RESEARCH ARTICLE

Roles of Fibroblast Growth Factor-inducible 14 in Hepatocellular Carcinoma

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Abstract

The prognostic value of the fibroblast growth factor-inducible 14 (Fn14) expression in hepatocellular carcinoma (HCC) is unknown. Real-time PCR (RT-PCR), western blot assays and immunohistochemistry analysis were here performed in order to compare Fn14 expressios in paired liver samples of HCC and normal liver tissue. Most of the tumor tissues expressed significantly higher levels of Fn14 compared to adjacent non-tumor tissues, with Fn14High accounting for 54.6% (142/260) of all patients. The Pearson χ^2 test indicated that Fn14 expression was closely associated with serum alpha fetal protein (AFP) (*P*=0.002) and tumor number (p=0.019). Univariate and multivariate analyses revealed that along with tumor diameter and portal vein tumor thrombosis (PVTT) type, Fn14 was an independent prognostic factor for both overall survival (OS) (HR=1.398, *p*=0.008) and recurrence (HR=1.541, *p*=0.001) rates. Fn14 overexpression HCC correlated with poor surgical outcome, and this molecule may be a candidate biomarker for prognosis as well as a target for therapy.

Keywords: Fibroblast growth factor-inducible 14 - hepatocellular carcinoma - portal vein tumor thrombosis - prognosis

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Introduction

Fibroblast growth factor-inducible 14 (Fn14) is a 14-kDA type I transmembrane receptor located on chromosome 16p13 (Meighan-Mantha et al., 1999; Feng et al., 2000) and belongs to the TNF receptor superfamily. It lacks a death domain and contains the PIEET canonical TRAF (tumor necrosis factor receptor associated factors)-binding sequence that can recruit TRAF adapter proteins via binding of TRAF-1, 2, 3, 5, and then activates downstream signaling pathways of NF-κB as well as the MAPK and AKT pathways (Brown et al., 2003; Saitoh et al., 2003; Ando et al., 2006). Fn14 and TNF-like weak inducer of apoptosis (TWEAK) are a receptor-ligand pair with pleiotropic effects, mediating pro-inflammatory and pro-angiogenic activities as well as stimulating invasion, migration, and survival (Michaelson and Burkly, 2009). It promotes the invasion of breast tumors by up-regulating matrix expression of MMP-9 (Michaelson et al., 2005) and can protect glioma cells cultured with cytotoxic agents from apoptosis by inducing Bcl-2 family members (Tran et al., 2005). In addition, the role of Fn14 in glioma cell motility was associated with the activation of Rac1 and NF-κB signaling pathway (Tran et al., 2003; Tran et al., 2006). Fn14 was one of 12 disease progression correlating genes, with highest expression levels in esophageal adenocarcinoma (Wang et al., 2006) and positive correlated with breast tumor metastasis, positive lymph node status and HER2-positive/ER-negative breast tumors (Willis et al., 2008). Generally, there is increasing evidence that Fn14 expression is elevated in a variety of human tumors, such as breast cancer, malignant glioma, esophageal adenocarcinoma, and pancreatic cancer (Han et al., 2002; Tran et al., 2006; Watts et al., 2007; Willis et al., 2008). Previous studies revealed, that Fn14 is also overexpressed in poorly differentiated HCC cell lines (Feng et al., 2000) and the TWEAK/Fn14 pathway promoted the proliferation of multiple HCC cell lines in vitro (Kawakita et al., 2005). A recent investigation demonstrated, that the TWEAK/Fn14 pathway can stimulate liver progenitor cell (LPC) mitosis in the rapid growth phase during response to choline-deficient, ethionine-supplemented (CDE) diet-induced injury and re-generation in vivo (Tirnitz-Parker et al., 2010). Nonetheless, the prognostic value of Fn14 for HCC has yet to be explored and this study evaluates the clinical significance of Fn14 in HCC patients after curative resection.

Materials and Methods

Patients and Tissue Samples

A total of 341 adult patients with HCC undergoing

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hepatectomy by the same surgical team at the Eastern Hepatobiliary Surgery Hospital between January, 2000 and May, 2011 were enrolled in the study. The preoperative clinical diagnosis of HCC met the diagnostic criteria of the American Association for the Study of Liver Diseases (Bruix and Sherman, 2005; Bridges et al., 2011). Among the study patients, 81 HCC patients were recruited between January, 2008 and May, 2011, and their resected samples were subjected to RNA extraction for quantitative real time PCR (qRT-PCR) analysis (77 samples) and protein extraction for western-blot verification (4 samples). A total of 260 HCC patients with different types of portal vein tumor thrombosis (PVTT) were recruited between January, 2000 and January, 2004, while these patients met the following inclusion criteria and thus underwent TMA analysis: (a) a distinctive pathological diagnosis of HCC, (b) surgical resection, which was defined as complete resection of all tumor nodules with the cut surface being free of cancer based on histologic examinations (Poon et al., 2002), (c) complete clinicopathologic and follow-up data. The exclusion criteria included (a) extra hepatic spread and (b) prior anticancer treatments before liver resection.

Curative resection of HCC was performed as described (Shi et al., 2010; Li et al., 2012). First, all detected lesions were resected, and intraoperative ultrasound examinations revealed no remnant tumor. Second, negative surgical margins were confirmed based on the histological examinations. Thrombectomy was performed according to the location and extent of PVTT. For patients with PVTT located within the resected area, the PVTT was resected en bloc with the tumor, while in patients with PVTT that extended into the main portal vein beyond the resection line, the PVTT was extracted from the opened stump of the portal vein. For patients with PVTT that extended into the main portal trunk and its primary branches on both sides, the main portal trunk was exposed and clamped distal to the PVTT. The portal vein was then incised at the bifurcation of the right and left portal veins, and the PVTT was extracted. After flushing with normal saline and confirmation that no PVTT remained, the stump was closed with a continuous suture.

All patients received the same postoperative care in the intensive care unit during the early postoperative period. Subsequent need for intensive care was determined based on the patient's condition, while liver function tests and clotting profiles were monitored. Ethical approval was obtained from the Eastern Hepatobiliary Surgery Hospital research ethics committee and informed consent was obtained from each patient.

Real-time PCR and western blot

Fresh tissue samples were collected in the operating room and processed within 30 minutes to minimize RNA and protein degradation. Each fresh sample was transferred to liquid nitrogen and stored at -80°C until use. qRT-PCR was performed for Fn14 gene expression detection in paired liver samples from 77 Chinese HCC patients. Total RNA was isolated using the Trizol reagent (15596-026, Invitrogen, Carlsbad, CA) and reverse-transcribed to cDNA using the PrimeScript RT reagent

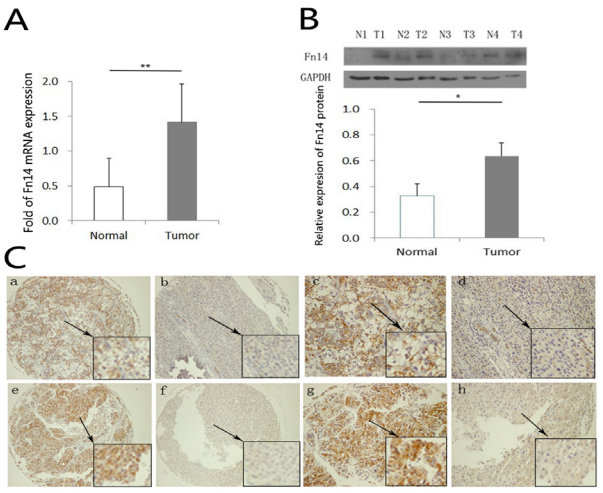

Figure 1. Overexpression of Fn14 in HCC Compared Normal Tissues. (A) qRT-PCR results of the relative Fn14 expression levels normalized by β-actin in 77 pairs of HCC and normal liver samples. (B) Western blot analysis of Fn14 in 4 paired HCC samples. (C) Immunohistochemical analysis of Fn14 expression in HCCs and adjacent non-tumor liver tissues. (a-d) Immunohistochemical staining of paired HCC and non-tumor tissues. Hepatocellular carcinoma cells in HCC were strongly Fn14 expression positive in the cytoplasm and cell membrane (a, c). Normal hepatocytes in the non-tumor tissue showed low Fn14 expression levels (b, d). (e-h) Immunohistochemical staining of paired HCC and non-tumor tissues. HCC cells were strongly Fn14 expression positive (e, g). Normal hepatocytes in the non-tumor tissue showed low Fn14 expression levels (f, h). Original magnifications: magnification×100 (a, b, e, f); magnification×200 (c, d, g, h)

Kit (DRR037A, Takara, Japan). The integrity of the total RNA was measured using Lab-on–a-Chip (Agilent, Palo Alto, CA). Only high-quality RNA with intact 18S and 28S RNA was used for sequent analysis. For RT-PCR, SYBR Premix Ex Taq (DRR081, Takara) was used according to the manufacturer's instructions. The primers were as follows: Fn14, forward: 5'-GCGCT CTGAG CCTGA CCTTCG-3' and reverse: 5'-GGTGG TGAAC TTCTC TCTCCTGC-3', 86bp; and β-actin, forward: 5'-GATCA TTGCT CCTCC TGAGC-3' and reverse: 5'-ACTCC TGCTT GCTGA TCCAC-3', 100bp. β-actin served as the internal control. A ratio of relative Fn14 messenger RNA (mRNA) levels in HCC samples/non-tumor liver samples of ≤1 was defined as under expression of the gene, whereas a ratio >1 was defined as overexpression. The relative expression levels of Fn14 in 77 pairs of HCC and normal liver samples is shown in Figure 1 A.

Cytoplasmic and nuclear protein were extracted using NE-PER Nuclear and Cytoplasmic Extractions Reagents (78833, Pierce, Rockford, IL) and Western blots were performed using a Fn14 specific polyclonal antibody (4403s, Cell Signaling Technology) and the GAPDH specific polyclonal antibody (KC-5G4, Kangcheng) as described previously (Dai et al., 2006). Images were captured using the Gel Dox XR system (Bio-Rad, Philadelphia, PA) (Figure 1 B).

TMAs and immunohistochemistry analysis

A tissue microarray composed of samples from 260 Chinese HCC patients with different types of PVTT

Table 1. Correlation Between Fn14 Staining and Clinicopathological Characteristics in 260 HCC Patients

Variables	Fn14 staining		
	High (n=142) n(%)	Low(n=118) n(%)	*p*
Age, years			
<60	129(90.8)	100(84.7)	0.131
≥60	13(9.2)	18(15.3)	
Gender			
Male	128(90.1)	106(89.8)	0.934
Female	14(9.9)	12(10.2)	
HBsAg			
Negative	18(12.7)	17(14.4)	0.684
Positive	124(87.3)	101(85.6)	
Liver cirrhosis			
No	23(16.2)	30(25.4)	0.066
Yes	119(83.8)	88(74.6)	
Serum AFP, ng/mL			
<20	10(7.0)	24(20.3)	0.002
≥20	132(93.0)	94(79.7)	
Tumor diameter(cm)			
≤5	20(14.1)	20(17.0)	0.736
>5, ≤10	71(50.0)	60(50.8)	
>10	51(35.9)	38(32.2)	
Tumor number			
Single	114(80.3)	107(90.7)	0.019
Multiple	28(19.7)	11(9.3)	
Tumor encapsulation			
No	105(73.9)	89(75.4)	0.785
Yes	37(26.1)	29(24.60	
PVTT type			
I	45(31.7)	44(37.3)	0.458
II	61(43.00	53(44.9)	
III	28(19.7)	15(12.7)	
IV	8(5.6)	6(5.1)	
Pathological satellites			
Absent	67(47.2)	69(58.8)	0.07
Present	75(52.8)	49(41.5)	
TNM stage			
II	15(10.6)	19(16.1)	0.192
III	125(88.0)	99(83.9)	
IV	2(1.4)	0	
cell differentiation			
II	5(3.5)	3(2.5)	0.284
III	127(89.4)	100(84.8)	
IV	10(7.1)	15(12.7)	

was used in this study. The classification of PVTT was as previously described (Shi et al., 2010). These patients underwent curative liver resections for primary tumors between January, 2000 and January, 2004 at the Eastern Hepatobiliary Surgery Hospital. The detailed clinicopathological characteristics of the patients are listed in Table 1.

Preoperative liver function of all patients was Child-Pugh A, and 225 (86.5%) had been infected with hepatitis B. The tumor stage was determined according to the 2010 International Union Against Cancer TNM classification and tumor differentiation was graded with the Edmondson grading system. After surgery, the patients were monitored until October 15, 2011 with a median follow-up of 8 months (range, 1-114 months). The resected specimens were paraffin-embedded and stored at 4°C and the construction of the tissue microarray (TMA) and protocol for immunohistochemistry were performed as previously described (Zhao et al., 2011). The expression of Fn14 was detected with an immunohistochemistry TMA assay, and the intensity of positive staining was measured as described elsewhere (Li et al., 2010); Fn14 expression was evaluated by two pathologists using a semi-quantitative scoring system. The intensity of staining was scored as 0 (negative), 1 (weak), 2 (medium), or 3 (strong). The extent of staining was scored as 0 (<5%), 1 (5%–24%), 2 (25%–49%), 3 (50%–74%), or 4 (≥75%), according to the percentages of the positive-staining tumor cells within each specimen in the tissue array. The sum of the scores for the intensity and extent of staining were determined. Tissues having a final staining score of less than 2 were considered ''negative'', and tissues having a score of greater than 2 were considered ''positive''. A sum score of 2 to 3 was considered ''weak'' (1+); 4 to 5 was ''moderate'' (2+); and 6 to 7 was ''marked'' (3+). The sum scores of most tumor tissues were determined as ''moderate'' or ''marked'', so 6 was used as the cut-off value to classify the intensity of Fn14 expression as either high or low (Fn14High: sum score≥6, and Fn14Low: sum score<6).

Follow-up

All patients had a uniform postoperative follow-up by the same team of surgeons and included a serum AFP assay, abdominal USG, and liver function test every month. A contrast CT scan was performed every 3 months for surveillance of recurrence and chest X-rays were also repeated every 3 months. When tumor recurrence or metastases were suspected, further investigations with CT scan, MRI, or positron emission tomography-CT scan were performed, while fine needle aspiration/biopsies were performed when necessary. The diagnosis of tumor recurrence was based on cytological/histological evidences or on the noninvasive diagnostic criteria for HCC used by the European Association for the Study of the Liver.

Patients with intrahepatic recurrence were treated aggressively with surgery, local ablative therapy, regional therapy, or systemic therapy, depending on the size, location and number of recurrent tumors as well as liver function status and presence of extra hepatic disease or tumor thrombus in the portal vein. Palliative treatment was administered to patients with advanced progress, poor liver function or poor general status.

Statistical analyses

Statistical analyses were performed with the SPSS 17.0 software (SPSS, Chicago, IL). The correlation between Fn14 expression and other clinicopathological characteristics was evaluated using χ^2 or the Fisher's exact tests for qualitative variables, and the Student t test or Mann-Whitney test for continuous variables. Overall survival (OS) was defined as the interval between HCC resection and death; patients who were alive at the end of follow-up were censored. The time to recurrence was calculated from the HCC resection to the first radiological evidence of recurrence. Patients experiencing death in the absence of recurrence were censored in determining recurrence (Llovet et al., 2008). Survival curves were calculated using the Kaplan-Meier method and compared using a log-rank test. The Cox proportional hazards model was used to determine the independent factors of

Table 2. Multivariate Analysis of Factors Associated with Overall Survival and Cumulative Recurrence in 260 HCC Patients

Variables[a]	OS	
	HR(95.0% CI)	*P*
Gender, Female vs. Male	1.667(1.076-2.583)	0.022
Age,<60 vs. ≥60 years	0.716(0.472-1.085)	0.115
Serum AFP,<20 vs. ≥20 ng/mL	0.943(0.633-1.403)	0.771
HBsAg, Negative vs. Positive	1.438(0.997-2.072)	0.052
Tumor diameter,<5, >5 and ≤10,>10cm	1.303(1.074-1.582)	0.007
Tumor encapsulation, Yes vs. No	1.438(1.069-1.934)	0.016
TNM stage, II, III, IV	1.145(0.754-1.740)	0.525
PVTT type, I, II, III, IV	1.256(1.072-1.472)	0.005
Fn14, low vs. high	1.398(1.090-1.793)	0.008
Variables[a]	Cumulative Recurrence	
	HR(95.0% CI)	*P*
Age,<60 vs. ≥60 years	0.723(0.486-1.076)	0.11
AST,≤37 vs. >37 U/L	1.094(0.783-1.527)	0.599
Serum AFP,<20 vs. ≥20 ng/mL	1.342(0.906-1.986)	0.142
HBsAg, Negative vs. Positive	1.508(1.039-2.191)	0.031
Tumor diameter,<5, >5 and ≤10,>10cm	1.259(1.044-1.519)	0.016
Tumor encapsulation, Yes vs. No	1.343(0.998-1.807)	0.052
Pathological satellites, Absent vs Present	1.121(0.865-1.453)	0.39
TNM stage, II, III, IV	1.026(0.697-1.511)	0.895
PVTT type, I, II, III, IV	1.194(1.028-1.386)	0.021
Fn14, low vs. high	1.541(1.187-2.002)	0.001

[a]Variables which were significant in the univariate analysis were adopted in the multivariate analysis using the forward LR method in a Cox proportional hazards regression model

survival and recurrence, based on the variables selected on the univariate analysis. $P<0.05$ was considered to be statistically significant.

Results

Overexpression of Fn14 in HCCs

The up-regulation of Fn14 mRNA was first confirmed in 77 paired tumor/non-tumor samples based on an qRT-PCR assays; 49 of the 77 (63.6%) HCC specimens showed Fn14 overexpression (tumor/non-tumor ratio >1.0) and the Fn14mRNA of tumor tissue was significant higher than the Fn14mRNA in normal tissue (Figure 1 A). To confirm a consistent expression model of Fn14 at the protein level, we next performed western blots on another 4 paired HCC samples. We found increased Fn14 expression in HCC samples compared with that in non-tumor tissues, and 3 (75%) patients were identified as Fn14 overexpressing (Figure 1 B). Finally, we assessed Fn14 expression in a TMA that included samples from 260 HCC patients. The immunohistochemistry results showed that Fn14 was primarily located in the membrane and the cytoplasm. Most of the tumor tissues expressed significantly higher levels of Fn14 compared to adjacent non-tumor tissues, with Fn14High accounting for 54.6% (142/260) of all the patients (Figure 1 C).

Association of Fn14 expression with clinicopathological characteristics

We next examined the relationship between

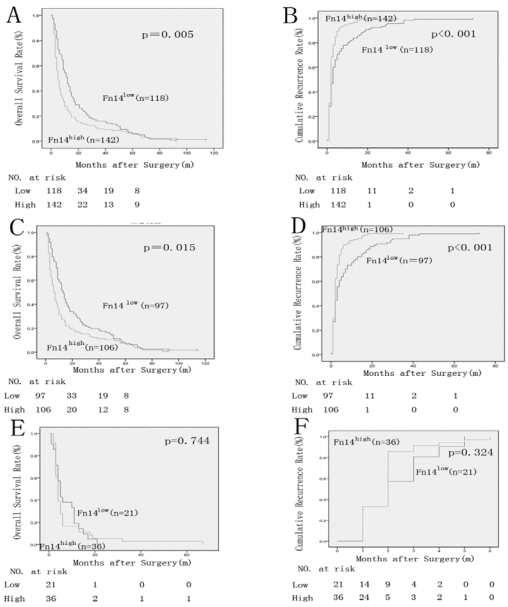

Figure 2. Prognostic Significance of Fn14 Assessed with Kaplan-Meier Analysis and Log-rank Tests. (A, B) Kaplan-Meier analysis of the correlation between Fn14 expression levels and overall survival or recurrence in 260 HCC patients. The overexpression of Fn14 predicts lower overall survival rate (A) and higher cumulative recurrence rate (B). (C-F) Kaplan-Meier analysis of overall survival and recurrence in the (C, D) PVTT type I+II group and (E, F) type III+IV group. The overexpression of Fn14 predicts higher cumulative recurrence rate in the PVTT type I+II group

Fn14 expression levels in tumor tissues and the clinicopathological characteristics of the 260 patients in the TMA analyses. The Pearson χ^2 test indicated that Fn14 expression was closely associated with serum AFP ($P=0.002$) and tumor number ($p=0.019$) (Table 1). These results suggest that tumors with higher serum AFP level and more tumor numbers are prone to higher Fn14 expression. Furthermore, we found that the expression of Fn14 did not correlate with other clinicopathological characteristics such as age, gender, HBsAg, liver cirrhosis, tumor diameter, tumor encapsulation, PVTT type, pathological satellites, TNM stage or tumor cell differentiation.

Association of Fn14 expression with prognosis

In the last follow-up in October, 2011, 100% (260/260) of the patients had suffered recurrence and 99.2% (258/260) had died with local or distant recurrence. The 1-, 3-, and 5-year OS and cumulative recurrence rates in the entire cohort were 34.2% and 89.2%, 12.7% and 98.1%, and 5.8% and 99.6%, respectively. Furthermore, the Fn14 expression levels were negatively correlated with the 1-, 3-, and 5-year survival rates (24.6%, 9.9% and 5.6%, respectively, for Fn14 overexpression versus 45.8%, 16.1% and 5.9%, respectively, for Fn14 non-overexpression). The 1-, 3- and 5-year cumulative recurrence rates in the Fn14 overexpression patients were higher than those in the Fn14 non-overexpression patients (95.8%, 100% and 100% versus 81.4%, 95.8% and 99.2%, respectively) (Figure 2 A and B). Univariate and multivariate analyses revealed, that along with tumor diameter and PVTT type, Fn14 was an independent

Table 3. Multivariate Analysis of Factors Associated with Overall Survival and Cumulative Recurrence in PVTT Type I and II Patients

Variables[a]	OS	
	HR(95.0% CI)	*P*
Gender, Female vs. Male	1.932(1.207-3.094)	0.006
Age,<60 vs. ≥60 years	0.665(0.424-1.043)	0.075
Serum AFP,<20 vs. ≥20 ng/mL	0.982(0.617-1.564)	0.939
Tumor diameter,<5, >5 and ≤10,>10cm	1.436(1.159-1.780)	0.001
Tumor encapsulation, Yes vs. No	1.631(1.169-2.276)	0.004
TNM stage, II, III	0.977(0.621-1.537)	0.919
Fn14, low vs. high	1.445(1.089-1.918)	0.011
Variables[a]	Cumulative Recurrence	
	HR(95.0% CI)	*P*
Age,<60 vs. ≥60 years	0.711(0.462-1.093)	0.120
Serum AFP,<20 vs ≥20 ng/mL	1.279(0.810-2.019)	0.290
HBsAg, Negative vs. Positive	1.548(1.028-2.331)	0.036
Tumor diameter,<5, >5 and ≤10,>10cm	1.373(1.112-1.694)	0.003
Tumor encapsulation, Yes vs. No	1.551(1.125-2.137)	0.007
TNM stage, II, III	1.117(0.714-1.748)	0.628
Fn14,low vs. high	1.647(1.226-2.212)	0.001

[a]Variables which were significant in the univariate analysis were adopted in the multivariate analysis using the forward LR method in a Cox proportional hazards regression model

prognostic factor for both OS (HR=1.398, *p*=0.008) and recurrence (HR=1.541, *p*=0.001) (Table 2).

Impact of Fn14 expression levels on prognosis of HCC patients with different PVTT types

All 260 patients were stratified according to a PVTT classification. Kaplan-Meier plots of patients with different PVTT types are shown in Figure 2 C-F. Of the 203 patients with type I and II, the 1-, 3-and 5-year cumulative recurrence rates were 86.7%, 97.5% and 99.5%, and the 1-, 3-and 5-year survival rates were 38.9%, 15.8% and 6.9%, respectively. Among these 203 patients, 106 were identified as overexpressing Fn14 and 97 were identified as non-overexpressing Fn14 in their tumors. The patients with Fn14 overexpression had a poorer surgical prognosis in contrast to those with Fn14 non-overexpression (94.3%, 100% and 100% versus 78.4%, 94.8% and 99.0% in the 1-, 3- and 5-year cumulative recurrence rates, respectively, *p*<0.001; 27.4%, 12.3% and 6.6% versus 51.5%, 19.6% and 7.2% in the 1-, 3- and 5-year survival rates, respectively, *p*=0.015) (Figure 2 C, D). Univariate and multivariate analyses revealed that along with tumor diameter and tumor encapsulation, Fn14 was an independent prognostic factor for both OS (HR=1.445, *p*=0.011) and recurrence (HR=1.647, *p*=0.001) (Table 3).

Of the 57 PVTT type III and IV patients, the comparison between the prognosis of patients with Fn14 overexpression (n=36) and those with Fn14 non-overexpression (n=21) (1-year cumulative recurrence rates, 100% versus 100%, *p*=0.324; 1-, 3- and 5-year survival rates, 16.7%, 2.8% and 2.8% versus 19.0%, 0% and 0%, *p*=0.744) did not show a statistical significance (Figure 2 E, F).

Discussion

In the present study, we showed that Fn14 was increased at both mRNA and protein levels in HCCs and associated with malignant clinicopathological characteristics. The correlation between Fn14 expression level and surgical outcomes was further investigated in a retrospective study of 260 HCC patients with different types of PVTT. We found that both tumor recurrence and survival rates substantially differed between patients with over and non-overexpression of Fn14 in tumor tissues. Multivariate analyses revealed that the Fn14 expression was an independent risk factor affecting recurrence and survival after curative resection, with the highest HR value for recurrence (HR 1.541, 95% CI 1.187-2.002; *P*=0.001) and the third highest value for survival (HR 1.398, 95% CI 1.090-1.793; *P*=0.008). Our data also showed, that high Fn14 expression in HCC tumors correlated with higher serum AFP levels, which might be explained by a RAR-AFP interaction and binding to the regulatory DNA region of the Fn14 gene in neoplastic tissues of HCC patients (Wang et al., 2012). HCC patients with various PVTT types had different prognoses. Patients with PVTT located in the segmental, sectoral, or right and or left portal veins (type I and II) showed significantly better survival rates than those with PVTT that extended to the main trunk of the portal vein or the superior mesenteric vein (type III and IV), which is in accordance with the literature (Shi et al., 2010). Therefore, we divided the 260 patients with different PVTT types in our study into two groups, using type II as a cutoff value. The analysis of 203 type I and II patients revealed that Fn14 overexpression also predicted shorter TTR (*P*<0.001) and survival (*p*=0.011). However, the impact of Fn14 expression level on the prognosis of the 57 PVTT type III and IV patients did not show statistical significance, what might be due to the fact that the number of type III and IV patients was small (n=57) and all of them had very short survival durations after surgical resection (≤6 months).

Otherwise, our data suggest that overexpression of Fn14 might be an indicator of poor outcome in HCC patients. Recently, also other research reported unfavorable Fn14 expressions in other carcinoma patients. Wang.et al. noted that there was a significant difference of Fn14 levels in patients categorized according to HER-2 expression rates, lymph node metastasis and clinical stage and suggested that Fn14 is a valuable marker of breast carcinoma progression (Wang et al., 2013) and others reported, that Fn14 was an important downstream regulator of HER2/HER3-driven breast cancer cell migration and invasion (Asrani et al., 2013). In addition, Fn14 overexpression was detected in human malignant ovarian tumors (Gu et al., 2013). Furthermore, Fn14 might be a novel therapeutic target for patients particularly with epidermal growth factor receptor (EGFR)-driven tumors, who suffer either primary or acquired resistance to EGFR tyrosine kinase inhibitors (TKIs) (Whitsett et al., 2012).

This study has some limitations. First, all of the patients had PVTT, which is a strong independent prognostic predictor for survival and recurrence, because all of them had relative shorter survival durations than

HCC patients without PVTT after surgical resection; Second, Figthe exact mechanism by which Fn14 functions in hepatocarcinogenesis is still unknown. In further studies, we intend to analyse correlations between clinicopathological parameters and clinical outcomes of Fn14 expression in HCC patients without PVTT and to investigate the mechanism Fn14 activity in hepatocarcinogenesis.

In conclusion, this study demonstrated that overexpression of Fn14 in HCC might be an indicator for more aggressive tumors and poorer clinical outcome. Fn14 could be a candidate biomarker for HCC prognosis and a target for therapy, especially in PVTT type I and II patients.

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