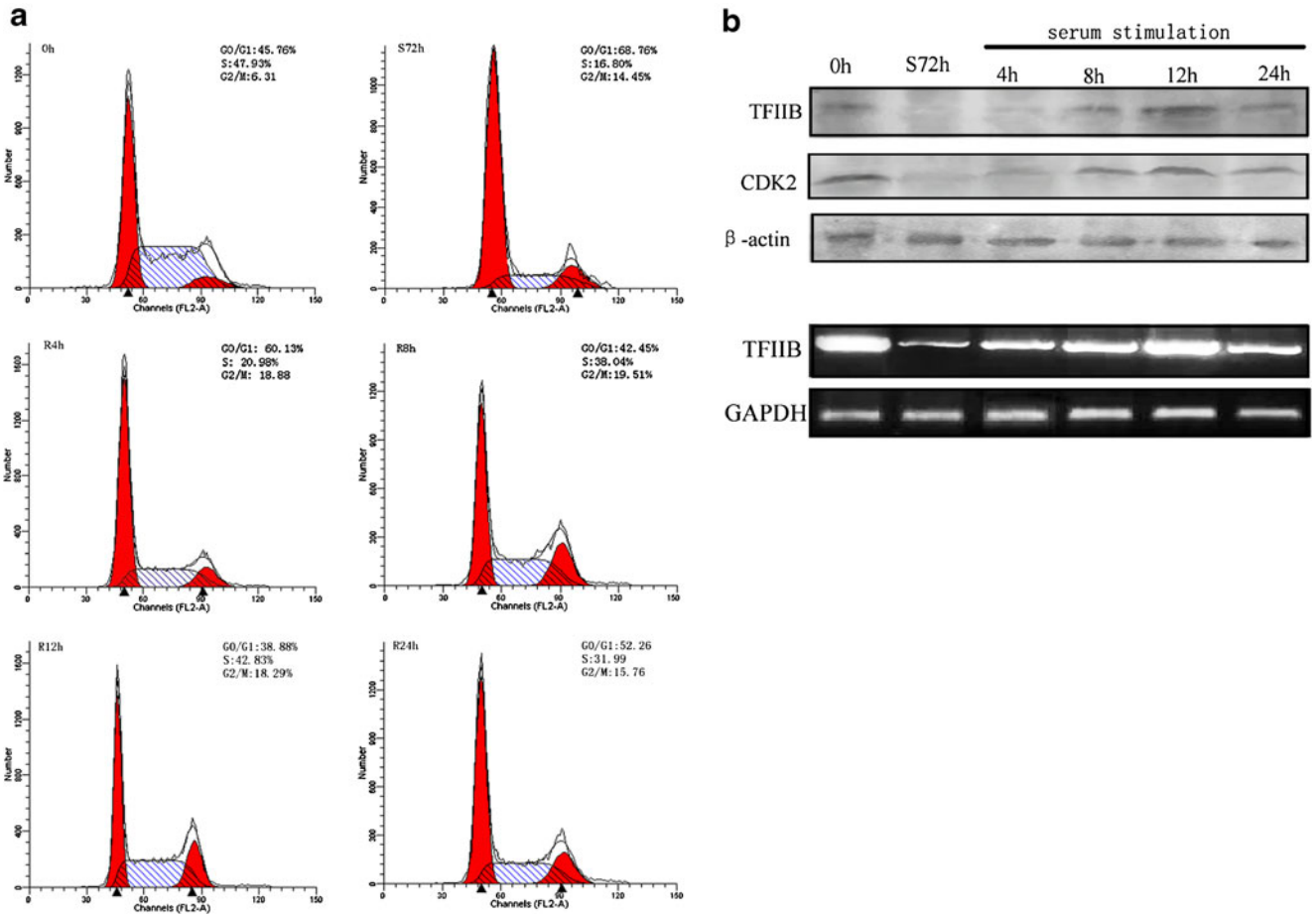

Fig. 3 TFIIB expression parallels the G1/S phase transition during the cell cycle. **a** Flow cytometry quantification of cell cycle progression in Huh7 cells: normal cells, cells that were serum starved for 72 h, and cells stimulated with medium containing 10 % FBS for the indicated time points. **b** Western blot and RT-PCR analysis showing the TFIIB and CDK2 in the normal, starved and serum stimulated Huh7 cells. β-actin and GAPDH were used as internal controls. Original magnification, ×400

TFIIB Expression is Elevated in Proliferating HCC Cells

Based on our results, we further detected the expression of TFIIB during cell cycle progression in HCC cells. Huh7 cells were arrested in G_1 phase through serum deprivation for 72 h, and the percentage of cells in G_1 phase was increased from 45.76 % to 68.76 %. Then, the G_1-arrested Huh7 cells were released through serum stimulation and harvested every 4 hours for 24 h. Upon serum addition, the cells reentered S phase (Fig. 3a). The Western blotting results showed that the G_1/S phase transition during 8–12 h coincided with the peak of TFIIB and CDK2 expression (Fig. 3b). The mRNA levels of TFIIB showed the same kinetics. Taken together, TFIIB was upregulated during HCC cell proliferation progression.

The Overexpression of TFIIB Promoted Hepatocellular Growth

To confirm that TFIIB induces cell proliferation, the TFIIB gene was transiently transfected into the normal Chang liver cell line (Fig. 4a), in which the endogenous expression of TFIIB is low. TFIIB expression was detected with Western blotting following transient transfections with different doses of the plasmid, and we found that 4 μg was the best transfection dose (Fig. 4b). Remarkably high cell viability was observed in the cells expressing TFIIB 36–48 h after transfection, whereas lower viability was observed in the cells that were not transfected or transfected with vector alone (Fig. 4c). Additionally, PCNA protein expression was also increased in cells transfected with TFIIB (Fig. 4d). Collectively, these data strongly demonstrate that TFIIB upregulates hepatocellular proliferation.

Discussion

HCC is the third most common cause of cancer-related death and is closely associated with chronic hepatitis virus infection. Although radiological liver imaging in combination with serum AFP has made it possible to diagnose HCC during the

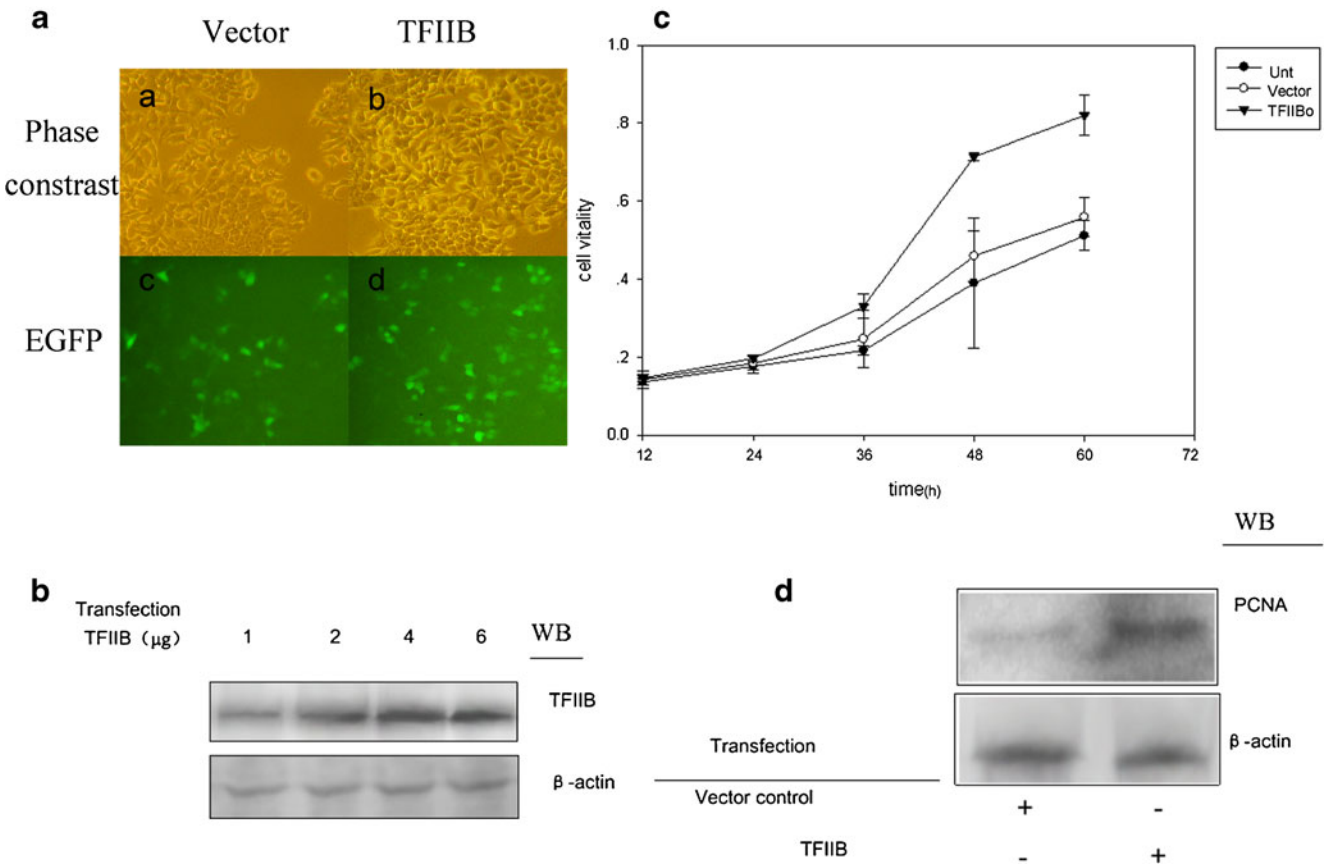

Fig. 4 The overexpression of TFIIB increases the proliferation of normal Chang liver cells. **a** Phase-contrast and fluorescent microscopy examination of Chang hepatocytes transiently transfected with TFIIB or vector alone at day 2 post-infection. **b** Western blot analysis of TFIIB expression in Chang hepatocytes transfected with different doses of pEGFP-N_3-TFIIB. β-actin was used as a control for protein load and integrity. **c** Chang cells were transiently transfected with pEGFP-N_3-TFIIB (TFIIBo) or vector alone pEGFP-N_3 (Vector). Untransfected Chang cells (Unt) were used as controls. The cell viability of these cells was measured with a commercial Cell Counting Kit at 5 time points after transfection. The results are reported as the means and standard deviation of three independent experiments. **d** Western blot analysis of PCNA expression in the Chang cells transfected with TFIIB. β-actin was used as a control for protein load and integrity

subclinical stage, the prognosis of HCC remains unsatisfactory, and the 5-year survival rate after surgery is limited to 25–39 % [18, 19]. Therefore, elucidating the mechanisms underlying the development of HCC and seeking new therapy targets are important.

TFIIB is one of the GTFs that are involved in the recognition of promoter sequences, the response to regulatory factors, and conformational changes essential to the activity of RNA polymerase during transcription [20]. TFIIB plays a central role in PIC assembly, providing a bridge between promoter-bound TFIID and RNAPII, and directly modulates the catalytic center of RNAPII [8]. Another general transcription factor, TFIID, has been proven to be upregulated in lung and breast carcinoma and is associated with poor differentiation and high mitotic activity [21]. Recently, more GTFs and RNAPII subunit proteins, such as RMP, have been reported to functionally counteract the transcriptional activation by HBx and be involved in viral oncogenesis or DNA repair in addition to transcription initiation [16, 22, 23]. TFIIB is a transactivation target of HBx, which competes with RMP for TFIIB [15]. Although much is known about the structure and transcription initiation function of TFIIB, the potential role of TFIIB in carcinogenesis has not been studied.

In this study, we used Western blot analysis to identify the expression of TFIIB in HCC both in vivo and in vitro. TFIIB expression was significantly increased in tumors compared with the adjacent normal tissues. The TFIIB transcript levels in HCC cells were also higher than normal liver cells. Immunohistological analysis verified the different TFIIB expression levels in the samples of HCC and benign normal tissue. In a series of 100 HCC tissues, high TFIIB expression was significantly correlated with the histological grade (P=0.030), the levels of AFP (P=0.011) and Ki-67 (P=0.0002) and poor survival (P=0.010). These findings suggested that aberrant TFIIB expression could arise in certain pathological states and may be associated with proliferation in HCC.

Further study confirmed the association of TFIIB with cell proliferation. We detected the expression of TFIIB during cell cycle progression in HCC cells and found that the protein expression and mRNA levels of TFIIB were upregulated during the transition from G1 to S phase. Consistent with a previous report, immunofluorescence and immunohistology analyses showed that TFIIB was mainly located in the nuclei of tumor cells (Figs. 1d and 2a). Furthermore, a cell growth assay demonstrated that the overexpression of TFIIB may promote cell proliferation. In addition, Western blotting indicated that PCNA expression was upregulated in TFIIB-overexpressed cells.

Taken together, our findings add a new function to the general transcription factor TFIIB and suggest that TFIIB is increased in HCC and may play a potential role in HCC cell proliferation. These data may add a new dimension to the mechanism of hepatocarcinogenesis because TFIIB is a target of many transcriptional regulatory factors. However, further studies are necessary to elucidate the proliferation pathway involving TFIIB in HCC pathogenesis.

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References

1. Feng H, Cheng AS, Tsang DP et al (2011) Cell cycle-related kinase is a direct androgen receptor-regulated gene that drives β-catenin/T cell factor-dependent hepatocarcinogenesis. J Clin Invest 121:3159–3175
2. Hsieh A, Kim HS, Lim SO et al (2011) Hepatitis B viral X protein interacts with tumor suppressor adenomatous polyposis coli to activate Wnt/β-catenin signaling. Cancer Lett 300:162–172
3. Lau WY (2000) Primary liver tumors. Semin Surg Oncol 19:135–144
4. Llovet JM, Bruix J (2008) Molecular targeted therapies in hepatocellular carcinoma. Hepatology 48:1312–1327
5. El-Serag HB, Rudolph KL (2007) Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 132:2557–2576
6. Ueda S, Basaki Y, Yoshie M et al (2006) PTEN/Akt signaling through epidermal growth factor receptor is prerequisite for angiogenesis by hepatocellular carcinoma cells that is susceptible to inhibition by gefitinib. Cancer Res 66:5346–5353
7. Baek HJ, Lim SC, Kitisin K et al (2008) Hepatocellular cancer arises from loss of transforming growth factor beta signaling adaptor protein embryonic liver fodrin through abnormal angiogenesis. Hepatology 48:1128–1137
8. Deng WS, Roberts SGE (2007) TFIIB and the regulation of transcription by RNA polymerase II. Chromosoma 116:417–429
9. Albert TK, Grote K, Boeing S et al (2010) Basal core promoters control the equilibrium between negative cofactor 2 and preinitiation complexes in human cells. Genome Biol 11:R33
10. Sevilimedu A, Shi H, Lis JT (2008) TFIIB aptamers inhibit transcription by perturbing PIC formation at distinct stages. Nucleic Acids Res 36:3118–3127
11. Wang YM, Fairley JA, Roberts SGE (2010) Phosphorylation of TFIIB links transcription initiation and termination. Curr Biol 20:548–553
12. Liu X, Bushnell DA, Wang D et al (2010) Structure of an RNA polymerase II–TFIIB complex and the transcription initiation mechanism. Science 327:206–209
13. Simone C, Robert OJ (2010) The linker domain of basal transcription factor TFIIB controls distinct recruitment and transcription stimulation functions. Nucleic Acids Res 39:464–474
14. Haviv I, Shamay M, Doitsh G et al (1998) Hepatitis B virus pX targets TFIIB in transcription coactivation. Mol Cell Biol 18:1562–1569
15. Yang Y, Zheng L, Chen Y (2000) Study of HBV X protein and RMP, an RPB5 mediate protein competitively interacting with

general transcription factor TF2B. Zhonghua Gan Zang Bing Za Zhi 8:15–17
16. Yang H, Gu J, Zheng Q et al (2011) RPB5-mediating protein is required for the proliferation of hepatocellular carcinoma cells. J Biol Chem 286:11865–11874
17. Liu Z, Wang D, Shao B et al (2011) Increased expression of transcription initiation factor IIB after rat traumatic brain injury. J Mol Histol 42:265–271
18. Lau WY, Lai EC (2008) Hepatocellular carcinoma: current management and recent advances. Hepatobiliary Pancreat 7:237–257
19. Li X, Pan Y, Fan R et al (2008) Adenovirus-delivered CIAPIN1 small interfering RNA inhibits HCC growth in vitro and in vivo. Carcinogenesis 29:1587–1593
20. Hahn S (2004) Structure and mechanism of the RNA polymerase II transcription machinery. Nat Struct Mol Biol 11:394–403
21. Wada C, Kasai K, Kameya T et al (1992) A general transcription initiation factor, human transcription factor IID, overexpressed in human lung and breast carcinoma and rapidly induced with serum stimulation. Cancer Res 52:307–313
22. Wei W, Gu JX, Zhu CQ et al (2003) Interaction with general transcription factor IIF (TFIIF) is required for the suppression of activated transcription by RPB5-mediating protein (RMP). Cell Res 13:111–120
23. Qadri I, Fatima K, AbdeL-Hafiz H (2011) Hepatitis B virus X protein impedes the DNA repair via its association with transcription factor, TFIIH. BMC Microbiol 11:48