RESEARCH COMMUNICATION

Talin1, a Valuable Marker for Diagnosis and Prognostic Assessment of Human Hepatocelluar Carcinomas

Jian-Lin Zhang, Ye-Ben Qian*, Li-Xin Zhu, Qi-Ru Xiong

Abstract

Background/Aims: Hepatocellular carcinoma (HCC) is one of the most lethal and prevalent cancers in the human population. Despite its significance, there is only limited understanding of pathological mechanisms and therapeutic options. Talin1, a focal adhesion complex protein that is required for cell adhesion and motility, regulates integrin interactions with extracellular matrix (ECM). In the present study, we aimed to study the possible role of Talin1 in diagnosis and prognosis of HCC. Methods: Expression of Talin1 protein was detected in normal liver tissues (n=10), HCC tissues (n=32) and adjacent non-cancerous tissues (n=32) by immunohistochemistry and real time PCR. Results: While Talin1 was observed in all tissues, the protein and mRNA expression of Talin1 in HCC tissues was significantly lower than that in the adjacent non-cancerous tissues and normal liver tissues($P<0.05$). In addition, the expression of Talin1 in HCCs was significantly correlated with pathological differentiation, integrity of the tumor capsule, portal vein tumor thrombus and tumor size ($P<0.05$). Conclusions: Talin1 is possibly involved in the process of the carcinogenesis, infiltration and metastasis of HCC and has potential as a marker for diagnosis and prognostic assessment.

Keywords: Hepatocellular carcinoma - Talin1 - diagnosis - prognosis

Asian Pacific J Cancer Prev, **12**, 3265-3269

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third most common cause of cancer mortality (Bosch et al., 2005; Farazi and DePinho, 2006). Despite its significance, there is only a limited understanding of its pathological mechanisms and therapeutic options (Farazi and DePinho, 2006). More and more researches have focused on the cellular and molecular mechanism of HCC for the purpose of improving the diagnosis and prognosis of this malignant tumor (Matsushima-Nishiwaki et al., 1996; 2001; 2003; Muto et al., 1999;).

The alteration of tumor cell adhesion is the initial step of the invasion and metastasis of tumor cells. Integrin receptors are cell surface receptors with critical functions in cell adhesion and migration, and integrin/FAK signaling pathway is thought to play a key role in integrin-mediated signaling transduction pathway leading to adhesion and metastasis of HCC (Liang et al., 2003). Talin was the first cytoplasmic protein partner of integrins to be identified (Horwitz et al., 1986). Talin binds to the NPXY motif of the integrin β cytoplasmic domains, which can lead to integrin activation and affect cell adhesion, spreading and motility (Tadokoro et al., 2003; Zhang et al., 2008). The expression of Talin is highly associated with endometrioid carcinoma (Slater et al., 2007) and prostate cancer (Sakamoto et al., 2010). Therefore, Talin is possibly involved in adhesion and metastasis of HCC by integrin-mediated signaling transduction pathway.

Vertebrates have two talin genes, TLN1 and TLN2, which encode Talin1 and Talin2, respectively. Talin1 is essential for cell adhesion and motility and is the primary talin component of focal adhesions. Talin1 is mainly -expressed in the kidney, liver, spleen, stomach, lung and vascular smooth muscle and its overexpression can promote prostate cancer cell adhesion, migration and invasion (Tsujioka et al., 2004; Senetar and McCann, 2005; Senetar et al., 2007). However, up to now, there are few available studies on the role of Talin1 expression in HCC tissues.

In the present study, we aimed to study the possible role of Talin1 in the diagnosis and prognosis of HCC by detecting its expression in HCC tissues. As a result, the protein and mRNA expression of Talin1 in HCC tissues was significantly lower than that in the adjacent non-cancerous tissues and normal liver tissues. Moreover, the expression of Talin1 in HCC tissues was significantly correlated with pathological differentiation of HCC, the integrity of tumor capsule, portal vein tumor thrombus and tumor size. Therefore, Talin1 is possibly involved in the process of the carcinogenesis, infiltration and metastasis of HCC and can serve as a valuable biomarker for the diagnosis and prognosis of HCC.

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Materials and Methods

Tissue samples

A total of 32 HCC samples and adjacent non-cancerous tissues (> 2 cm away from tumor) from July 2007 to July 2008 were collected at the Department of Surgery, First Affiliated Hospital of Anhui Medical University. None of the patients had received radiotherapy or chemotherapy prior to surgery. Both HCC and adjacent non-cancerous tissues were proved by pathological examination. The 32 patients included 26 males and 6 females with a median age of 60 years (range, 29-67 years), 12 patients were Edmonson stage I, 15 patients were stage II, and 5 patients were stage III. The diameter of the tumor was smaller than 5 cm in 11 patients, and ≥ 5 cm in 21 patients. Portal vein tumor thrombus was found in 8 patients. 10 cases of normal liver tissues samples were obtained from patients with hemangiomas or liver traumatic rupture as controls. One part of tissue sample was immediately frozen in liquid nitrogen and subsequently stored at -80 °C for Real Time RT-PCR, and the remain part of the same tissue sample was fixed in 10% formalin and embedded in paraffin.

Immunohistochemistry

Tissues were fixed in 10% formalin and embedded in paraffin in 24 hours. Serial 4-μm paraffin sections were used for immunochemical staining. The sections were deparaffined in xylene and rehydrated in graded ethanol. After antigen retrieval of microwave irradiation, slides were incubated with IHC Biotin Block Kit (BLK-0001, Maixin Biotechnology, China) to inhibit endogenous peroxidase activity. Then, mouse anti-Talin1 monoclonal primary antibody (SC-81805, 1:100 dilution, Santa Cruz Biotechnology, CA, USA) or mouse IgG at the same dilution, used as a negative control, was added to the two serial sections using a drop-wise technique. Sections were then incubated with the appropriate secondary antibodies (SP-9002, Zhongshan Golden Bridge Biotechnology, China). Finally, all the slides were counterstained with hematoxylin after DAB color development.

Semi-quantitative method

Blinded analysis of positive immunostained sections was performed with the image-analysis program (Image Pro Plus, Media Cybernetics). The total Talin1 immunostaining score was calculated as the staining intensity and the sum of the percent positivity of stained tumor cells. The staining intensity was scored as "0" (no staining), "I" (weakly stained), "II" (moderately stained), and "III" (strongly stained); The percent positivity was scored as "0" (< 5%), "I" (5%-25%), "II" (26%-50%), "III" (51%-75%), "IV" (> 75%). Talin1 expression level was defined as follow: "-" (score 0-1), "+" (score 2-3), "++" (score 4-5) and "+++" (score ≥ 6)(Ma et al, 2009).
RNA preparation and real-time quantitative RT-PCR

Total RNA was extracted from HCC tissues, adjacent non-cancerous and normal control tissues using Trizol (Invitrogen) according to its manufacturer's instructions. The concentration of RNA was determined with a spectrophotometer and their integrity was assessed by gel electrophoresis. Total RNA (2 μg) was reverse-transcribed with RevertAid™ First Strand cDNA Synthesis Kit (Fermentas #k1622) in a 20 μL reaction system containing 1 μL of Oligo(dT)18, 4 μL of 5 × reaction buffer, 1 μL of RiboLock™ RNase Inhibitor, 2 μL of dNTP Mix, 1 μL of RevertAid™ M-Mulv Reverse Transcriptase, and DEPC-treated Water up to 20 μL. The mixture was incubated for 60 min at 42°C and then for an additional 5 min at 70°C. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The primers were as follows: Talin1 (TLN1): (sense) 5'-TGT GCC AAT GGC TAC CTG GA-3' and (anti-sense) 5'-GAA CCA GCC ACA CGC TTT GA-3' (product size, 110 bp); GAPDH: (sense) 5'-CCA CTC CTC CAC CTT TGA C-3' and (antisense) 5'-ACC CTG TTG CTG TAG CCA-3' (product size, 102 bp). Real-time quantitative PCR was done on the Rotor-Gene 3000 sequence detection system in combination with the SYBR® Premix Ex TaqTM II (TaKaRa Biotechnology, China). Melting curve analyses following amplification were performed to assure the product specificity. Samples were assayed in a 20 μL reaction mixture containing 2 μL of cDNA, 10 μL of 2 × SYBR® Premix Ex TaqTM II, 0.8 μL of PCR Forward Primer, 0.8 μL of PCR Reverse Primer and 6.4 μL of DEPC-treated Water. The following PCR cycling parameters were employed: at 95 centigrade for 30 s, followed by 40 cycles at 95 centigrade for 5 s, 57 centigrade for 15 s and then at 72 centigrade for 20 s. Fluorescence detection point was setted at 72 centigrade, The PCR products were resolved on a 1.5% agarose gel. All experiments were carried out in triplicate. Relative expression of Talin1 mRNA was normalized to the housekeeping gene GAPDH in the same cDNA. The normalized $^{\Delta}$Ct (Times Ct = Ct, Talin1 - Ct, GAPDH) values were used to calculate the relative gene expression for the three tissues.

Statistical analysis

Quantitative values were presented as mean ± SD; categorical variables were enumeration data from counting the number of samples. Non-parameter Kruskal-Wallis test, Wilcoxon's test and Spearman test were used to compare Talin1 protein expression among groups; Differences of Talin1 mRNA expression among groups were assessed by Independent Student's t-test, one-way ANOVA (one-way analysis of variance) and SNK (Student-Newman-Keuls) test; Variables associated with One-year disease-free survival rate were tested by Kaplan-Meier estimates and compared by log-rank test. The SPSS 17.0 software was used for all statistical analyses. A statistically significant P value was defined as < 0.05.

Results

Expression of Talin1 in HCC tissues

To preliminarily investigate the role of Talin1 in HCC, we detected the expression of Talin1 in normal liver tissues, HCC tissues and its adjacent non-cancerous tissues by immunohistochemistry and real time PCR. The expressions of Talin1 in the three tissues were shown in Table 1 and Figure 1. The brown granules of positive cells were mainly distributed in the cytoplasm and cell membrane. In the normal liver tissues, HCC tissues and

Table 1. Expression of Talin1 at the Protein Level in HCC Tissues, Adjacent Non-cancerous Tissues and the Normal Liver Tissues

Group	n	Talin1 expression			
		-	+	++	+++
HCC tissues	32	12	16	4	0
adjacent non-cancerous tissues	32	1	9	9	13
normal liver tissues	10	0	1	3	6

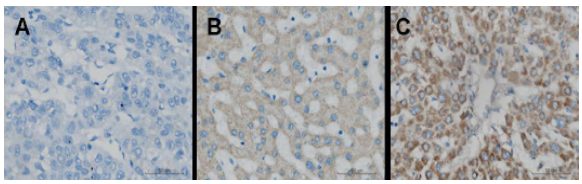

Figure 1. The Expression of Talin1 Protein in HCC Tissues, Adjacent Non-cancerous Tissues and the Normal Liver Tissues. A: Negative staining of Talin1 in HCC tissues(× 200); B: Moderate staining in adjacent non-cancerous tissues(×200); C: Strong staining in the normal liver tissues(× 200)

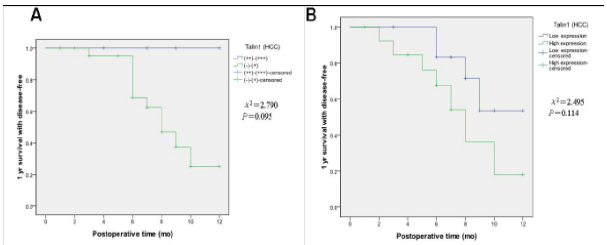

Figure 2. Relationship Between Talin1 Protein Expression. (A) and mRNA expression (B) and One -year disease-free survival in HCC tissues

its adjacent non-cancerous tissues, the positive rate of Talin1 expression was 100% (10/10), 62.5% (20/32), and 96.9% (31/32), respectively. The expression of Talin1 protein in HCC tissues was significantly lower than that in the adjacent non-cancerous tissues and normal liver tissues (P=0.000), but there was no significant difference between adjacent non-cancerous tissues and normal liver tissues (P=0.258). In addition, as shown in Table 2, the relative expression of Talin1 mRNA in HCC tissues was significantly lower than that of the adjacent non-cancerous tissues and normal control tissues (F=46.551, P<0.01). There was no significant difference between adjacent non-cancerous tissues and normal liver tissues (P>0.05).

Relationship of Talin1 expression in HCC tissues and clinical data

To further investigate the possible role of Talin in HCC, we were designed to analyze the relationship between Talin1 expression in HCC tissues and clinical data such as gender, age, tumor size, number of tumor, portal vein tumor thrombus, the expression of AFP, HBsAg, the integrity of tumor capsule and pathological differentiation of HCC by statistical analysis. As shown in Table 3, there was no significant difference between Talin1 expression and gender, age, number of tumor, the expression of AFP and HBsAg (P>0.05); the signal intensity of Talin1 expression was stronger in smaller (<5 cm) than that in larger (≥5 cm) tumors, stronger in patients without portal vein tumor thrombus than that with

Table 2. Relative Expression of Talin1 mRNA in HCC Tissues, Adjacent Non-cancerous Tissues and the Normal Liver Tissues

Group	n	ΔCt	P
HCC tissues	32	7.886±0.803[a]	[a]P=0.000
adjacent non-cancerous tissues	32	6.143±0.811[b]	[b]P=0.000
normal liver tissues	10	5.936±0.697[c]	[c]P=0.473

Data are mean±S.D. vs adjacent non-cancerous tissues, aP<0.01; vs normal liver tissues, bP<0.01; vs normal liver tissues, cP>0.05

Table 4. The Correlation Between Talin1 mRNA Expression and Clinical Data in HCC Tissues

Clinical data	n	ΔCt	t	P
Gender				
Male	26	7.817±0.778	-1.013	0.319
Female	6	8.185±0.912		
Age(y)				
≥60	17	7.812±0.707	-0.546	0.589
<60	15	7.969±0.917		
Tumor size(cm)				
<5	11	7.415±0.562	-2.616	0.014
≥5	21	8.132±0.809		
Number of tumor				
Single	20	7.687±0.849	-1.883	0.069
Multiple	12	8.218±0.615		
Venous invasion				
-	24	7.636±0.659	-3.583	0.001
+	8	8.635±0.757		
AFP(μg/L)				
<400	22	7.705±0.732	-1.973	0.058
≥400	10	8.283±0.845		
HBsAg				
-	7	7.456±0.973	-1.648	0.110
+	25	8.006±0.725		
Tumor capsule				
with integral	26	7.688±0.746	-3.338	0.002
Wi without integral	6	8.742±0.365		
Edmondson stage				
I-II	27	7.707±0.737	-3.390	0.002
III	5	8.852±0.275		

venous invasion, stronger in tumor without an integral capsule than that with an integral capsule and stronger in well-differentiated tumor grade(I-II grade) cancer tissues than in poor differentiated (III grade) (P<0.05).

Relationship between Talin1 expression and One-year disease-free survival

To investigate the possible role of Talin in the prognosis of HCC, we further analyzed the relationship between Talin1 expression and One-year disease-free survival by statistical analysis. Complete follow-up data was obtained from 27 (27/32) cases of HCC, and the follow-up time was >1 year. 11 cases showed disease recurrence or died in the 27 cases. Variables associated with One-year disease-free survival rate were tested by Kaplan-Meier estimates and compared by log-rank test. One-year disease-free survival rate was 100% in patients in groups with Talin1 protein expression (++) - (+++), One-year disease-free survival rate decreased as time passed in groups with Talin1 protein expression (-) - (+), but the expression of

Table 3. The Correlation Between Talin1 Protein Expression and Clinical Data in HCC Tissues

Clinical data		Talin1 expression					n	χ2	P	r
		-	+	++	+++	P				
Gender	Male	9	14	3	0	26	-0.426	0.670	-0.077	0.677
	Female	3	2	1	0	6				
Age(y)	≥60	8	5	4	0	17	-0.167	0.868	0.030	0.871
	<60	4	11	0	0	15				
Tumor size(cm)	<5	1	6	4	0	11	-3.065	0.002	-0.550	0.001
	≥5	11	10	0	0	21				
Number of tumor	Single	6	11	3	0	20	-1.117	0.264	-0.201	0.271
	Multiple	6	5	1	0	12				
Venous invasion	-	6	14	4	0	24	-2.497	0.013	-0.449	0.010
	+	6	2	0	0	8				
AFP(μg/L)	<400	7	11	4	0	22	-1.346	0.178	-0.242	0.183
	≥400	5	5	0	0	10				
HBsAg	-	2	3	2	0	7	-1.006	0.314	-0.181	0.322
	+	10	13	2	0	25				
Tumor capsule	with integral	7	15	4	0	26	-2.451	0.014	-0.440	0.012
	Wi without integral	5	1	0	0	6				
Edmondson stage	I	2	7	3	0	12	-6.651	0.036	-0.456	0.009
	II	6	8	1	0	15				
	III	4	1	0	0	5				

Talin1 in HCC tissues had no correlation with the One-year disease-free survival of patients (χ^2=2.790, P>0.05) (Figure 2A). The expression of Talin1 gene mRNA in HCC tissues was divided into two groups: higher expression group (>average expression) and lower expression group (≤average expression). Kaplan-Meier curve showed that the disease-free survival was decreased. One-year disease-free survival rate was 53.1% in higher expression group, the survival rate was 18.1% in lower expression group, but without significant difference between two groups (χ^2=2.495, P>0.05) (Figure 2B).

Discussion

HCC is one of the most lethal and prevalent human carcinomas. Even with improved diagnosis and compositive therapy, the prognosis of HCC is still dismal(Qin and Tang, 2002). Therefore, to identify valuable tumor biomarkers is important for early diagnosis and therapy of HCC. In this study, our data indicates that Talin1 is possibly involved in the processs of the carcinogenesis, infiltration and metastasis of HCC and can serve as a potential marker for the diagnosis and prognosis of HCC.

Invasion into surrounding tissues is a predominant phenotype of cancer cells, and regulatory mechanisms of cell motility is critical in this process. Cell adhesion is essential for motility in embryogenesis, wound healing, inflammation, and tumor metastasis (Cheng et al., 2007). The formation of focal adhesions is one result induced by cell adhesion and is a regulated process which is involved in cellular movement. The focal adhesions contain multiple proteins such as vinculin, talin and tensin. Talin is a major link between integrin and the cytoskeleton. Moreover, it plays a crucial role in focal adhesion assembly and in integrin-mediated signaling since it is able to recruit focal adhesion kinase (Martel et al., 2001; Scibelli et al., 2003; Chen et al., 2006), while integrin-mediated signaling transduction pathway can lead to adhesion and metastasis of HCC (Liang et al., 2003). Therefore, Talin is possibly involved in adhesion and metastasis of HCC and become a potential biomarker for the diagnosis and prognosis of HCC. In vertebrates, there are two talin genes, namely Talin1 and Talin2. Talin1 is essential for cell adhesion and motility and is the primary talin component of focal adhesions. Thus, we firstly focused on the relationship between Talin 1 and HCC.

To our knowledge, there are few available studies on the role of Talin1 expression in HCC tissues. In our study, the expression of Talin1 protein in HCC tissues was significantly lower than that in the adjacent non-cancerous tissues and normal liver tissues. In addition, the expression of Talin1 is lower in the tumor tissues with larger, poor differentiated and HCC tissue without an integral capsule. These results indicate that Talin1 may play a crucial role in the carcinogenesis, proliferation and infiltration of HCC. Metastasis is one of the characteristics of progression of HCC. Our further study shows that the expression of Talin1 is stronger in patients without portal vein tumor thrombus than that with portal vein tumor thrombus; The data indicates that the down-regulation of Talin1 expression can provide the HCC cells more chances to invade and metastasize.

In the retrospective study on banked tissue, Slater et al (Slater et al., 2007) found that alpha-actinin and talin were completely de-expressed in both endometriosis and endometrioid carcinoma tissue. Our data also demonstrated that talin expression is lower in HCC tissues compared with that in control. The loss of talin in tumor tissue indicates a significant change in the integrity of these cancer tissues and the possibility that individual cells may break away from the parent histology due to loss of cell adhesion, ultimately leading to tumor angiogenesis and development.

Talin1 is an integrin regulatory protein that mediates integrin interactions with ECM. Sakamoto et al. (2010) recently reported that the overexpression of Talin1 enhanced prostate cancer cell adhesion, migration and

invasion by activating survival signals and anoikis resistance. However, our results suggest that lower expression of Talin1 may contribute to HCC invasion and metastasis. One possible explain is that Talin1 exerts different effects in the formation of different cancer, which are the multi-step and complex processes associated with accumulation of genetic and epigenetic changes (Aravalli et al., 2008).

In this study, our data also indicates that One-year disease-free survival rate is direct correlated to Talin 1. These results suggest that Talin1 may serve as a potential prognostic factor for HCC. However, since the number of samples in the present study was relatively small, the prognostic significance of Talin1 expression needs to be further investigated in a larger sample of patients with long term follow-up.

In summary, Talin1 is possibly involved in the processs of the carcinogenesis, infiltration and metastasis of HCC and may serve as a valuable biomarker for the diagnosis and prognosis of HCC. However, the concrete molecular mechanism of Talin1 in the formation and metastasis of HCC remains to be further investigated.

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