Int J Clin Exp Pathol 2017;10(1):760-769
www.ijcep.com /ISSN:1936-2625/IJCEP0039294

Original Article

Low expression level of Dickkopf 4 in hepatocellular carcinoma and its clinical significance

Dan Cui, Zhi Wang, Tao Wang, Xiaoyin Tang, Bo Zhai

Department of Interventional Oncology, Renji Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai, China

Received September 2, 2016; Accepted October 31, 2016; Epub January 1, 2017; Published January 15, 2017

Abstract: Purpose: To study the clinical significance of Dickkopf 4 in hepatocellular Carcinoma. Methods: The expression of DKK4 and beta-catenin was analyzed by real-time qPCR and immunohistochemistry. Patient's prognosis was evaluated through clinical follow-up. Results: DKK4 was expressed at a low level in HCC tissues, while beta-catenin was highly expressed ($P < 0.05$). The expression of beta-catenin and that of DKK4 at the protein level was negatively correlated, with $r = -0.4$, $P = 0.006$. The expression of DKK4 in enveloped tumor tissues with a diameter. Results: DKK4 was expressed at a low level in HCC tissues, while beta-catenin was h > 5 cm. The comparison of overall survival time between patients with high levels of DKK4 and those with low levels of DKK4 indicated that the former group had a longer total cumulative postoperative survival time ($P = 0.03$). Conclusion: The total survival time of patients with high levels of DKK4 was longer than those with the low levels. This suggested that DKK4 might be a tumor suppressor gene that might play a role in the proliferation and metastasis of HCC. And the expression level of DKK4 might be considered as a prognostic indicator for HCC.

Keywords: Hepatocellular carcinoma, DKK4, β-catenin, prognosis

Introduction

Liver cancer ranks the fifth common cancer among males and the ninth most frequent cancer among females [1]. Hepatocellular carcinoma (HCC) is the most common form of liver cancer. The incidence and mortality rates of HCC have increased significantly over the past two decades [2]. The lack of effective diagnostic methods for early detections and treatment options for advanced HCC might be the main reasons. Widely used markers, such as alpha-fetoprotein (AFP), have showed low sensitivity and poor diagnostic value [3]. Accordingly, new markers, which could be used for diagnosis at the early stage and potentially predicting prognosis, are needed.

Recently, numerous protein pathways have been found to involve in the development and progression of HCC. And Wnt/β-catenin signal transduction pathway is one pathway that plays a prominent role in HCC [4]. The Wnt/β-catenin signaling pathway is conserved throughout the animal kingdom and is involved in embryonic development. It also plays an important role in the maintenance of dynamic homeostasis in adult tissues [5] and participates in stem cell differentiation and self-renewal [6]. Abnormal activation of the Wnt signaling pathway can turn on many downstream oncogenes, which can lead to the development of cancerous cells. It is this pathway that is believed to play an important role in the development of HCC. In recent years, some studies have found that Dickkopf (DKK) proteins were related to HCC incidence through the Wnt/β-catenin signal transduction pathway. DKK1, 2, and 4 belong to the DKK protein family and participate in the regulation of the Wnt/β-catenin signaling pathway, but it is still not clear that whether or not DKK3 also plays a similar role [7]. Up until now, there was little research on how DKK4 specifically regulated the Wnt/β-catenin signaling pathway, although it was known to be an antagonist of the pathway.

Therefore, in this study, we performed a preliminary study on DKK4 mRNA and protein expression in HCC tissues to develop an initial understanding of the relationship between DKK4 and HCC. Our aim was to establish a foundation for

further studies on the molecular mechanisms of Wnt and to establish a theoretical basis for the development of serological assays and clinical applications.

Material and methods

Samples and reagents

Fresh tissue specimens from 36 male (median age 53.31 years) and 9 female (median age 50.83 years) patients were obtained from Renji Hospital of the Shanghai Jiaotong University School of Medicine. HCC and adjacent tissue (at least 2 cm from the edge of the cancerous tissue) specimens came from liver cancer hepatectomies from 2008-2011 (all tissues were hepatocellular with hepatitis B-related cirrhosis). Ten normal liver tissue samples were taken from 10 male patients (median age 30.01 years) with hepatic vascular tumor diseases in the absence of hepatitis B-related cirrhosis. All sample collection was approved by the hospital ethics committee and accompanied by signed informed consent. In addition to the fresh tissue samples, 224 liver cancer paraffin samples (tissue microarrays) from 192 male (median age 50.9 years) and 32 female patients (median age 46.4 years) were obtained from our hospital's pathology department. Anti-DKK4 polyclonal antibody (rabbit anti-human) was purchased from Abcam. The goat anti-rabbit secondary antibody kit (Zhongshan Golden Bridge Biotechnology Co. Ltd, Beijing), total RNA extraction kit D9108A, reverse transcription kit DRR037A, real-time PCR probe kit DRR390A, DKK4 gene probes, and primers were all purchased from Takara Company.

Real-time quantitative PCR monitoring of the relative expression of Dkk4 mRNA

Total RNA was extracted from tissues using the D9108A total RNA extraction kit. The purity and concentration of the RNA was measured by UV spectrophotometer and electrophoresis was used to verify its integrity. Two micrograms of total RNA was used for reverse transcription under the following conditions: 37°C for 15 min and 85°C for 5 sec. Two microliters of the reverse transcribed product was used as the template for real-time PCR. The upstream primer for the DKK4 gene was 5'-GACTGCAATACCAG AAAG-3', and the downstream primer was 5'-GCATCTTCCATCGTAGTA-3'. The DKK4 probe sequence was 5' (FAM)-CCGCGATGAGAAGCC-GTTCT (Eclipse)-3'. The upstream primer used for the reference gene β-actin was 5'-GAC-TACCTCATGA AGATCCTCACC-3', and the downstream primer was 5'-TCTCCTTAATGTCACGCA CGATT-3'. The beta-actin probe sequence was 5' (FAM)-CGGCTACAGCTTCA CACCACGGC (Eclipse)-3'. The reactions were set up as follows: Premix Ex Tap 10 μl, 0.4 μl each of the upstream and downstream primers for DKK4, 0.8 μl probe solution, 2 μl cDNA template, and sterile distilled water to a total reaction volume of 20 μl. The two-step reaction conditions were: 95°C denaturation for 30 sec, 40 reaction cycles of 95°C for 5 sec, 60°C for 30-31 sec. The PCR reaction was carried out using a CFX96 real-time quantitative PCR instrument for real-time monitoring of the intensity of fluorescence during each cycle's extension phase. Data analysis was automatically performed by the accompanying software. The housekeeping gene β-actin was used to determine the quality of the sample cDNA and as the internal reference.

IHC determination of DKK4 and β-catenin protein expression

IHC was performed using the streptavidin-biotin-peroxidase complex (SP) method. Paraffin sections were baked for an hour at 60°C and a LEIKA semiautomatic machine was used to carry out dehydration and dewaxing treatments for 40 min. A 3% hydrogen peroxide solution was used for 15 minutes to eliminate any endogenous peroxidase activity. Citrate was used to hot fix the antigen for 18 min in a microwave. After blocking with goat serum blocking solution, PBS was added to dilute the rabbit anti-human DKK4 polyclonal antibody (working concentration 1:100) and the rabbit anti-human β-catenin monoclonal antibody (working concentration 1:300). Both primary antibodies were allowed to bind overnight to samples. Secondary antibody and subsequent staining procedures were performed according to the goat anti-rabbit secondary antibody kit instructions. Staining times were controlled under an optical microscope to prevent non-specific background staining. PBS was used in lieu of primary antibody in the negative control sample. Five representative areas were selected at 200× magnification and each area was scored based on the intensity of cytoplasmic staining and the percentage of the area occupied by stained cells. DKK4 staining was evaluated based on the following criteria (5): no visible stain, 0 points; light yellow stain, 1 point; brownish yellow stain, 2 points; brown stain, 3

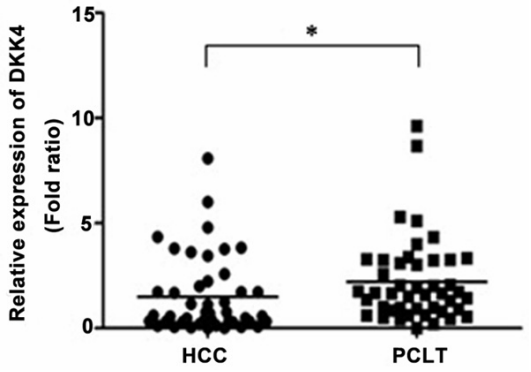

Figure 1. Comparison of the relative expression levels of DKK4 mRNA in different tissues. HCC: Hepatocellular Carcinoma, PCLT: Periphery of Cancerous Liver Tissue, *P < 0.05 is considered statistically significant.

Table 1. DKK4 expression in HCC, PCLT and NL samples by IHC

Group	Positive	Negative	Total	Positive Expression Rate PCL	*P* Value
HCC	12	32	44	27.2	< 7.2l
PCLT	21	23	44	47.7	
NL	6	4	10	60	

HCC: hepatocellular carcinoma, PCLT: periphery of cancerous liver tissue, NL: normal liver tissue.

points. To score the percentage of the area occupied by stained cells: no stained cells was scored as 0 points, < 20% stained cells was scored as 1 point, over 20% but less than 30% stained cells was scored as 2 points, more than 30% stained cells was scored as 3 points. If the total score was 5 or above, DKK4 was defined as highly expressed. A total score < 5 was defined as low DKK4 expression level. Beta-catenin was identified mainly by the location of stain in the cells.

Clinical studies and follow-up

Fresh biopsy specimens and paraffin-embedded sections from the pathology department were HE stained and evaluated independently by two pathologists. The pathologists looked for the presence of an envelope and the integrity of the envelope, whether the sample exhibited cirrhosis or microscopic venous invasion, and the degree of tumor differentiation (Edmondson grade, grade I-II cancer cells are morphologically similar to normal liver cells with a high degree of differentiation; grade III-IV cancer cells have large and deeply stained nuclei, little cytoplasm, and are poorly differentiated). Samples with no observable envelope (by eye) or incomplete envelope (under optical microscope) were defined as having no envelope. Venous invasion was defined as perivascular infiltration of cancerous cells with discontinuous staining of vascular endothelial cells under the light microscope, or the presence of thrombus within the lumen. Tumor diameter reflects the maximum diameter of the tumor tissue. Patient survival time was calculated from the first day after surgery and subsequent patient information was obtained through clinical visits, telephone, email, etc. In the event of patient death or if a patient was lost to follow-up, the case was considered to have ended. The final follow-up date was December 2015, and patient follow-up time ranged from 48-84 months.

Statistical analysis

SPSS 19.0 statistical software was used for statistical analysis. Data are shown as mean ± standard deviation. Variance analysis was used to test the difference in expression of the DKK4 mRNA in liver tumors and adjacent tissues. The non-parametric Mann-Whitney U test was used to analyze the correlation between DKK4 mRNA and the pathological features of liver cancer, as well as the correlation between the expression of DKK4 protein and β-catenin protein. The chi-square test was used to test the relationship between DKK4 protein expression and clinical pathological features of liver cancer. The Kaplan-Meier test was used to calculate the post-surgery survival rate. The log-rank test was used to calculate the difference between groups. Correlation analyses were performed using Pearson's test. All statistical methods used two-tailed P < 0.05 as the boundaries for statistical significance.

Results

Expression of Dkk4 mRNA in liver tumors and adjacent tissues

A comparison between 45 samples of freshly biopsied HCC tissues and their adjacent tissues revealed that the expression of DKK4 was undetectable in only 1 sample. Of the remaining 44 samples, 56.8% (25 of 44 samples) of the liver cancer tissues had comparatively decreased DKK4 mRNA expression when compared to a corresponding sample of adja-

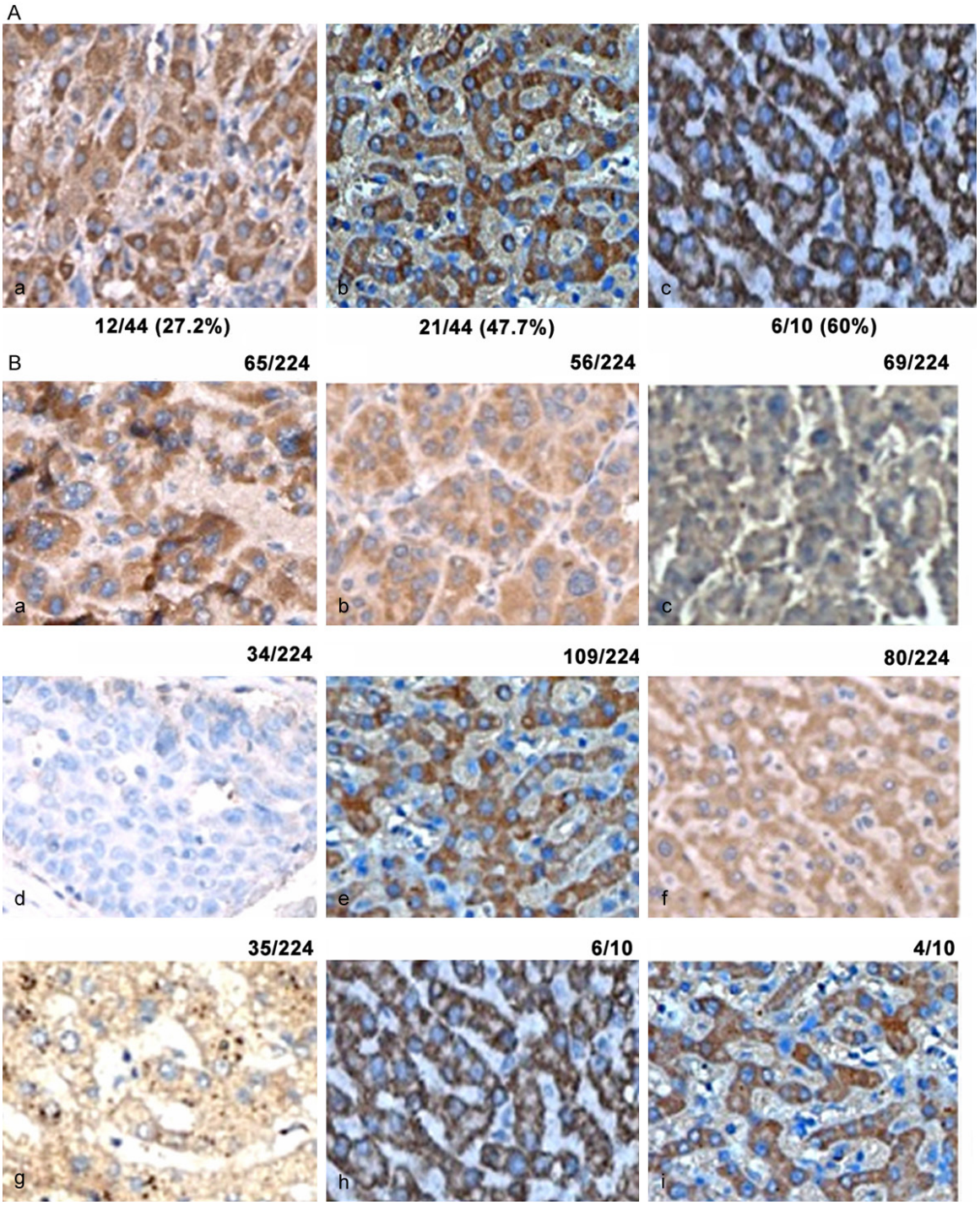

Figure 2. A: Comparison of DKK4 protein expression in liver cancer tissues, peripheral tissues, and normal tissues. a: Liver cancer tissue; b: Periphery of cancerous liver tissue, c: Normal liver tissue. B: IHC detection of DKK4 protein expression in liver cancer tissues and adjacent tissues in 224 patient samples (200× magnification). a, b, c, d: Liver cancer tissues; e, f, g: Periphery of cancerous liver tissue; h, i: Normal liver tissue. a, e, h: Show high expression; b, c, d, f, g, i: Show low expression.

cent tissue. Overall, DKK4 expression at the mRNA level was lower in HCC tissues than in corresponding adjacent tissues ($P < 0.05$, **Figure 1**).

Table 2. DKK4 expression in HCC, PCLT and NL from tissue microarray

Group	Positive	Negative	Total	Positive Expression Rate (%)	*P* Value
HCC	65	159	224	29.1	< 9.11
PCLT	109	115	224	48.5	
NL	6	4	10	60	

HCC: hepatocellular Carcinoma, PCLT: periphery of cancerous liver tissue, NL: normal liver tissue.

Table 3. Expression of β-catenin protein in HCC and PCLT

Group	Positive	Negative	Total	Positive Expression Rate osi	*P* Value
HCC	25	19	44	56.8	< 6.81
PCLT	14	30	44	31.8	

HCC: hepatocellular carcinoma, PCLT: periphery of cancerous liver tissue.

Table 4. Correlation between DKK4 and β-catenin protein expression in HCC

DKK4	Cases	β-catenin	
		Positive	Negative
Positive	12	3	9
Negative	32	22	10
Total	44	25	19

Expression of DKK4 protein in liver cancer tissues, adjacent tissues, and normal liver tissues

Using our IHC setup, the presence of brown stain indicated that the sample was positive for DKK4 protein, which is mainly expressed in the cytoplasm. Of the 44 HCC samples that were positive for DKK4 mRNA, 12 HCC samples were positive for DKK4 protein (27.2% positive expression rate). Of the samples obtained from areas adjacent to the tumor, 21 were positive for DKK4 protein (47.7% positive expression rate), while 10 samples of normal liver tissues also tested positive (60% positive expression rate). DKK4 protein showed lower expression in liver cancer tissues when compared to neighboring tissues and normal liver tissues (P < 0.05, **Table 1**; **Figure 2A**).

Further analysis of tissue microarrays from 224 liver cancer samples and their adjacent tissues showed 65 cases in which DKK4 was positively expressed in cancerous tissues (29.1% positive rate) and 109 cases in which DKK4 was positively expressed in adjacent liver tissues (48.5% positive rate). There were 6 cases in which DKK4 was positively expressed in normal liver tissues (60% positive rate). As a whole, the expression of DKK4 protein was lower in liver cancer tissues than in neighboring and normal liver tissues. (P < 0.05, **Table 2**; **Figure 2B**).

Expression of β-catenin protein in liver cancer tissues and adjacent tissues

In normal liver tissues, our IHC protocol stains β-catenin as brown particles located on the cell membrane. There was very little cytoplasmic staining and no nuclear staining. In liver cancer tissues, β-catenin was normally located in the cell nucleus or cytoplasm, with very little found at the cell membrane. Of the 44 cases of freshly biopsied liver cancer samples, β-catenin protein expression was found in 56.8% (25/44) of the samples, of which 20 samples showed nuclear staining and 5 cases had cytoplasmic staining. The rate of β-catenin protein expression in adjacent liver tissues was 31.8% (14/44), of which 11 samples showed cell membrane staining, 3 samples had cytoplasmic staining, and there was no samples with nuclear staining. The β-catenin protein was expressed at a higher level than in normal liver tissues peripheral (P < 0.05, **Table 3**).

Correlation between DKK4 and β-catenin protein expression in HCC

By comparing IHC results of DKK4 and β-catenin protein expression from the above-described samples of liver cancer tissues, we found that the expression level of DKK4 and β-catenin in the cell nucleus was negatively correlated (P = 0.006, r = -0.4, **Table 4**). Cases in which DKK4 positively expressed at high levels had low β-catenin expression in the nucleus, while cases in which DKK4 low expressed showed higher β-catenin expression. This further demonstrated that DKK4 might play a role in modulating β-catenin nuclear entry (**Figure 3**).

Relationship between DKK4 mRNA expression and HCC clinical pathology

HCC tissue samples were grouped according to different clinical pathological features and

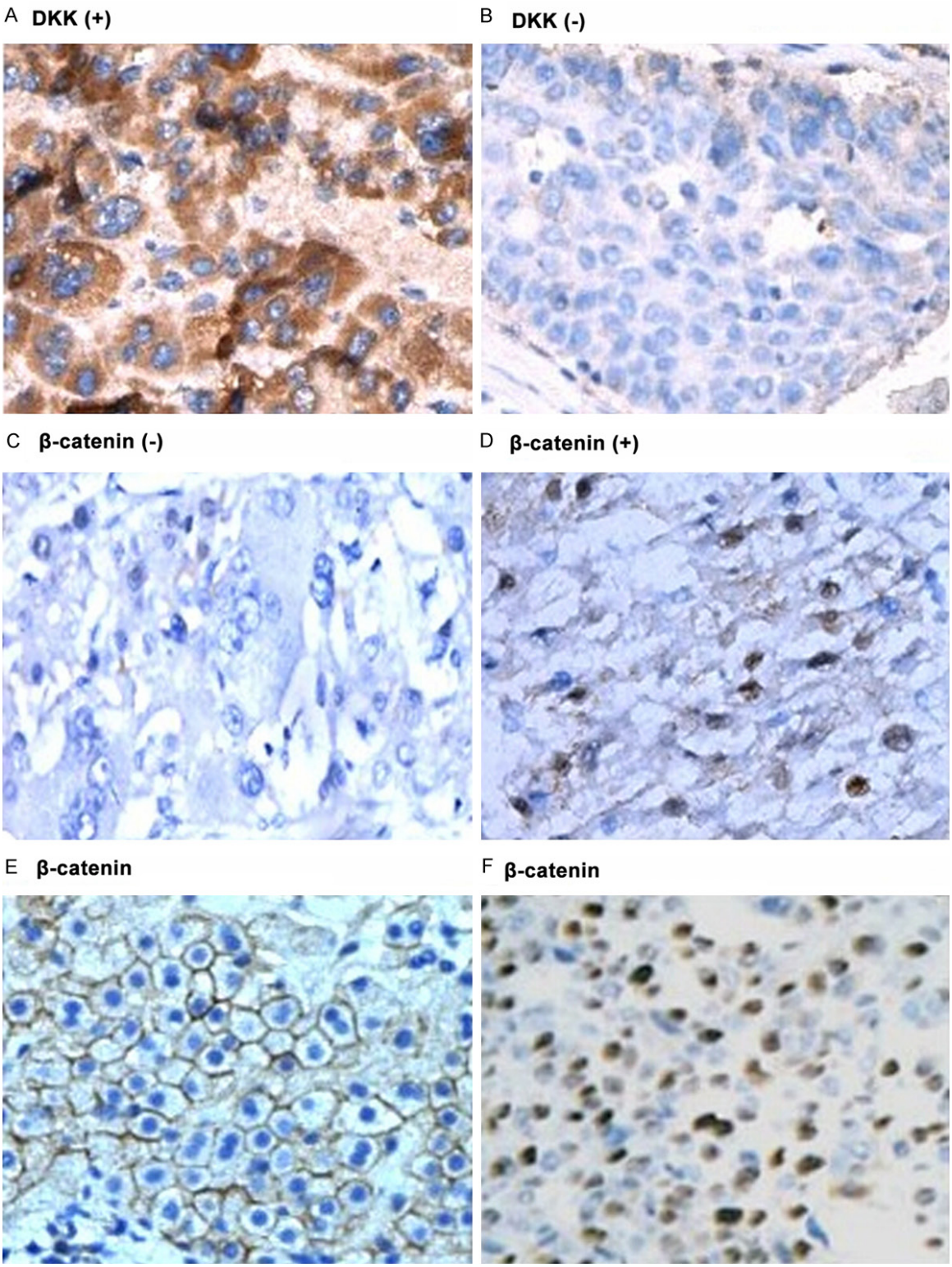

Figure 3. DKK4 and β-catenin protein expression in the same tissue type. A and C: Are from the same type of liver cancer tissue; B and D: Are from the same type of liver cancer tissue; E: Normal liver tissue; F: Liver cancer tissue.

compared for the expression of DKK4 mRNA between groups. The results showed that the level of expression of DKK4 mRNA was not significantly related to gender, age, tumor size,

Table 5. Correlation between level of DKK4 mRNA expression and clinical pathological features of hcc patients

Clinical Pathological Indicator	Cases	Median Expression level	*P* Value
Gender			0.819
Men	35	0.076	
Women	9	0.074	
Age (year)			0.074
< 55	21	0.2611	
≥ 55	23	0.144	
Tumor Size (cm)			0.486
≥ 5	20	0.2143	
< 5	24	0.1465	
AFP (ng/ml)			0.602
Positive (< 10)	19	0.1738	
Negative (≥ 10)	25	0.1871	
Envelope			0.322
Yes	32	0.1519	
No	12	0.2803	
Degree of Differentiation			0.07
Low Differentiation	27	0.1243	
High Differentiation	17	0.2926	
Vascular Invasion			0.865
Yes	9	0.216	
No	35	0.1733	

AFP, degree of completion of the tumor envelope, degree of differentiation, or vascular invasion (P > 0.05, **Table 5**).

Correlation between the expression of DKK4 protein in HCC tissues and clinical pathology

HCC tissue specimens were grouped according to their clinical pathological features and compared with the level of DKK4 protein expression. Our results showed that DKK4 protein expression was higher in tumors with diameters ≤ 5 cm (P = 0.037) and in HCC tissues with no envelope (P = 0.025). DKK4 protein expression was not significantly related to gender, age, liver cirrhosis, number of tumors, tumor differentiation, vascular invasion, TNM classification, or AFP (P > 0.05, **Table 6**).

Analysis and significance of DKK4 protein expression and clinical prognosis

We compared the post-operative survival time between the group (N = 65) that expressed high levels of DKK4 to the low DKK4 expression group (N = 159). The result showed that the cumulative post-surgical survival time in the high DKK4 expression group was longer than in the low expression group (P = 0.03). The median survival times were 51 months and 39 months for the high expression and low expression groups, respectively (**Figure 4**).

Discussion

Recurrence and metastasis were the major difficulties in treating HCC in patients, as the recurrence rate could be as high as 60.8%, which seriously affected the clinical efficacy [8, 9]. Research on the mechanism of HCC recurrence and metastasis was very important for the improvement of HCC prognosis.

Initiation and development of HCC were multistep processes that involved many biological and pathological events. It had been found in recent years that the activation of the Wnt signaling pathway played an important role in the initiation and development of HCC. Wnt was a family of secreted glycoproteins with autocrine and paracrine functions. When Wnt binded to the Frizzled receptor and its co-receptor LRP5 or LRP6 on the cell membrane, the intracellular phosphoprotein disheveled (Dsh) was phosphorylated and inhibits the kinase activity of glycogen synthase kinase-3β (GSK3β).

The protein β-catenin was not easy to phosphorylate because the target serine and threonine residues were not easy to access. Therefore, cytoplasmic levels of β-catenin were relatively stable. Increased levels of cytoplasmic β-catenin resulted in nuclear entry, where β-catenin forms complexes with T-cell factor (TCF) and lymphocyte enhancer factor (LEF) in the nucleus, then binded to the regulatory regions of the Wnt signaling pathway target genes, turning on the transcription and translation of downstream genes. In the absence of Wnt, the proteins Axin, GSK3β, and the tumor suppressor protein APC would form a ternary complex that activates GSK3β, which then phosphorylates the serine and threonine in β-catenin, leading to ubiquitination and degradation of β-catenin in the proteasome, ultimately resulting in the shutdown of the Wnt signaling pathway.

As an antagonist of the Wnt signaling pathway, DKK4 could compete for the binding of LRP5/6 with Wnt and prevent the formation of the

Table 6. Relationship between DKK4 protein expression and HCC clinical pathological features

Clinical Pathological Indicator	High Expression	Low Expression	*P* Value
Gender			0.519
Men	47	108	
Women	18	51	
Age (year)			0.082
< 50	24	79	
≥ 50	41	80	
Liver Cirrhosis			0.743
Accompanied with	50	119	
Not accompanied with	15	40	
Tumor Diametermpa (cm)			0.037*
< 5	35	109	
≥ 5	30	50	
Number of Tumors			0.097
Multiple	16	30	
Single	49	129	
Envelope			0.025*
Yes	29	97	
No	36	62	
Degree of Differentiation			0.458
High Differentiation	37	99	
Low Differentiation	28	60	
Vascular Invasion			0.667
Yes	19	42	
No	46	117	
AFP (ng/ml)			0.524
> 400	35	93	
≤ 400	30	66	
TNH			0.934
I-II	38	92	
III-IV	27	67	

LRP6, Wnt and Frizzled complex. This then blocked downstream signal transduction. DKK4 might also form a new ternary complex with Kremen and LRP6, resulting in the removal of LRP6 from the cell membrane through endocytosis, which blocked further Wnt-mediated signal transduction. DKK4 was also part of a negative feedback regulatory mechanism that was often defective in primary liver cancers [10]. The specific molecular mechanisms were poorly understood. Also, the expression of DKK4 could be promoted through activation of the JNK pathway, suggesting that there may be other pathways through which DKK4 can function.

Our results showed that there was a decrease in the expression of DKK4 mRNA in 56.8% of liver cancer tissues when compared with adjacent normal tissues. Fatima et al studied the expression of DKK4 in vitro cells and murine HCC cell models and found a decrease in the expression of DKK4 in both models. The decreased expression had a significant effect on the proliferation, migration, and growth of in vitro and in vivo murine HCC cells. These results had been confirmed by Liao et al [11]. These studies indicated that DKK4 might be a tumor suppressor gene and that its overexpression inhibited the growth of cancer cells. However, the specific mechanism driving the reduced expression of DKK4 was still not clear. Also, the relationship between the expression of DKK4 and β-catenin was still not well-defined in clinical samples.

DNA methylation played an important role in the initiation of liver cancer. Methylation of the DKK1-DKK3 genes played a role in gastrointestinal tumor formation, but so far, there had few studies on DKK4 [12, 13]. Chromosomal fragments 8p21.3-922 and 8p23 were often missing in HCC, and the 8p11.2 region had been shown to contain a tumor suppressor gene [14]. The location of the DKK4 gene in the chromosome was 8p11.2-p11.1 [15]. Our IHC experiments showed that the percentage of positive DKK4 expression in HCC tissues is 27.2%, while it is 47.7% in adjacent normal tissues. However, the percentage of positive DKK4 expression was 60% in normal liver tissues. This indicated that DKK4 protein was expressed at a significantly lower level in HCC tissues than in corresponding adjacent tissues and normal liver tissues.

In addition, our study also found that in HCC tissue, 56.8% of cells was positive for β-catenin protein expression and that β-catenin was mostly found in the nucleus (80%). In adjacent tissues, 31.8% of cells expressed β-catenin, with no nuclear staining. Therefore, β-catenin expression was higher in liver cancer tissues than in corresponding adjacent tissues. Interestingly, the expression of DKK4 protein was negatively correlated to the expression of β-catenin protein, where samples with no DKK4

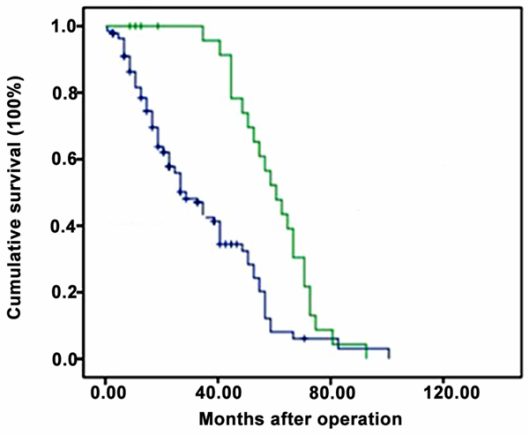

Figure 4. Comparison of post-operative total cumulative survival time between groups of patients who showed high or low expression of DKK4. Green represents over expression, Blue represents down expression.

protein expression had high β-catenin protein expression and nuclear staining. This further suggested that DKK4 could inhibit the entry of β-catenin into the nucleus.

Following activation of the Wnt/β-catenin signaling pathway, the quantity of β-catenin did not necessarily increase. It was the subcellular localization of the protein that enables β-catenin to play its regulatory roles. There might be other factors involved in the regulation of β-catenin by DKK4 in the Wnt/β-catenin pathway, such as WIF-1 [16], SFRPs [17], and others which abnormally silenced in HCC. So far, it was unclear whether these other factors affected the expression of β-catenin. Hirata et al [18] found that DKK4 activated the JNK signaling pathway while inhibiting the Wnt/β-catenin signaling pathway in renal carcinoma, suggesting that DKK4 might be involved in both pathways in the genesis of liver cancer.

DKK4 function differed between tissues. Unlike in liver cancer, DKK4 was highly expressed in renal cancer tissues. This might be due to the regulatory activity of DKK4 was not limited to the Wnt/β-catenin signal transduction pathway.

Our study also compared the relationship between DKK4 mRNA levels, protein expression, and clinical pathological features. In addition, we also showed that DKK4 might be a tumor suppressor gene that might play a role in the recurrence and metastasis of liver cancer. DDK4 might be relevant for disease prognosis and might be used as a clinical index for HCC.

In summary, our study showed that DKK4 mRNA was low expressed in HCC tissues. The expression of DKK4 protein was negatively correlated to the expression of β-catenin protein, where samples with no DKK4 protein expression had high β-catenin protein expression and nuclear staining. Also the total survival time of patients who expressed high levels of DKK4 was longer than those with low levels. This suggested that DKK4 was a tumor suppressor gene that might play a role in the proliferation and metastasis of HCC. And DKK4 expression levels might be considered as a prognostic indicator for HCC.

Acknowledgements

This research was supported by a grant from The National Natural Science Foundation of China (No. 81472845).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Bo Zhai, Department of Interventional Oncology, Renji Hospital, School of Medicine, Shanghai Jiaotong University, 160# Pujian Road, Shanghai 200127, China. Tel: 0086-21-68383131; Fax: 0086-21-68383131; E-mail: zhaiboshi@sina.com

References

[1] Siegel R, Ma J, Zou Z and Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014; 64: 9-29.

[2] Ferenci P, Fried M, Labrecque D, Bruix J, Sherman M, Omata M, Heathcote J, Piratsivuth T, Kew M, Otegbayo JA, Zheng SS, Sarin S, Hamid SS, Modawi SB, Fleig W, Fedail S, Thomson A, Khan A, Malfertheiner P, Lau G, Carillo FJ, Krabshuis J and Le Mair A. Hepatocellular carcinoma (HCC): a global perspective. J Clin Gastroenterol 2010; 44: 239-245.

[3] Gupta S, Bent S and Kohlwes J. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C. A systematic review and critical analysis. Ann Intern Med 2003; 139: 46-50.

[4] Whittaker S, Marais R and Zhu AX. The role of signaling pathways in the development and treatment of hepatocellular carcinoma. Oncogene 2010; 29: 4989-5005.

[5] Logan CY and Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 2004; 20: 781-810.
[6] Reguart N, He B, Taron M, You L, Jablons DM and Rosell R. The role of Wnt signaling in cancer and stem cells. Future Oncol 2005; 1: 787-797.
[7] Fatima S, Lee NP and Luk JM. Dickkopfs and Wnt/beta-catenin signalling in liver cancer. World J Clin Oncol 2011; 2: 311-325.
[8] Belghiti J, Regimbeau JM, Durand F, Kianmanesh AR, Dondero F, Terris B, Sauvanet A, Farges O and Degos F. Resection of hepatocellular carcinoma: a European experience on 328 cases. Hepatogastroenterology 2002; 49: 41-46.
[9] Kew MC. Epidemiology of hepatocellular carcinoma. Toxicology 2002; 181-182: 35-38.
[10] Krupnik VE, Sharp JD, Jiang C, Robison K, Chickering TW, Amaravadi L, Brown DE, Guyot D, Mays G, Leiby K, Chang B, Duong T, Goodearl AD, Gearing DP, Sokol SY and McCarthy SA. Functional and structural diversity of the human Dickkopf gene family. Gene 1999; 238: 301-313.
[11] Liao CH, Yeh CT, Huang YH, Wu SM, Chi HC, Tsai MM, Tsai CY, Liao CJ, Tseng YH, Lin YH, Chen CY, Chung IH, Cheng WL, Chen WJ and Lin KH. Dickkopf 4 positively regulated by the thyroid hormone receptor suppresses cell invasion in human hepatoma cells. Hepatology 2012; 55: 910-920.
[12] Yang B, Du Z, Gao YT, Lou C, Zhang SG, Bai T, Wang YJ and Song WQ. Methylation of Dickkopf-3 as a prognostic factor in cirrhosis-related hepatocellular carcinoma. World J Gastroenterol 2010; 16: 755-763.
[13] Hirata H, Hinoda Y, Nakajima K, Kawamoto K, Kikuno N, Kawakami K, Yamamura S, Ueno K, Majid S, Saini S, Ishii N and Dahiya R. Wnt antagonist gene DKK2 is epigenetically silenced and inhibits renal cancer progression through apoptotic and cell cycle pathways. Clin Cancer Res 2009; 15: 5678-5687.
[14] Zhang LH, Qin LX, Ma ZC, Ye SL, Liu YK, Ye QH, Wu X, Huang W and Tang ZY. Allelic imbalance regions on chromosomes 8p, 17p and 19p related to metastasis of hepatocellular carcinoma: comparison between matched primary and metastatic lesions in 22 patients by genome-wide microsatellite analysis. J Cancer Res Clin Oncol 2003; 129: 279-286.
[15] Pineau P, Nagai H, Prigent S, Wei Y, Gyapay G, Weissenbach J, Tiollais P, Buendia MA and Dejean A. Identification of three distinct regions of allelic deletions on the short arm of chromosome 8 in hepatocellular carcinoma. Oncogene 1999; 18: 3127-3134.
[16] Ding Z, Qian YB, Zhu LX and Xiong QR. Promoter methylation and mRNA expression of DKK-3 and WIF-1 in hepatocellular carcinoma. World J Gastroenterol 2009; 15: 2595-2601.
[17] Takagi H, Sasaki S, Suzuki H, Toyota M, Maruyama R, Nojima M, Yamamoto H, Omata M, Tokino T, Imai K and Shinomura Y. Frequent epigenetic inactivation of SFRP genes in hepatocellular carcinoma. J Gastroenterol 2008; 43: 378-389.
[18] Hirata H, Hinoda Y, Majid S, Chen Y, Zaman MS, Ueno K, Nakajima K, Tabatabai ZL, Ishii N and Dahiya R. DICKKOPF-4 activates the noncanonical c-Jun-NH2 kinase signaling pathway while inhibiting the Wnt-canonical pathway in human renal cell carcinoma. Cancer 2011; 117: 1649-1660.