RETRACTED ARTICLE: Expression tendency and prognostic value of TCF21 in hepatocellular carcinoma

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ABSTRACT

Background: Transcription factor 21 (TCF21) is identified as a tumor suppressor in a variety of human tumors. The purpose of the study was to examine its expression tendency and prognostic value in hepatocellular carcinoma (HCC).

Methods: Relative expression of TCF21 mRNA in tissue samples from HCC patients and healthy volunteers were detected through quantitative real-time polymerase chain reaction (qRT-PCR) while its protein level was examined via immunohistochemistry analysis. Chi-square test was adopted to assess the association of TCF21 expression with the clinicopathological characteristic of the patients. Then Kaplan–Meier analysis was employed to analyze the function of TCF21 expression on overall survival among HCC patients.

Results: Both the mRNA and protein levels of TCF21 were significantly reduced in HCC tissue samples compared with healthy controls (p < .05). Also, its expression was obviously affected by the classification of tissue pathology, metastasis, T stage, N stage and pathological grading. According to Kaplan–Meier analysis, patients with higher expression of TCF21 experienced dramatically longer overall survival time than those with lower expression (log rank test, p < .001).

Conclusions: TCF21 expression was decreased in HCC patients and it could act as a prognostic marker.

Abbreviations: TCF21: Transcription factor 21; HCC: hepatocellular carcinoma; qRT-PCR: quantitative real-time polymerase chain reaction; bHLH: basic helix-loop-helix; CT: cycle threshold; DAB: 3,3-diaminobenzidine; PBS: phosphate-buffered; SD: standard deviation; HR: hazard ratio; CI: confidence interval

Introduction

Hepatocellular carcinoma (HCC), a most crucial primary liver cancer, is the sixth most frequent cancer worldwide and the second leading cause of cancer mortality [1]. Increasing researches have focused on molecular mechanisms of HCC, including activation of oncogenic pathways, abrogation of cell-cycle checkpoints and Notch [2–4]. Although surgical resection acts as the most effective therapeutic method for treating HCC, high-recurrence rate and early distant metastasis still lead to poor prognosis [5]. Thus, it is necessary to search for a useful and promising marker for predicting clinical outcome of HCC.

Transcription factor 21 (TCF21), located on chromosome 6q23–q24, is a number of the basic helix-loop-helix (bHLH) TF family and during embryogenesis, is critical for the development of numerous cell types for the heart, lung, kidney, and spleen [6–9]. It encodes a cell type specific class II basic helix-loop-helix transcription factor and controls cell differentiation fate [10]. Expression level of TCF21 is proved to be highest in embryonic development but rapidly decreased in postnatal tissues [11]. It also functions as a tumor suppressor and is deregulated in several cancers [12–14]. Tan et al. found that TCF21 over-expression inhibited the growth of HCC cell lines [15]. However, the prognostic performance of TCF21 has never been reported in HCC.

In our research, we mainly detected TCF21 expression levels and their correlation with clinicopathological features in HCC patients. Besides, the clinical significance of TCF21 in HCC prognosis was also estimated.

Methods and materials

Ethics statement

The research was permitted by the Research Ethics Committee of Chinese PLA General Hospital-Sixth Medical Center. Each participant signed written informed consent prior to sampling.

Patients and tissue samples

One hundred eighty nine patients who were diagnosed with HCC and underwent surgery at the Chinese PLA General Hospital-Sixth Medical Center were selected for this study, including 45 females and 144 males. Their age ranged from 20 to 70 years. Meanwhile, 83 healthy volunteers with matched age, gender, and residential area to the cases were
taken as healthy controls, who had no cancer history. The diagnoses for the patients were confirmed by at least two cancer experts. Tumor stage was confirmed by two pathologists separately following the Seventh American Joint Committee on Cancer guidelines. We completed a follow-up lasting for at least 5 years. Exclusion criteria were as follows: dying from unexpected incidents or other diseases.

Tumor tissues from all patients and healthy tissues from healthy volunteers were extracted and immediately frozen in liquid nitrogen, respectively. Then all specimens were maintained at −80 °C until use.

**RNA isolation and quantitative real-time PCR (qRT-PCR)**

Total RNA was isolated from all samples using Trizol reagent (Invitrogen, Carlsbad, California, America) based on the protocol from the manufacturer and reverse transcription was conducted to synthesize the first chain of cDNA with an Expand Reverse Transcriptase Kit (Takara, Japan). RT-PCR reaction was performed on the Applied Biosystems 7900 Fast Real-Time PCR system (Applied Biosystems, Foster City, California, USA). Relative quantitative expression of TCF21 mRNA was determined with the comparative cycle threshold (CT) approach. qRT-PCR primer sequences for TCF21 were designed: sense-5′-'CATTTACCCAGTCAACCTGA-3′ and antisense-5′-'CCACTTCCCTCGGTTACGTCTC-3′. A system of 20 μl was used for RT-qPCR reaction and the procedure was in line with the description in previous studies [14]. Each sample was in triplicate.

**Immunohistochemistry analysis**

HCC tissues and adjacent normal tissues were fixed, dehydrated, and embedded in paraffin. Then, the tissue sections were dewaxed in 100% xylene and rehydrated through a grade series of ethanol solutions (100, 90, 80, 70% ethanol) and water with standard agreements. Then, the sections received antigen retrieval in citrate buffer (10 mM) for 2 min at 100 °C. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol. Afterward, 10% normal goat serum was applied to decrease nonspecific staining. After washing with phosphate-buffered saline (PBS), the sections were incubated with biotinylated rabbit anti-goat secondary antibody for 10 min at 37 °C, followed by cultivation with streptavidin-horseradish peroxidase complex (Maixin Inc., Fuzhou, China), and colorized with 3, 3-diaminobenzidine (DAB) chromogen solution. These sections were counterstained with hematoxylin, mounted in neutral gum, and analyzed using a bright field microscope. Positive expression of TCF21 was defined as brown staining in the cytoplasm, determined independently by two experienced pathologists. The intensity of TCF21 staining was scored from 0 to 3: 0, no staining; 1, weakly positive staining; 2, moderately staining to modest granular staining; and 3, strongly diffuse and homogeneous positive staining. Positive expression of TCF21 presented the scores were higher 4 while the others were a negative expression. For statistical purposes, specimens with a sum score between 0 and 3 were considered to display low TCF21 expression, while others belonged to high TCF21 expression.

**Statistical analysis**

All computations were conducted with SPSS software system for Windows (version 19.0; SPSS Inc., Chicago, IL, USA). Each experiment was repeated at least three times and all continuous data were summarized and reported as mean ± standard deviation (SD). Difference between the two groups was compared with students’ t-test. The association of TCF21 expression level with clinical pathological features was estimated via the chi-square test. Survival curves were compared using Kaplan–Meier survival method. Univariate and multivariate cox proportional hazard model were used to analyze potential role to influence factors in HCC prognosis. The difference was considered statistical significance when p values were less than .05.

**Results**

**TCF21 expression in HCC patients and healthy volunteers**

To investigate the function of TCF21 expression on HCC, qRT-PCR assay was performed. As indicated in Figure 1, in comparison to healthy tissues, TCF21 mRNA expression was obviously reduced in HCC tissues (p < .001).

Meanwhile, the same tendency for TCF21 protein was also observed. In HCC tissue sections, TCF21 expression was relatively weak and the percentage of the cells with positive staining was only 38.98% (68/189). Yet, the TCF21 expression level in adjacent normal tissues was high and the proportion for the cells with positive staining was as high as 78.84% (149/189). The difference was significant between HCC tissues and adjacent normal tissues (p < .001, Figure 2).

![Figure 1](image-url)
Association between TCF21 expression and clinicopathological factors in patients with HCC

To explore whether TCF21 was involved in HCC progression, the association between its expression and clinicopathological characteristics was analyzed via $\chi^2$ test. As a result, the expression of TCF21 was distinctly related to the classification of tissue pathology ($p = .032$), metastasis ($p = .004$), T-stage ($p = .03$), N-stage ($p = .017$) and pathological grading ($p = .039$) (Table 1). However, there was no significant association between TCF21 expression and other variables, such as gender, age, location and tumor size.

Prognostic value of TCF21 in HCC

To explore the prognostic value of TCF21 in HCC, a 5-years follow-up was completed. According to the Kaplan–Meier survival method, a positive association was discovered between TCF21 expression and overall survival time (Figure 3, log rank test, $p < .001$). Then, univariate and multivariate cox regression analyses were performed to investigate prognostic roles of clinical parameters and TCF21 expression in HCC. As shown in Table 2, both univariate and multivariate analysis manifested that low expression of TCF21 (HR = 2.365, 95% CI = 1.458–8.837, $p = .000$) was an independent factor for the clinical outcome of HCC.

Discussion

HCC represents approximately 6% of total new malignancy cases all over the world and a majority of primary liver cancer, annually seeing more than 5 million new cases globally [16–18]. Even worse, clinical outcomes of HCC cases are frequently dismal [19]. Accurate prognostic biomarkers are meaningful for predicting HCC prognosis, which may improve the management of the disease. In our study, we investigated the prognostic values of TCF21 in HCC. Analysis results demonstrated that the expression of TCF21 was decreased in HCC cases at both protein and mRNA levels. Moreover, the down-regulation of TCF21 predicted aggressive progression and low-survival rate in this malignancy. TCF21 might be a potential prognostic biomarker for HCC.

Recently, in order to improve the prognosis of HCC, many researchers have been devoted to explore biological markers for cancer. For instance, Yu et al. found that the combination of miR-224 and pAKT expressions can be an indicator predicting the prognosis of HCC [20]. According to Yu et al., kidney-type glutaminase was up-regulated in HCC and its expression showed high value in the diagnosis and prognosis of these diseases [21]. HOTAIR exhibited over-expression in HCC and was associated with cell migration and invasion, but its prognostic value was unsatisfactory [22,23]. Despite numerous proposed biomarkers, prognosis evaluation for HCC remains...
a great challenge in the clinic. Also, it is necessary to find out novel and specific biomarkers for this malignancy.

In our research, we examined the prognostic significance of TCF21 in HCC. TCF21 was down-regulated in HCC tissues compared to healthy controls. Furthermore, TCF21 down-regulation showed a tight correlation with the classification of tissue pathology, metastasis, T-stage, N-stage and pathological grading. All the data revealed that TCF21 might act as a tumor suppressor in HCC and its expression deficiency contributed to the malignancy progression. The study carried out by Tan et al. reported that enforced expression of TCF21 could reduce the proliferation and migration of HCC cells and enhance apoptosis in vitro [15]. Dai et al. revealed that TCF21 might inhibit tumor progression in colorectal cancer through suppressing PI3K/AKT signaling pathway [24]. Besides, two published studies have demonstrated that the down-regulation of TCF21 could inactivate metastasis suppressor KISS1, thus leading to cancer metastasis [25,26].

The findings revealed that TCF21 might regulate the biological behaviors of HCC cells through multiple signaling pathways.

Accumulated studies have verified that TCF21 might be employed as a biomarker for prognosis evaluation in several cancers [25,27,28]. Given the association of TCG21 with cancer progression, we speculated that TCF21 might be useful in prognosis estimation for HCC. To confirm the hypothesis, Kaplan–Meier method and Cox regression analysis were performed. We found that HCC patients with high TCF21 expression had prolonged overall survival time. According to Cox regression analysis, low expression of TCF21 was confirmed to be an independent biomarker for poor prognosis of HCC patients. The present study, possibly for the first time, explored the prognostic value of TCF21 in HCC.

Several limitations in our study should be stated. First, the relatively small sample size might reduce the statistical power of our results. Second, cell experiments and animal models...
were urgently needed to explore the molecular mechanisms of TCF21 affecting HCC. Therefore, further investigations are required to explore related molecular mechanisms.

**Conclusion**

In summary, TCF21 expression level is significantly decreased in HCC and shows a negative association with aggressive progression of the disease. TCF21 may be a predictive biomarker for HCC prognosis.

**Disclosure statement**

The authors declare that they do not have any conflicts of interest.

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