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Original Article

Upregulation of colonic and hepatic tumor overexpressed gene is significantly associated with the unfavorable prognosis marker of human hepatocellular carcinoma

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Abstract: Colonic hepatic tumor overexpressed gene (ch-TOG), a member of the highly conserved XMAP215 family of microtubule-associated proteins (MAPs), plays a crucial role in bipolar mitotic spindle assembly. Here, we performed proof-of-principle studies targeting ch-TOG for the development of HCC and further compared its prognostic significance with the clinicopathologic features of HCC. Quantitative real-time PCR was used to measure the expression level of ch-TOG mRNA in 207 cases of paired HCC and adjacent noncancerous liver tissues (ANLT). Additionally, immunohistochemistry was employed to identify ch-TOG protein in 71 HCC tissues. All HCC patients were divided into two groups according to the expression level of ch-TOG. Cumulative progression-free survival (PFS) and overall survival (OS) curves were estimated using the Kaplan-Meier method, and the prognostic value of ch-TOG was further evaluated using the Cox proportional hazards regression model. Our studies suggested that ch-TOG is overexpressed in HCC tissues compared with ANLT. The ch-TOG level was correlated with other prognostic factors, including the hepatitis B surface antigen (HBsAg) (p = 0.030), median size (p = 0.008), clinical tumor-node-metastasis (TNM) stage (p = 0.002), and alpha-fetoprotein (AFP) level (p = 0.030). Kaplan-Meier survival analysis showed that increased ch-TOG was associated with reduced PFS (p = 0.002) and OS (p = 0.004). Multivariate Cox proportional hazards analysis identified ch-TOG as an independent prognostic factor for the PFS (hazard ratio [HR] = 1.479, 95% confidence interval [CI] = 1.028-2.127, p = 0.035) and OS (HR = 1.609, 95% CI = 1.114-2.325, p = 0.011) of the HCC patients. Increased ch-TOG represents a powerful marker for predicting poorer prognosis in the clinical management of HCC, and may serve as a potential molecular target for HCC therapies in the future.

Keywords: Hepatocellular carcinoma, ch-TOG, prognosis, marker

Introduction

Hepatocellular carcinoma (HCC) is the most aggressive lethal neoplasm that causes an estimated 700,000 deaths worldwide [1, 2]. Hepatic resection represents an effective therapeutic strategy that largely improves survival; however, most patients show relapse within 5 years, and their prognosis is not good [3, 4]. Recently, many lines of compelling evidence have supported the idea that widespread genomic alterations may disrupt the normal signaling pathway in host cells and contribute directly to the evolution of HCC [5-7]. Thus, a better understanding of the underlying mechanisms of cancer-related genes in HCC progression is central to identify potential candidates for the therapeutic management of HCC.

Proper and accurate segregation of duplicated chromosomes by the spindle apparatus is essential for a high-quality life in animals, especially human beings, which effectively prevent the increasingly proportions of numerous cancers [8, 9]. The spindle apparatus is a dynamic array of microtubules (MTs), motors and non-motor proteins [10, 11]. Among them, non-motor proteins were found to be crucial for promoting spindle assembly and maintaining spindle function [12]. Transforming acidic coiled-coil protein 3 (TACC3), colonic and hepatic tumor overexpressed gene (ch-TOG) and clathrin

are a complex of non-motor proteins that markedly stabilizes MTs in the kinetochore fibers (K-fibers) by forming inter-MT bridges [13].

As one of the key structures of TACC3-ch-TOG-clathrin complexes, ch-TOG is highly conserved from yeast to humans, and participates in a series of mitotic events in human cells because it stabilizes spindle microtubules, regulates centrosomal MT dynamics, and promotes bipolar spindle formation [14, 15]. The dysregulation of ch-TOG may dramatically reduce the centrosomal MT polymer mass, resulting in a multipolar spindle formation and contributing to the development of cancer [16, 17]. It has been reported that ch-TOG is overexpressed in colonic and hepatic tumors compared with corresponding healthy tissues [18].

However, the functional role of ch-TOG in HCC progression has not been well characterized. In the present study, we investigated the expression levels and prognostic values of the ch-TOG gene in HCC patients. Our data revealed that ch-TOG is highly expressed in human HCC specimens compared with adjacent noncancerous liver tissues (ANLT) and represents an independent prognostic factor of progression-free survival (PFS) and overall survival (OS) in HCC patients who have undergone surgical intervention.

Materials and methods

Ethics

The study protocol strictly conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institutional ethics committee of the Affiliated Hospital of Guilin Medical University. All participants signed informed consents and allowed experiments to be conducted with their samples, and analysed their clinicopathologic data.

Patients and tissue samples

In this study, we investigated 207 patients who were diagnosed with HCC and were treated with hepatectomy at the Affiliated Hospital of Guilin Medical University, Guilin, Guangxi Province, China between March 2001 and December 2007. Routine workup was performed before surgery and included typical physical examination, hematologic and biochemistry profiles, ultrasonography (US), computed tomography (CT), and magnetic resonance imaging (MRI). The final diagnosis of HCC was confirmed by histopathological examination. The tumor stage was determined according to the tumor-node-metastasis (TNM) classification system of the International Union Against Cancer, 7th Edition [19]. The conventional clinicopathological variables of HCC patients, including age, gender, family history, hepatitis B surface antigen (HBsAg), median size, cirrhosis, tumor number, wine drinking, smoking, TNM stage, alpha-fetoprotein (AFP), presence of portal vein tumor thrombus (PVTT), distant or lymph node metastasis (DLNM), and recurrence are summarized in **Table 1**. In addition, twelve specimens of normal liver tissues surrounding hepatic hemangioma tissues were used as normal liver tissues, which were confirmed by pathology. Next, 207 pairs of HCC samples and matched ANLT were used for quantitative real-time PCR (qRT-PCR) analysis, transported in liquid nitrogen, and then stored at -80°C. In addition, 71 cases of paired HCC and adjacent liver tissues used for immunohistochemical analysis were fixed in 10% formalin and subsequently embedded in paraffin.

The mean follow-up period of the 207 HCC patients was 36.2 months (range, 2.0 to 84.0 months). Postoperative surveillance included abdominal ultrasonography, chest radiography and serum AFP testing every 6 months for the first 2 years, and the same testing every 3-6 months thereafter. Recurrence was diagnosed with the emergence of new hepatic lesions by ultrasonography, dynamic CT or MRI. PFS was defined as the time interval between the date of surgery and the date that disease progression or any cause of death was first observed. OS was measured as the time interval from the date of surgery to death or the last follow-up visit.

Quantitative real-time PCR (qRT-PCR) assay

Total RNA was extracted from fresh or frozen HCC samples using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and was reverse transcribed into first-strand cDNA using the Prime Script RT Reagent Kit (TaKaRa, Otsu, Japan). *qRT-PCR* analysis was performed using an ABI Prism 7500 Sequence Detector System (Applied Biosystems, Foster City, CA, USA). Each well (20 μl reaction volume) contained 10 μl of SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 0.2 μl of each primer, and 1 μl of template. The primer sequences used

Table 1. Correlation between the clinicopathologic variables and ch-TOG in HCC

Clinical character	Clinical variable	No. of patients	ch-TOG		x^2	*p* value
			Low n (%)	High n (%)		
Gender	Female	32	11 (34.4)	21 (65.6)	0.056	0.812
	Male	175	64 (36.6)	111 (63.4)		
Age (years)	≤ 55	141	48 (34.0)	93 (66.0)	0.917	0.338
	> 55	66	27 (40.9)	39 (59.1)		
Family history	No	174	64 (36.8)	110 (63.2)	0.143	0.706
	Yes	33	11 (33.3)	22 (66.7)		
HBsAg	Negative	39	20 (51.3)	19 (48.7)	4.711	0.030
	Positive	168	55 (32.70)	113 (67.3)		
Median size (range, cm)	≤ 5	70	34 (48.6)	36 (51.4)	6.970	0.008
	> 5	137	41 (29.9)	96 (70.1)		
Liver cirrhosis	No	20	4 (20.0)	16 (80.0)	2.525	0.112
	Yes	187	71 (38.0)	116 (62.0)		
Tumor number	Single	139	55 (39.6)	84 (60.4)	2.039	0.153
	Multiple	68	20 (29.4)	48 (70.6)		
Wine drinking	No	101	33 (32.7)	68 (67.3)	1.081	0.298
	Yes	106	42 (39.6)	64 (60.4)		
Smoking	No	106	35 (33.0)	71 (67.0)	0.971	0.325
	Yes	101	40 (39.6)	61 (60.4)		
TNM stage	I-II	89	43 (48.3)	46 (51.7)	9.865	0.002
	III-IV	118	32 (27.1)	86 (72.9)		
AFP (ng/ml)	≤ 100	74	34 (45.9)	40 (54.1)	4.704	0.030
	> 100	133	41 (30.8)	92 (69.2)		
PVTT	No	163	60 (36.8)	103 (63.2)	0.111	0.739
	Yes	44	15 (34.1)	29 (65.9)		
DLNM	No	179	66 (36.9)	113 (63.1)	0.234	0.628
	Yes	28	9 (32.1)	19 (67.9)		
Recurrence	No	166	64 (38.6)	102 (61.4)	1.956	0.162
	Yes	41	11 (26.8)	30 (73.2)		

HBsAg, hepatitis B surface antigen; TNM, tumor-node-metastasis; AFP, alpha-fetoprotein; PVTT, portal vein tumor thrombus; DLNM, distant or lymph node metastasis.

were as follows: ch-TOG (forward: 5'-CTCCTGAAAGGCTTGGACAAT-3'; reverse: 5'-GCATCCCGAATCCATCTGTAAA-3'). The length of the amplified fragment was 209 bp. The levels of ch-TOG genes were normalized to levels of the expression of the β-actin gene (forward: 5'-GACAGGATGCAGAAGGAGATTACT-3'; reverse: 5'-TGATCCACATCTGCTGGAAGGT-3'), which generated a 142 bp fragment. Relative mRNA expression and the cut-off of high or low of ch-TOG were calculated according to our previous report [20].

Immunohistochemistry (IHC) assay

Sections were first deparaffinized with xylene and then rehydrated with graded ethanol and distilled water. Next, sections were heated in a microwave for 3 minutes with citrate antigenic retrieval buffer at pH = 6.0. Then, endogenous peroxidase activity was blocked with 3% H_2O_2 for 10 min. Next, the sections were treated with 10% goat serum at room temperature for 30 minutes. Anti-ch-TOG rabbit antibody (catalog R37031, ATLAS ANTIBODIES Company, 1:200 dilution) was incubated with the sections overnight at 4°C. The following day, after washing, the sections were incubated with peroxidase-conjugated goat anti-rabbit antibody at room temperature for 1 hour. Finally, all of the sections were stained with 3,3-diaminobenzidine tetrahydrochloride (DAB) and then lightly counterstained with hematoxylin. For the negative controls, the antibody was replaced with nor-

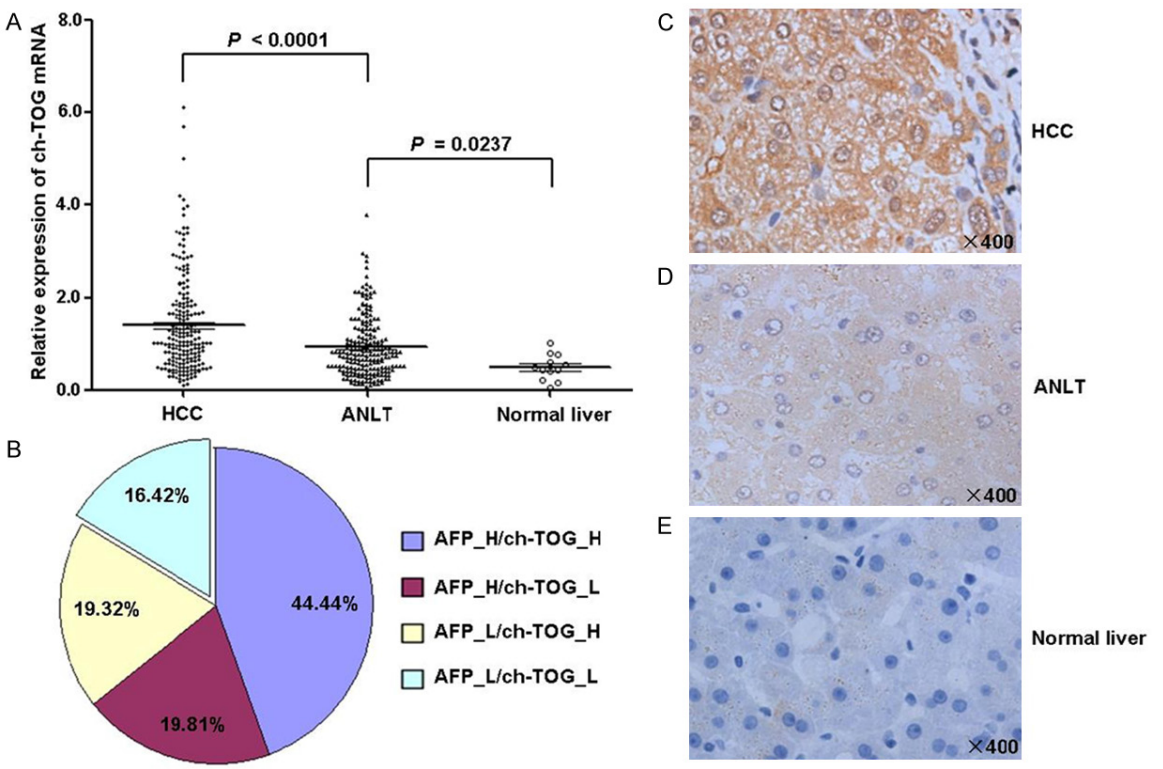

Figure 1. ch-TOG expression is determined in HCC by qRT-PCR and immunohistochemistry. A. The relative expression of ch-TOG mRNA in 207 paired HCC tissues and matched adjacent noncancerous liver tissues (ANLT) and 12 normal liver tissues was evaluated by qRT-PCR. The relative ch-TOG mRNA expression was normalized to the internal reference β-actin. B. The distribution of both ch-TOG mRNA expression and serum AFP level in 207 HCC patients; the numbers in the pie indicated the percentages of ch-TOG and/or AFP whose level is high (H) or low (L) in HCC specimens. C-E. Representative immunohistochemical expression patterns of ch-TOG in HCC specimen and corresponding ANLT and normal liver tissues are shown. The nuclei were counterstained with hematoxylin. Original magnification: ×400.

mal rabbit serum. The immunohistochemically stained tissue sections were scored by two independent pathologists who were blinded to the patients' clinical and biochemical information. The ch-TOG immunostaining intensities were scored as follows: the percentage of positive cells, grades 0-3 (0, no positive cells; 1, < 25% positive cells; 2, 25%-50% positive cells; 3, > 50% positive cells).

Statistical analysis

All clinical statistical analyses were performed with SPSS 13.0 (SPSS Inc, Chicago, IL). The correlation between ch-TOG expression and clinicopathologic parameters was evaluated using the Pearson χ^2 test. PFS and OS curves were obtained using the Kaplan-Meier method and were compared using the log-rank test. The Cox proportional hazards model was performed to determine independent prognostic factors based on the variables affecting survival in univariate analysis, and the adjusted hazard ratio (HR) and the 95% confidence interval (CI) were estimated. Differences were considered statistically significant when the *p* values were less than 0.05.

Results

Patient characteristics

The baseline characteristics of the enrolled participants are shown in **Table 1**. Of the 207 HCC patients, 175 were male, and 32 were female; 141 were ≤ 55 years old, and 66 were > 55 years old; there were 41 HCC patients who showed recurrence during the follow-up period. The HBsAg was positive in 168 patients, and 187 patients with liver cirrhosis. Serum AFP testing showed 133 patients with an increased AFP level (> 100 ng/ml), and 74 patients had an AFP level lower than 100 ng/ml. There were 70 patients with a tumor ≤ 5.0 cm in diameter, and the remainder had a tumor > 5.0 cm in diameter; 139 patients had one nodule, and 68

patients had multiple nodules. According to the TNM staging system, 89 patients were stage I-II, and 118 were stage III-IV. Tumor thrombi in the portal vein were found in 44 patients.

mRNA and protein levels of ch-TOG in HCC tissues

To determine whether ch-TOG might be involved in HCC, we first examined the relative ch-TOG mRNA levels in 207 pairs of HCC tissues and their matched ANLT as well as in 12 normal liver tissues from hepatic hemangiomas surrounding the liver tissues by qRT-PCR. The HCC specimens showed relative expression of ch-TOG compared with their corresponding ANLT samples and the 12 normal liver tissues (mean ± SD, 1.396 ± 0.073, 0.921 ± 0.045, and 0.494 ± 0.081, respectively; $p < 0.0001$ and $p = 0.0237$, respectively) (**Figure 1A**). We next analyzed the ch-TOG protein expression in 71 surgical specimens of HCC and the 6 normal liver tissues samples by IHC. The resulting data revealed that ch-TOG protein was strongly expressed in 53 (74.65%) surgical HCC specimens (**Figure 1C**); however, only 22 (30.98%) ANLT had positive ch-TOG staining (**Figure 1D**), and the difference in ch-TOG staining between the HCC and ANLT was statistically significant ($p < 0.001$), and ch-TOG protein expression was weak or negative in normal liver tissue (**Figure 1E**).

The combination of ch-TOG and AFP improves the diagnostic power for HCC

We determined whether the combination of ch-TOG and AFP represented a more powerful criterion for diagnosing HCC by correlation analysis. As shown in **Table 1**, the percentage rates for individuals with ch-TOG^{high} AFP^{high} were 44.44% (92/207), those for groups with ch-TOG^{low} AFP^{high} were 19.81% (41/207), and those for patients with ch-TOG^{high} AFP^{low} were 19.32% (40/207) (**Figure 1B**), suggesting the combination of ch-TOG and AFP greatly increased the diagnostic accuracy of HCC, and that simultaneous detection of AFP and ch-TOG could improve sensitivity of diagnosis to 83.58%.

Correlation between the expression of ch-TOG in tumor tissues and clinicopathologic characteristics in HCC patients

We next investigated the relationship between ch-TOG expression and clinicopathological features of HCC patients. Compared with patients without the HBsAg negative, the expression of ch-TOG was significantly higher in patients with the HBsAg positive ($\chi^2 = 4.711$; $p = 0.030$). Moreover, the relative expression of ch-TOG in the HCC groups with a tumor diameter > 5 cm was remarkably higher than in those with a tumor diameter ≤ 5 cm ($\chi^2 = 6.970$; $p = 0.008$). Moreover, the expression of ch-TOG in advanced TNM stage (III-IV) patients was notably increased compared with that in early TNM stage (I-II) patients ($\chi^2 = 9.865$; $p = 0.002$). Furthermore, the ch-TOG levels were significantly higher in groups with AFP > 100 ng/ml than in those with AFP ≤ 100 ng/ml ($\chi^2 = 4.704$; $p = 0.030$). By contrast, no significant relationship was observed between the expression of ch-TOG and some commonly clinical parameters, such as age, gender, family history, cirrhosis, tumor numbers, wine drinking, smoking, presence of PVTT, DLNM, and recurrence (all $p > 0.05$, **Table 1**).

Univariate and multivariate analyses of prognostic parameters in HCC patients

To identify the variables with potential prognostic power, the impact of traditional clinicopathological variables on HCC survival outcomes was investigated by univariate analysis. Next, the independent predictors of PFS and OS were determined by multivariate Cox proportional hazards model analysis. The HR, 95% CI, and *p* value for each parameter were calculated.

Univariate analysis results indicated that the high ch-TOG expression group ($p = 0.002$), as well as the median tumor size > 5 cm ($p < 0.001$), multiple tumors ($p < 0.001$), TNM stage III-IV ($p < 0.001$), and presence of PVTT ($p < 0.001$) were significant prognostic factors for PFS in HCC patients (**Table 2**). Additionally, some clinicopathologic features, including the high ch-TOG expression group ($p = 0.004$), as well as the median tumor size > 5 cm ($p < 0.001$), multiple tumors ($p < 0.001$), TNM stage III-IV ($p < 0.001$), and presence of PVTT ($p < 0.001$) significantly affected the OS of HCC patients (**Table 3**).

Multivariate analysis results showed that the high ch-TOG expression group (HR, 1.479; 95% CI, 1.028-2.127; $p = 0.035$) as well as a tumor size > 5 cm (HR, 2.771; 95% CI, 1.673-4.589; $p < 0.001$) and a TNM stage III-IV (HR, 2.025; 95% CI, 1.285-3.190; $p = 0.002$) were signifi-

Table 2. Univariate and multivariate analysis of progression-free survival

Clinical character	Category	No. of patients	Univariate analysis			Multivariate analysis	
			Mean	95% CI	*p* value	HR (95% CI)	*p* value
ch-TOG	Low	75	50.01	41.73-58.27	0.002	1.479 (1.028-2.127)	0.035
	High	132	33.61	27.63-39.59			
Gender	Female	32	49.96	36.66-63.26	0.154		
	Male	175	38.17	32.87-43.47			
Age (years)	≤ 55	144	38.64	32.59-44.68	0.543		
	> 55	66	41.98	33.29-50.67			
Family history	No	174	37.85	32.51-43.18	0.129		
	Yes	33	49.24	36.63-61.85			
HBsAg	Negative	39	35.97	24.93-47.01	0.643		
	Positive	168	40.49	34.94-46.04			
Median size (range, cm)	≤ 5	70	61.48	53.94-69.03	< 0.001	2.771 (1.673-4.589)	<0.001
	> 5	137	28.23	22.66-33.79			
Liver cirrhosis	No	20	31.03	15.97-46.09	0.237		
	Yes	187	40.57	35.33-45.81			
Tumor number	Single	139	45.84	39.71-51.96	< 0.001	1.153 (0.787-1.690)	0.464
	Multiple	68	26.61	19.08-34.14			
Wine drinking	No	101	44.91	37.42-52.40	0.102		
	Yes	106	35.21	28.76-41.65			
Smoking	No	106	43.58	36.40-50.76	0.101		
	Yes	101	35.79	28.97-42.61			
TNM stage	I-II	89	55.84	48.63-63.07	< 0.001	2.025 (1.285-3.190)	0.002
	III-IV	118	27.44	21.53-33.35			
AFP (ng/ml)	≤ 100	74	44.22	36.88-51.57	0.126		
	> 100	133	36.85	30.53-43.38			
PVTT	No	163	44.43	38.72-50.14	< 0.001	1.184 (0.775-1.809)	0.436
	Yes	44	22.91	14.70-31.12			
DLNM	No	179	41.51	36.12-46.90	0.065		
	Yes	28	25.59	15.79-35.38			

HR, hazard ratio; CI, confidence interval.

cant independent prognostic factors for the PFS of HCC patients (**Table 2**). Furthermore, the high ch-TOG expression group (HR, 1.609; 95% CI, 1.114-2.325; $p = 0.011$) as well as a tumor size > 5 cm (HR, 2.804; 95% CI, 1.690-4.652); $p < 0.001$) and a TNM stage III-IV (HR, 1.993; 95% CI, 1.267-3.134; $p = 0.003$) could independently predict the OS of HCC patients (**Table 3**).

Increased ch-TOG predicts poor survival

While dividing 207 HCC patients into two subgroups-"low ch-TOG expression" and "high ch-TOG expression"-we investigated the relationship between ch-TOG expression and patient survival. Kaplan-Meier survival analysis revealed that patients with high ch-TOG expression was associated with a shorter PFS (mean, 33.61 months) (95% CI, 27.63-39.59) than patients exhibiting low ch-TOG expression (mean, 50.01 months) (95% CI, 41.73-58.27) ($p = 0.002$, **Figure 2A**). In patients with a high ch-TOG level, the mean OS rates were only 38.68 months (95% CI, 33.00-44.36) compared with 53.84 months in patients with a low ch-TOG level (95% CI, 46.16-61.51) ($p = 0.004$, **Figure 2B**).

Discussion

Mitosis plays an important role in mediating proper and accurate chromosome segregation into two daughter cells [21]. Mis-regulation of

Table 3. Univariate and multivariate analysis of overall survival

Clinical character	Category	No. of patients	Univariate analysis			Multivariate analysis	
			Mean	95% CI	*p* value	HR (95% CI)	*p* value
ch-TOG	Low	75	53.84	46.16-61.51	0.004	1.609 (1.114-2.325)	0.011
	High	132	38.68	33.00-44.36			
Gender	Female	32	54.92	43.10-66.74	0.074		
	Male	175	42.17	37.14-47.21			
Age (years)	≤ 55	144	43.04	37.35-48.73	0.541		
	> 55	66	46.47	38.31-54.62			
Family history	No	174	42.32	37.27-47.37	0.092		
	Yes	33	53.82	42.12-65.51			
HBsAg	Negative	39	41.38	30.96-51.81	0.590		
	Positive	168	44.78	39.56-49.99			
Median size (range, cm)	≤ 5	70	66.15	59.83-72.48	< 0.001	2.804 (1.690-4.652)	< 0.001
	> 5	137	32.93	27.56-38.31			
Liver cirrhosis	No	20	36.95	22.92-50.98	0.345		
	Yes	187	44.92	39.98-49.85			
Tumor number	Single	139	50.17	44.51-55.82	< 0.001	1.138 (0.776-1.672)	0.506
	Multiple	68	31.90	24.42-39.38			
Wine drinking	No	101	48.86	41.88-55.83	0.058		
	Yes	106	39.70	33.57-45.83			
Smoking	No	106	48.74	42.15-55.33	0.058		
	Yes	101	39.36	32.87-45.86			
TNM stage	I-II	89	60.67	54.37-66.97	< 0.001	1.993 (1.267-3.134)	0.003
	III-IV	118	31.68	25.95-37.41			
AFP (ng/ml)	≤ 100	74	48.89	41.76-56.04	0.092		
	> 100	133	40.79	34.89-47.12			
PVTT	No	163	49.13	43.87-54.40	< 0.001	1.376 (0.899-2.107)	0.142
	Yes	44	25.75	17.71-33.79			
DLNM	No	179	45.62	40.55-50.69	0.106		
	Yes	28	34.82	23.53-46.11			
Recurrence	No	166	42.82	37.47-48.16	0.272		
	Yes	41	49.63	40.42-58.85			

mitotic genes has been associated with genome instability, which is one of the dominating pathogeneses that leads to tumorigenesis [22-24]. Additionally, the up-regulation of mitotic protein may sequester its binding partners to some extent, disrupting the collaborative relationships, and thereby causing mitotic catastrophe and defective chromosome segregation [25]. More importantly, cancer patients with chromosome instability were positively correlated with an unfavorable prognosis [26]. Thus, a complete investigation of mitotic genes can provide new insights into the mechanistic details of HCC.

ch-TOG is an evolutionarily conserved centrosomal protein that is crucial for a diverse array of cellular processes. First, ch-TOG proteins promote the rapid elongation of short MTs by polymerase activity [27, 28]. Second, ch-TOG proteins couple with TACC3 and clathrin, which remarkably stabilize K-fibers by physical cross-linking and by antagonizing MT catastrophe [29]. More importantly, ch-TOG is sufficient for regulating centrosomal MT assemble and bipolar spindle formation [14, 30]. Strong evidence has suggested that ch-TOG mutants greatly influenced their binding to tubulin, an activity that may not motivate the incorporation of tubulin into a growing microtubule end, leading to interruption of normal mitosis [15, 31]. However, the contribution of ch-TOG to HCC development and progression has remained poorly understood.

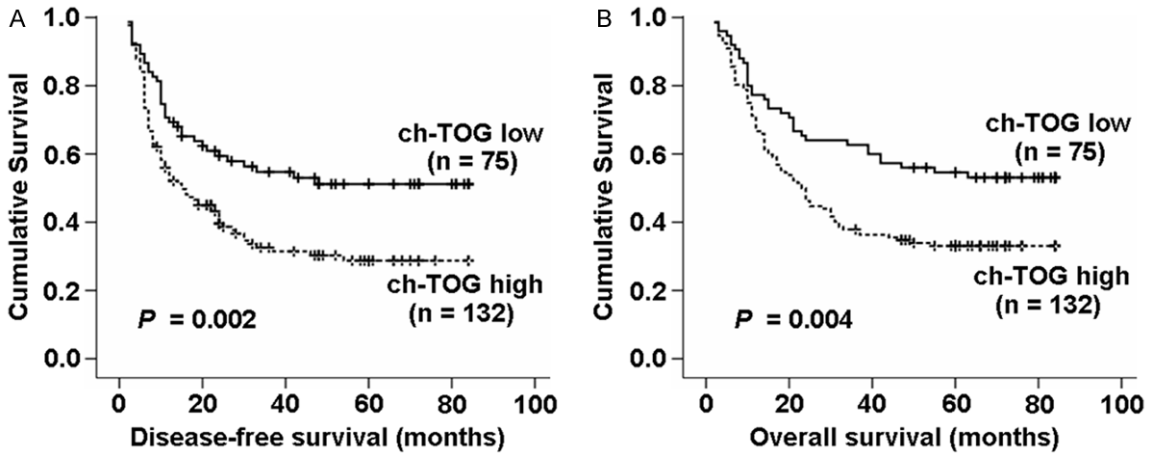

Figure 2. ch-TOG expression is positively correlated with poor outcome in HCC patients. The 207 HCC patients were divided into high ch-TOG expression groups (n = 132) and low ch-TOG expression groups (n = 75). Kaplan-Meier analysis was conducted to disclose the relationship of ch-TOG expression and the PFS (A) and OS (B) of all patients in the study cohort according to ch-TOG expression status.

To our knowledge, the present study is the first to reveal the clinical implications and biological relevance of ch-TOG in the development and progression of HCC. Our qRT-PCR and IHC results confirmed that ch-TOG is expressed much more strongly in HCC specimens than in corresponding ANLT. In addition, we correlated clinicopathological features of the patients with ch-TOG expression and observed that up-regulation of ch-TOG was positively correlated with HBsAg, tumor size, clinical TNM stage, and serum AFP level, strongly indicating that the over-expression of ch-TOG is necessary for rapid MT rearrangements and remarkably promoting tumor cell division and proliferation.

The HCC cohort of large tumors (> 5 cm) showed a high ch-TOG expression level compared with the HCC cohort of small tumors (≤ 5 cm), a finding that could be interpreted as the pro-oncogenic role of ch-TOG in tumor progression. Mutations in the ch-TOG gene result in defective chromosome segregation, leading to aneuploid cells and the further promotion of tumorigenesis. In addition, tumor size was found to be an independent prognostic factor for both the PFS and OS of HCC patients as determined by our Cox proportional hazards regression model, a finding that was consistent with that in the literature: patients with a tumor size ≥ 5 cm had an unfavorable prognosis compared with those with a tumor size < 5 cm [32, 33]. This observation is consistent with tumors greater than 5.0 cm having a high incidence of microscopic vascular invasion [34, 35]. Moreover, tumors greater than 5 cm in diameter were also a significant risk factor for recurrence in HCC patients [36].

Of note, comparison of the ch-TOG expression level between the early (I-II) and late (III-IV) TNM stages showed higher ch-TOG expression in the advanced TNM stage, further substantiating that ch-TOG is involved in the development of HCC due largely to its vital role in controlling proper DNA replication and in the series of events following mitotic progression. The overexpression of ch-TOG may induce similar overexpression of its functional partners, leading to an altered cell division program and the promotion of the tumorigenesis of HCC. We also found that clinical TNM stage has independent prognostic significance for both the PFS and OS of HCC patients in the Cox proportional hazards regression model, a finding that is in agreement with that of our previous studies [20].

Generally, serum AFP level has been regarded as having not only diagnostic value but also predictive value in HCC patients in clinical practice [37, 38]. However, the diagnostic and prognostic value of AFP is poor in most early stage HCC patients, particularly when used alone; 30-40% patients with a normal AFP level are often missed, and those patients often then develop advanced-stage HCC [39, 40]. We found that the percentage of HCC patients with high ch-TOG expression coupled with an elevated serum AFP level was significantly higher than the percentage of HCC patients with either alone in our study cohort, highlighting that ch-

TOG may be an effective tumor marker complementary to serum AFP level for improving HCC diagnostic accuracy.

More importantly, Kaplan-Meier survival analysis demonstrated that ch-TOG expression was significantly associated with the clinical outcome of HCC patients in our study. Patients with low ch-TOG expression levels had significantly longer PFS and OS rates than those with high ch-TOG expression levels. Furthermore, multivariate Cox analysis proved that ch-TOG is an independent prognostic indicator for both the PFS and OS of patients with HCC, suggesting that ch-TOG has clinical implications in predicting the clinical outcomes of HCC patients who have undergone surgical resection.

In conclusion, our data significantly propose that the up-regulation of ch-TOG in HCC is a valid biomarker for the tumorigenesis of HCC, and could predict a poorer clinical outcome. Uncovering the potential functions of ch-TOG in HCC may provide a new avenue to exploit ch-TOG as a molecular therapeutic target for HCC treatment. We hope that further investigations in a larger population will clearly confirm our results.

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Disclosure of conflict of interest

None.

Abbreviations

HCC, Hepatocellular carcinoma; ch-TOG, Colonic hepatic tumor overexpressed gene; ROC, receiver operating characteristic; HR, Hazard ratios; CI, confidence intervals; PFS, progression-free survival; OS, overall survival; TNM, tumor-node-metastasis; AFP, alpha-fetoprotein; HBsAg, hepatitis B surface antigen; PVTT, portal vein tumor thrombus; DLNM, distant or lymph node metastasis; ANLT, adjacent non-cancerous liver tissues; qRT-PCR, quantitative real-time PCR; IHC, Immunohistochemistry.

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References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
[2] Aihara A, Huang CK, Olsen MJ, Lin Q, Chung W, Tang Q, Dong X and Wands JR. A cell-surface beta-hydroxylase is a biomarker and therapeutic target for hepatocellular carcinoma. Hepatology 2014; 60: 1302-1313.
[3] El-Serag HB. Hepatocellular carcinoma. N Engl J Med 2011; 365: 1118-1127.
[4] Wang N, Feng Y, Zhu M, Tsang CM, Man K, Tong Y and Tsao SW. Berberine induces autophagic cell death and mitochondrial apoptosis in liver cancer cells: the cellular mechanism. J Cell Biochem 2010; 111: 1426-1436.
[5] Nishida N and Goel A. Genetic and epigenetic signatures in human hepatocellular carcinoma: a systematic review. Curr Genomics 2011; 12: 130-137.
[6] Nakagawa H and Shibata T. Comprehensive genome sequencing of the liver cancer genome. Cancer Lett 2013; 340: 234-240.
[7] Nishida N and Kudo M. Recent advancements in comprehensive genetic analyses for human hepatocellular carcinoma. Oncology 2013; 84 Suppl 1: 93-97.
[8] Barr AR and Bakal C. A sensitised RNAi screen reveals a ch-TOG genetic interaction network required for spindle assembly. Sci Rep 2015; 5: 10564.
[9] Murray AW. A brief history of error. Nat Cell Biol 2011; 13: 1178-1182.
[10] Manning AL and Compton DA. Structural and regulatory roles of nonmotor spindle proteins. Curr Opin Cell Biol 2008; 20: 101-106.
[11] Peterman EJ and Scholey JM. Mitotic microtubule crosslinkers: insights from mechanistic studies. Curr Biol 2009; 19: R1089-1094.
[12] Cheeseman LP, Harry EF, McAinsh AD, Prior IA and Royle SJ. Specific removal of TACC3-ch-TOG-clathrin at metaphase deregulates kinetochore fiber tension. J Cell Sci 2013; 126: 2102-2113.

[13] Booth DG, Hood FE, Prior IA and Royle SJ. A TACC3/ch-TOG/clathrin complex stabilises kinetochore fibres by inter-microtubule bridging. EMBO J 2011; 30: 906-919.
[14] Barr AR and Gergely F. MCAK-independent functions of ch-Tog/XMAP215 in microtubule plus-end dynamics. Mol Cell Biol 2008; 28: 7199-7211.
[15] Widlund PO, Stear JH, Pozniakovsky A, Zanic M, Reber S, Brouhard GJ, Hyman AA and Howard J. XMAP215 polymerase activity is built by combining multiple tubulin-binding TOG domains and a basic lattice-binding region. Proc Natl Acad Sci U S A 2011; 108: 2741-2746.
[16] Cassimeris L, Becker B and Carney B. TOGp regulates microtubule assembly and density during mitosis and contributes to chromosome directional instability. Cell Motil Cytoskeleton 2009; 66: 535-545.
[17] Gergely F, Draviam VM and Raff JW. The ch-TOG/XMAP215 protein is essential for spindle pole organization in human somatic cells. Genes Dev 2003; 17: 336-341.
[18] Charrasse S, Schroeder M, Gauthier-Rouviere C, Ango F, Cassimeris L, Gard DL and Larroque C. The TOGp protein is a new human microtubule-associated protein homologous to the Xenopus XMAP215. J Cell Sci 1998; 111: 1371-1383.
[19] Sobin LH and Compton CC. TNM seventh edition: what's new, what's changed: communication from the International Union Against Cancer and the American Joint Committee on Cancer. Cancer 2010; 116: 5336-5339.
[20] Liao W, Huang G, Liao Y, Yang J, Chen Q, Xiao S, Jin J, He S and Wang C. High KIF18A expression correlates with unfavorable prognosis in primary hepatocellular carcinoma. Oncotarget 2014; 5: 10271-10279.
[21] Bakhoum SF, Silkworth WT, Nardi IK, Nicholson JM, Compton DA and Cimini D. The mitotic origin of chromosomal instability. Curr Biol 2014; 24: R148-149.
[22] Nagahara M, Nishida N, Iwatsuki M, Ishimaru S, Mimori K, Tanaka F, Nakagawa T, Sato T, Sugihara K, Hoon DS and Mori M. Kinesin 18A expression: clinical relevance to colorectal cancer progression. Int J Cancer 2011; 129: 2543-2552.
[23] Ricke RM, Jeganathan KB and van Deursen JM. Bub1 overexpression induces aneuploidy and tumor formation through Aurora B kinase hyperactivation. J Cell Biol 2011; 193: 1049-1064.
[24] Diaz-Rodriguez E, Sotillo R, Schvartzman JM and Benezra R. Hec1 overexpression hyperactivates the mitotic checkpoint and induces tumor formation in vivo. Proc Natl Acad Sci U S A 2008; 105: 16719-16724.
[25] Tang NH and Toda T. MAPping the Ndc80 loop in cancer: A possible link between Ndc80/Hec1 overproduction and cancer formation. Bioessays 2015; 37: 248-256.
[26] Swanton C, Nicke B, Schuett M, Eklund AC, Ng C, Li Q, Hardcastle T, Lee A, Roy R, East P, Kschischo M, Endesfelder D, Wylie P, Kim SN, Chen JG, Howell M, Ried T, Habermann JK, Auer G, Brenton JD, Szallasi Z and Downward J. Chromosomal instability determines taxane response. Proc Natl Acad Sci U S A 2009; 106: 8671-8676.
[27] Brouhard GJ, Stear JH, Noetzel TL, Al-Bassam J, Kinoshita K, Harrison SC, Howard J and Hyman AA. XMAP215 is a processive microtubule polymerase. Cell 2008; 132: 79-88.
[28] Podolski M, Mahamdeh M and Howard J. Stu2, the budding yeast XMAP215/Dis1 homolog, promotes assembly of yeast microtubules by increasing growth rate and decreasing catastrophe frequency. J Biol Chem 2014; 289: 28087-28093.
[29] Hood FE, Williams SJ, Burgess SG, Richards MW, Roth D, Straube A, Pfuhl M, Bayliss R and Royle SJ. Coordination of adjacent domains mediates TACC3-ch-TOG-clathrin assembly and mitotic spindle binding. J Cell Biol 2013; 202: 463-478.
[30] Popov AV, Severin F and Karsenti E. XMAP215 is required for the microtubule-nucleating activity of centrosomes. Curr Biol 2002; 12: 1326-1330.
[31] Al-Bassam J, Larsen NA, Hyman AA and Harrison SC. Crystal structure of a TOG domain: conserved features of XMAP215/Dis1-family TOG domains and implications for tubulin binding. Structure 2007; 15: 355-362.
[32] Chen YL, Ko CJ, Chien SY, Chen LS, Chen ML, Chi CW and Lai HW. Tumor size as a prognostic factor in resected small hepatocellular carcinoma: a controversy revisited. J Gastroenterol Hepatol 2011; 26: 851-857.
[33] Kim SJ, Lee KK and Kim DG. Tumor size predicts the biological behavior and influence of operative modalities in hepatocellular carcinoma. Hepatogastroenterology 2010; 57: 121-126.
[34] Zhou L, Rui JA, Wang SB, Chen SG and Qu Q. Prognostic factors of solitary large hepatocellular carcinoma: the importance of differentiation grade. Eur J Surg Oncol 2011; 37: 521-525.
[35] Pawlik TM, Delman KA, Vauthey JN, Nagorney DM, Ng IO, Ikai I, Yamaoka Y, Belghiti J, Lauwers GY, Poon RT and Abdalla EK. Tumor size predicts vascular invasion and histologic grade: Implications for selection of surgical treatment for hepatocellular carcinoma. Liver Transpl 2005; 11: 1086-1092.

[36] Kow AW, Kwon CH, Song S, Shin M, Kim JM and Joh JW. Risk factors of peritoneal recurrence and outcome of resected peritoneal recurrence after liver resection in hepatocellular carcinoma: review of 1222 cases of hepatectomy in a tertiary institution. Ann Surg Oncol 2012; 19: 2246-2255.

[37] Sun Z, Zhu Y, Xia J, Sawakami T, Kokudo N and Zhang N. Status of and prospects for cancer vaccines against hepatocellular carcinoma in clinical trials. Biosci Trends 2015; [Epub ahead of print].

[38] Ma WJ, Wang HY and Teng LS. Correlation analysis of preoperative serum alpha-fetoprotein (AFP) level and prognosis of hepatocellular carcinoma (HCC) after hepatectomy. World J Surg Oncol 2013; 11: 212.

[39] Saffroy R, Pham P, Reffas M, Takka M, Lemoine A and Debuire B. New perspectives and strategy research biomarkers for hepatocellular carcinoma. Clin Chem Lab Med 2007; 45: 1169-1179.

[40] Toyoda H, Kumada T, Tada T, Kaneoka Y, Maeda A, Kanke F and Satomura S. Clinical utility of highly sensitive Lens culinaris agglutinin-reactive alpha-fetoprotein in hepatocellular carcinoma patients with alpha-fetoprotein <20 ng/mL. Cancer Sci 2011; 102: 1025-1031.