Connection Between EIF3S3 Polymorphisms and Hepatocellular Carcinoma Prognosis

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Abstract

Background: *EIF3S3* that encodes a p40 subunit of eukaryotic initiation factor 3 (eIF3), has been reported overexpression in several human malignancies. The expression of *EIF3S3* in hepatocellular carcinoma (HCC) and its clinical role in the disease progression is still unclear. Here we aimed to study the effect of *EIF3S3* on HCC prognosis.

Methods: Quantitative real-time polymerase chain-reaction (qRT-PCR) was performed to assess the mRNA levels of *EIF3S3* in 120 HCC tissues samples and 60 adjacent noncancerous specimens. Kaplan-Meier and Cox regression analysis was used to study the prognostic value of *EIF3S3*.

Results: The expression of *EIF3S3* was significantly increased in HCC compared to the expression found in adjacent tissues (P<0.001). Furthermore, high *EIF3S3* expression was positively correlated with vascular invasion, tumor size, cirrhosis, AFP levels and TNM stage (all P<0.05). Kaplan-Meier survival analysis showed that patients with high *EIF3S3* expression had worse overall survival (OS) (P<0.001) and disease-free survival (DFS) (P<0.001) than those with low *EIF3S3* expression. In addition, multivariable analysis revealed that *EIF3S3* could be an independent prognostic factor of OS (P<0.001) and DFS (P<0.001) for patients with HCC.

Conclusion: The results suggested that *EIF3S3* might serve as a promising prognostic factor for OS and DFS of HCC patients.

Background

Hepatocellular carcinoma (HCC) is the fifth most common human cancer and the third most common cause of cancer-related death worldwide [1, 2], due to its high invasive and metastatic features. As the results of the prevalence of hepatitis B and C viral infections, the morbidity and mortality of HCC is more likely discovered in China [3]. Actually, most HCC patients have lost the best treatment opportunity when they were diagnosed for the first time, because of the absence of the specific symptoms in the early stage. Despite improvements in the treatment of HCC such as surgical techniques, radiotherapy, chemotherapy, drug intervention and molecular targeted therapy, the prognosis of HCC still remains poor with a 5-year survival rate of 11% [4, 5]. Moreover, the recurrence and metastasis are the main factors for the frustrating outcome of HCC [6]. Therefore, identification of new potential biomarkers improving the outcome of patients with HCC is urgent.

EIF3 is the largest of eukaryotic initiation factors (*eIF*) with a molecular mass of 800 kDa, which is composed of 13 subunits (*EIF3a* to *eIF3m*) and plays an essential role in cellular and viral initiation of translation [7-9]. *EIF3S3* encodes for a p40 subunit of *eIF3*, which is the largest initiation factor of the protein synthesis. *EIF3S3* was identified by Kittler et al., who screened over 5000 genes for the necessity on Hela cell division and found 37 genes that were required for cell division, and two of them were *eIF3* subunits, *EIF3S3* and *EIF3S10* [10-12]. Amplification and over expression of. *EIF3S3* have been observed in prostate and breast cancers [13, 14], and its amplification has been associated with advanced stages

of prostate cancer [15]. These findings suggested that *EIF3S3* has been involved in cancer development. However, to date, there have been few reports on the role of *EIF3S3* in patients with HCC, and the clinical significance of *EIF3S3* in the progression of HCC is also unclear.

Therefore, the the present study was designed to investigate whether the expression level of *EIF3S3* could be used as prognostic biomarker for HCC patients.

Methods

Patients and samples

A total of 120 fresh tumor tissue samples and 60 randomly selected adjacent noncancerous tissue samples were obtained from HCC patients who underwent curative resection at Chinese PLA General Hospital-Sixth Medical Center. HCC was confirmed by pathological examination and none of these patients had received any radiotherapy or chemotherapy prior to surgical treatment. The stages of tumor were classified according to the TNM staging system of UICC. All clinicopathological characteristics of 120 patients were collected and summarized in **Table 1**. A 5-year follow-up was performed in all patients by telephone or questionnaires. The study was approved by the Research Ethics Committee of Chinese PLA General Hospital-Sixth Medical Center, and written informed consents were provided by all participants in the study.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA from snap-frozen samples were extracted using TRIzol (Invitrogen, USA) reagent according to the manufacturer's protocol. Then, the concentration of isolated RNA was measured by the NanoDrop ND-1000 spectrophometer (NanoDrop Technologies, USA). Complementary DNA (cDNA) was synthesized from total RNA using Superscript Reverse Transcriptase (Invitrogen) following the manufacturer's directions. Afterwards, the cDNA was subjected to real-time PCR that was carried out using an Applied Biosystems 7500 real-time PCR system. For standardization of RNA quality control, the *GAPDH* was used as an internal control. The primer sequences were as follows: *EIF3S3* sense 5'-AAGGAGTTCACTGCCCAAAA-3' and antisense 5'-AAGAGTTGCCCTGGTGTGAC-3'; *GAPDH* sense 5'-TGACTTCAACAGCGACACCCA-3' and 5'-CACCCTGTTGCTGTAGCCAAA-3'. Each sample was examined in triplicate.

Statistical analysis

The statistical analyses were performed using SPSS statistics software (Version 18.0, Chicago) and Prism 5.0 software (GraphPad, La Jolla, CA, USA). The correlation of *EIF3S3* expression with clinicopathological features was assessed by Chi-square test, and quantitative data were analyzed by Student's t-test. Survival rate was constructed by the KaplanMeier with logrank test, and the Cox regression model was used for univariate and multivariate survival analysis. All data in this study are presented as mean±standard deviation (SD). $P<0.05$ was considered statistically significant.

Results

The expression of *EIF3S3* is upregulated in HCC

To investigate the role of *EIF3S3* in HCC, we explored the *EIF3S3* mRNA levels in 120 HCC and 60 adjacent non-cancerous tissues samples using qRT-PCR. As shown in **Figure 1**, the mRNA expression of *EIF3S3* was significantly increased in HCC tissues in comparison to adjacent nontumor tissues (mean±SD: 0.61 ± 0.56 vs 0.22 ± 0.37, $P<0.05$). The results suggested that *EIF3S3* might serve as an oncogene in HCC.

Correlation of *EIF3S3* expression with clinicopathological features of HCC patients

To further verify this observation that *EIF3S3* might play a role in the progression of HCC, the patients were divided into two groups according to *EIF3S3* expression (high and low) as defined by the mean. Associations between *EIF3S3* expression and the clinicopathological parameters in HCC were shown in **Table 1**. The expression of *EIF3S3* was found to correlate closely with vascular invasion ($P=0.007$), tumor size ($P=0.033$), cirrhosis ($P=0.007$), AFP levels ($P=0.012$) and TNM stage ($P=0.001$). However, no statistical correlations were discovered between *EIF3S3* expression and age ($P=0.841$), sex ($P=0.869$), and differentiation ($P=0.570$).

Relationship between *EIF3S3* expression and survival

Next, Kaplan-Meier curve analysis was applied to evaluate the relationship between *EIF3S3* expression and survival of patients with HCC. The log-rank test revealed that patients with high *EIF3S3* expression had a significantly shower overall survival (OS) when compared with those of low *EIF3S3* expression ($P<0.001$, **Figure 2A**). Likewise, the disease free survival (DFS) time of HCC patients with high *EIF3S3* expression was markedly shorter than those with low expression of *EIF3S3* ($P<0.001$; **Figure 2B**).

Furthermore, univariate analysis of prognostic factors revealed that the following variables including vascular invasion, cirrhosis, AFP level, TNM stage and *EIF3S3* expression correlated significantly with OS (all $P<0.05$; **Table 2**). Multivariate analysis indicated that *EIF3S3* expression was an independent prognostic factor for OS (HR=4.810, 95% CI 2.233-10.361; $P<0.001$), along with tumor size, cirrhosis, AFP level and differentiation (all $P<0.05$). In addition, univariate analysis of prognostic factors showed that *EIF3S3* expression, cirrhosis, AFP level and TNM stage had significant prognostic influences on DFS (all $P<0.05$; **Table 3**). Moreover, multivariate survival analysis revealed that *EIF3S3* expression and cirrhosis were independent factors that affected the DFS (both $P<0.05$).

Discussion

Because of the high incidence of recurrence and metastasis after hepatic resection, the long-term survival of HCC patients still remains poor. Currently, despite the BCLC staging system and CLIP score of patients with HCC is critical for the guidance of therapy selection, HCC patients with the same stage are diverse

[16, 17]. Early diagnosis of HCC is difficult due to its high-grade malignancy and tumor invasion [4]. Nowadays, AFP has been used as a diagnostic and prognostic marker for HCC [18], moreover, gene therapy and molecular targeted therapy are used in clinical research and various genes for targeted therapy of HCC are effective than the traditional forms of treatments [19]. Therefore, it is important to find effective molecular biomarkers with high sensitivity and specificity for HCC treatment and prognosis.

EIF3 is the largest initiation factor of the protein synthesis. Originally, *eIF3* was identified as a factor that binds to the 40S ribosomal subunit and thereby prevents the association of the 40 and 60S subunits with one another. *eIF3* complex has been the essential for initiation of protein synthesis for both cells and virus [20]. *EIF3S3* located at 8q23, encodes the p40 subunit of *eIF3*. Previous studies have indicated that *EIF3S3* gene was abundantly expressed in several cancers. For example, Quti Saramaki et al. showed that high level amplification of *EIF3S3* was found in prostate tumors and it was associated with poor cancer-specific survival of the carcinoma [21]. Moreover, the effect of *EIF3S3* on cell proliferation was also reported. In the study of Savinainen et al., they found that *EIF3S3* overexpression regulated cell growth and viability and the overexpression of the gene may provide growth advantage to the cancer cells [22]. What's more, a study carried out by Okamoto et al. revealed that *EIF3S3* was amplified in HCCs, and the expression of *EIF3S3* was significantly associated with large tumor size,and hepatitis B virus infection. They concluded that *EIF3S3* encodes the p40 subunit of the *eIF3* and may be involved in the progression of HCC [23]. However, the clinical role of *EIF3S3* in human HCC prognosis was unclear.

In the present study, the expression of *EIF3S3* was significantly upregulated in HCC tissues compared with in adjacent noncancerous liver tissues. Moreover, correlation analysis between *EIF3S3* expression and clinical parameters of HCC patients revealed that high expression of *EIF3S3* were significantly related to vascular invasion, tumor size, cirrhosis, AFP levels and TNM stage, indicating that *EIF3S3* expression may be responsible for tumor progression in HCC. These results were consistent with the previous study [23]. Moreover, the data suggested that *EIF3S3* presented an unfavorable outcome in HCC, and might be a potential prognostic marker. Our data revealed that HCC patients with high *EIF3S3* expression had a significantly shorter OS and DFS than those with low expression. In addition, multivariate analysis identified *EIF3S3* as an independent prognostic factor for HCC patients. However, the molecular mechanisms of *EIF3S3* in HCC that high expression of *EIF3S3* results in poor outcome still remain unclear.

Until now, little is known about the actual role of *EIF3S3* in carcinogenesis as well as the mechanisms *EIF3S3* on the regulation of cancer. Dysregulated translation of the gene may be involved because several other initiation factors such as eIF4E, eIF4G, and eIF5A2 are also amplified and over expressed in various types of cancers [24-26]. Therefore, the specific mechanism by which *EIF3S3* overexpression could improve the growth of HCC cells still remains to be elucidated.

Conclusion

In summary, the present study suggest that increased expression of *EIF3S3* could be involved in the progression of HCC and might be a novel biomarker of poor prognosis for patients with HCC.

Abbreviations

Eukaryotic initiation factor 3 (eIF3)

Hepatocellular carcinoma (HCC)

Quantitative real-time polymerase chain-reaction (qRT-PCR)

Overall survival (OS)

Disease-free survival (DFS)

Complementary DNA (cDNA)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Chinese PLA General Hospital-Sixth Medical Center and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials All data generated or analysed during this study are included in this published article.

Competing interests The authors declare that they have no competing interests.

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Authors' contributions C.Y. design of the work; P.D. the acquisition, analysis, W.L. interpretation of data; P.D. the creation of new software used in the work; C.Y. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Tables

Table 1. Correlation between *EIF3S3* expression and clinicopathologic characteristics of HCC patients.

Characteristic	Number	*EIF3S3* expression		*P*
	N=120	High	Low	
Age				0.841
≥50 years	62	32	30	
<50 years	58	31	27	
Sex				0.869
Male	62	33	29	
Female	58	30	28	
Vascular invasion				0.007
No	66	42	24	
Yes	54	21	33	
Tumor size, cm				0.033
<5 cm	70	31	39	
≥5 cm	50	32	18	
Cirrhosis				0.007
Presence	70	44	26	
Absence	50	19	31	
AFP (ng/ml)				0.012
≤20	47	18	29	
>20	73	45	28	
Differentiation				0.570
Well-moderate	79	40	39	
Poor-undifferentiated	41	23	18	
TNM stage				0.001
I-II	61	23	38	
III-IV	59	40	19	

Table 2. Univariate and multivariate analysis of variables associated with OS in patients with HCC

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
EIF3S3 expression	4.725 (2.300-9.708)	<0.001	4.810 (2.233-10.361)	<0.001
Age	1.449 (0.777-2.703)	0.244	-	-
Sex	1.009 (0.539-1.887)	0.978	-	-
Vascular invasion	2.058 (1.082-3.913)	0.028	-	-
Tumor size	1.189 (0.566-2.496)	0.648	2.477 (1.118-5.487)	0.025
Cirrhosis	3.708 (1.830-7.512)	<0.001	6.720 (2.939-15.365)	<0.001
AFP (ng/ml)	2.308 (1.192-4.466)	0.013	2.204 (1.103-4.402)	0.025
Differentiation	1.729 (0.926-3.228)	0.086	2.119 (1.113-4.036)	0.022
TNM stage	2.420 (1.254-4.672)	0.008	-	-

Table 3. Univariate and multivariate analysis of variables associated with DFS in patients with HCC

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
EIF3S3 expression	4.530 (2.179-9.421)	<0.001	4.582 (2.135-9.835)	<0.001
Age	1.420 (0.761-2.650)	0.270	-	-
Sex	1.047 (0.560-1.1.959)	0.885	-	-
Vascular invasion	1.852 (0.972-3528)	0.061	-	-
Tumor size	1.099 (0.522-2.314)	0.804	-	-
Cirrhosis	3.275 (1.617-6.633)	0.001	3.286 (1.557-6.6932)	0.002
AFP (ng/ml)	2.141 (1.108-4.137)	0.024	-	-
Differentiation	1.698 (0.908-3.177)	0.098	-	-
TNM stage	2.037 (1.058-3.923)	0.033	-	-

Figures

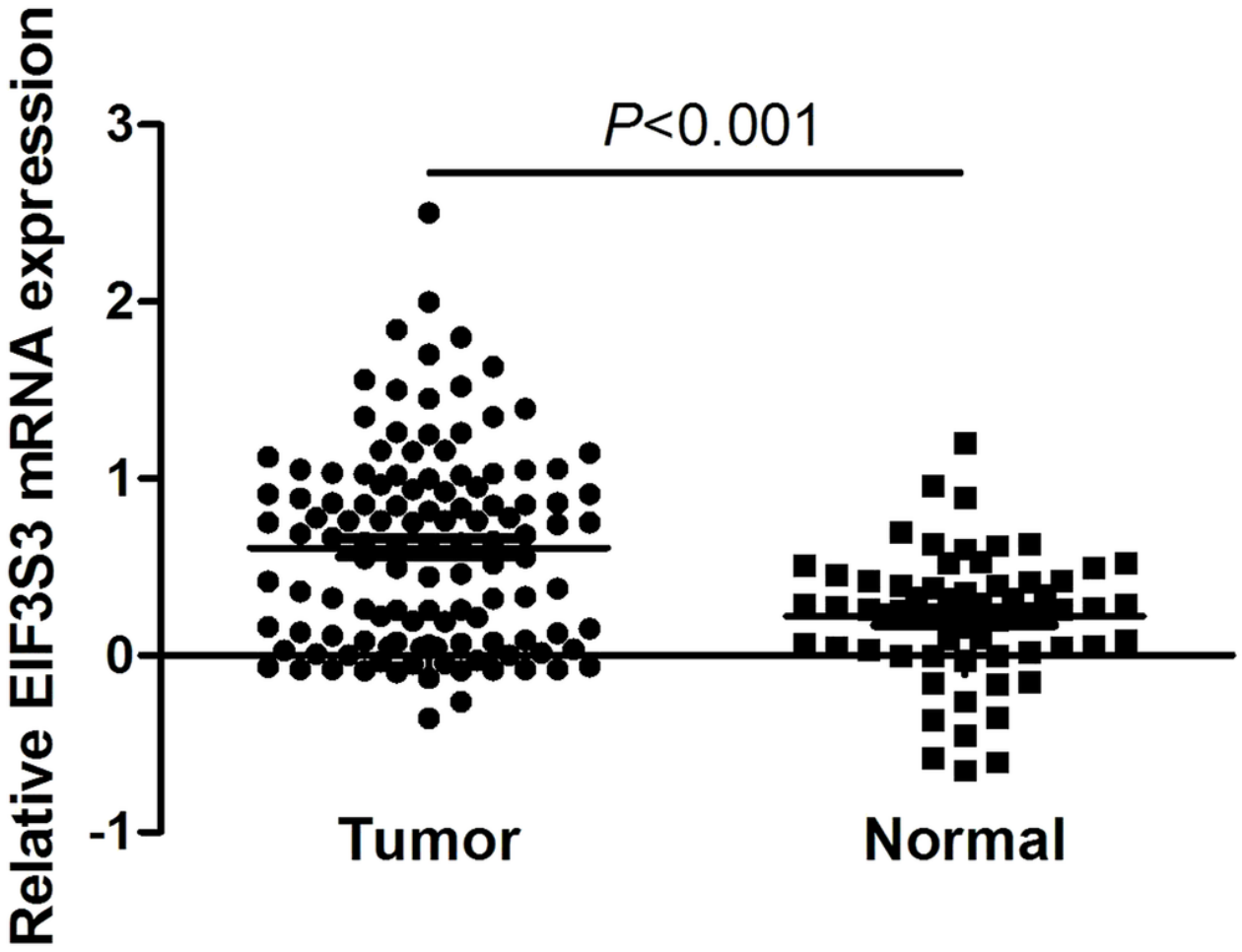

Figure 1

Relative EIF3S3 mRNA levels. The expression of EIF3S3 in HCC tissues was significantly higher than in adjacent normal specimens (P<0.001).

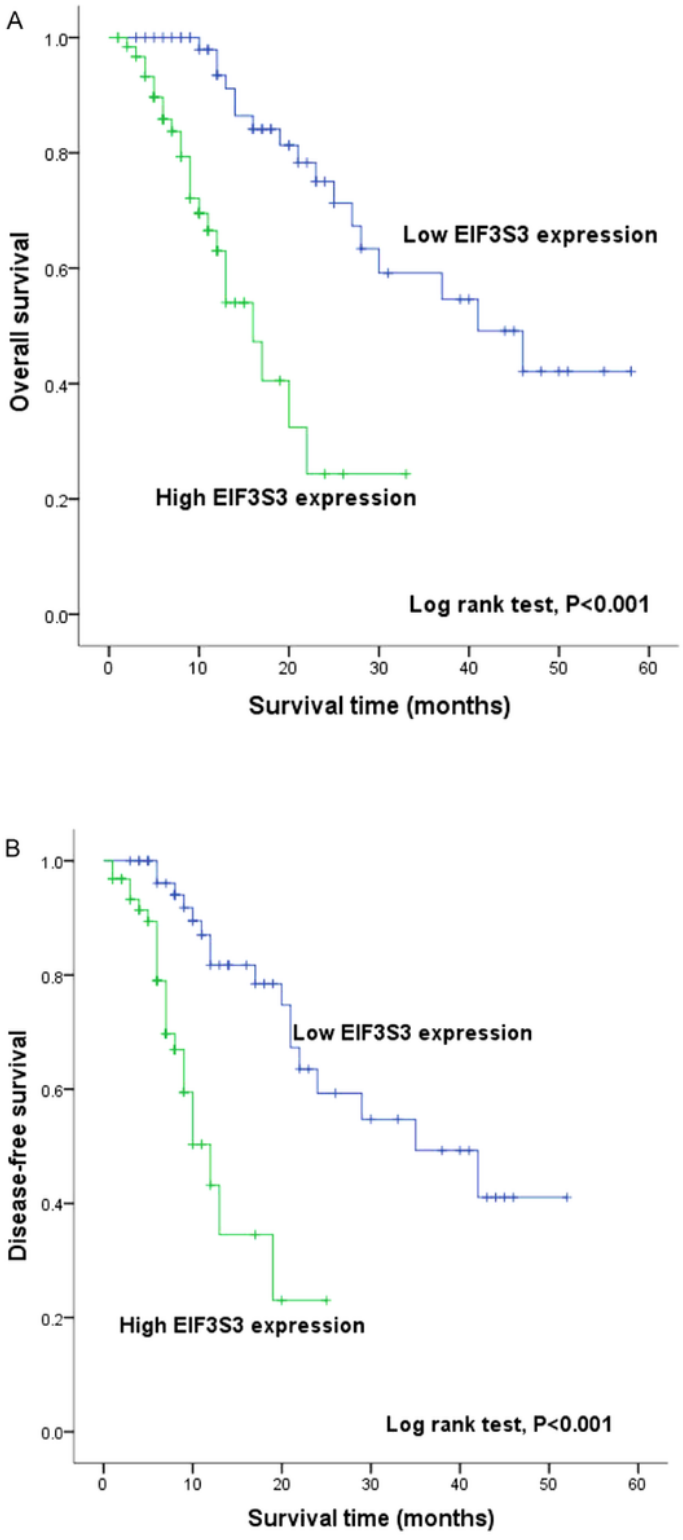

Figure 2

Survival analysis. Patients with high EIF3S3 expression had shorter OS (A) and DFS (B) compared to those with low EIF3S3 expression (both, P<0.001).