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Original Paper

The Prognostic Significance of NEK2 in Hepatocellular Carcinoma: Evidence from a Meta-Analysis and Retrospective Cohort Study

YuSheng Cheng[a,b] XiaoLong Chen[a,b] LinSen Ye[a,b] YinCai Zhang[a,b] Jing Liang[c] Wei Liu[a,b] BoXuan Zhou[a] ShiYang Zheng[a] Yiming Huang[a,b] GuiHua Chen[a,b] YiNan Deng[a,b] Qi Zhang[b,d] Yang Yang[a,b]

[a]Department of Hepatic Surgery, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, [b]Guangdong Provincial Key Laboratory of Liver Disease Research, Guangzhou, [c]Department of Pathology, Guangdong Key Laboratory of Liver Disease Research, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, [d]Cell-gene Therapy Translational Medicine Research Center, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China

Key Words
NEK2 • Hepatocellular carcinoma • Prognosis • Meta-analysis

Abstract
Background/Aims: Numerous studies have shown that *NIMA-related kinase 2* (*NEK2*) expression in hepatocellular carcinoma (HCC) tissue is associated with survival and clinicopathological features; however, the evidence remains inconclusive. Thus, we aimed to further explore the prognostic and clinicopathological significance of *NEK2* expression in HCC using a two-part study consisting of a retrospective cohort study and a meta-analysis. ***Methods:*** In the cohort study, *NEK2* expression in 206 HCC samples and adjacent normal liver tissues was detected by immunohistochemistry (IHC). Patients were divided into a high *NEK2* expression group and a low *NEK2* expression group by the median value of the immunohistochemical scores. The Kaplan–Meier method with the log-rank test was used to analyze survival outcomes in the two groups, and multivariate analysis based on Cox proportional hazard regression models was applied to identify independent prognostic factors. In the meta-analysis, eligible studies were searched in PubMed, EMBASE, Web of Science, and CNKI databases. STATA version 12.0 (Stata Corporation, College Station, TX) was used for statistical analyses. ***Results:*** The IHC results of our cohort study showed higher *NEK2* expression in HCC tissues compared with adjacent normal liver tissues. Multivariate analysis revealed that high *NEK2* expression was an independent risk factor for poor overall survival (OS) [hazard ratio (HR) = 1.763; 95% CI, 1.060–2.935; P = 0.029] and disease-free survival (DFS) [hazard ratio (HR) = 1.687; 95% CI,

Y.S. Cheng, X. Chen and L. Ye contributed equally to this work.

Yang Yang, Qi Zhang and YiNan Deng

Guangdong Provincial Key Laboratory of Liver Disease Research
Guangzhou (China); Tel. +86 20 85253106, Fax +86 20 85252276,
E-Mail yysysu@163.com; kee_kee@126.com; dengyinan2010@163.com

1.102–2.584; P = 0.016] in HCC patients. A total of 11 studies with 1,698 patients were enrolled in the meta-analysis, consisting of 10 studies from the database search and our cohort study. The pooled results revealed that high *NEK2* expression correlated closely with poor OS among HCC patients (HR = 1.47; 95% CI, 1.21–1.80; P < 0.01), and DFS/recurrence-free survival (RFS) (HR = 1.92; 95% CI, 1.41–2.63; P < 0.01). Additionally, our meta-analysis also showed that the proportion of HCC patients with high *NEK2* expression was greater in the group with larger tumors (>5 cm) than in the group with smaller tumors (≤ 5 cm) [odds ratio (OR) = 2.02; 95% CI, 1.13–3.64; P < 0.01). ***Conclusion:*** Our study demonstrated that high *NEK2* expression is a risk factor for poor survival in HCC patients. More prospective, homogeneous, and multi-ethnic studies are required to validate our findings.

Introduction

Hepatocellular carcinoma (HCC) is the second most common cause of cancer-related death in men and is the sixth most common cause in women worldwide [1]. East Asia and Africa have the highest rates of HCC in the world [2]. Nearly 55% of all HCC cases in the world occur in China [3]. Moreover, the incidence of HCC is growing worldwide, especially in the United States and Europe [2, 4]. HCC is characterized by fast infiltrating growth, poor differentiation, and early metastasis [5]. Despite substantial improvements in terms of early diagnosis and therapy, HCC patients still have an unfavorable oncological prognosis [6, 7]. Thus, it is essential that the mechanisms responsible for the progression of HCC are further elucidated to enable the development of novel therapeutic targets for HCC.

NIMA-related kinase 2 (*NEK2*), a member of the *NIMA* family of serine/threonine kinases located in the centrosome, plays a crucial part in modulating the cell cycle and mitosis [8]. In addition, *NEK2* is also involved in B cell development and immunological responses [9], as well as chromatin condensation, spindle assembly checkpoint, microtubule organization and stabilization, kinetochore attachment, and centrosome duplication [10-12]. Greater *NEK2* activity could cause chromosomal instability and abnormal chromosomal replication and rearrangement, which are closely linked with the initiation and progression of cancer [13, 14]. A body of evidence indicates that *NEK2* is overexpressed in many malignancies and its expression level inversely correlates with prognosis, including in colorectal cancer, non-Hodgkin lymphoma, multiple myeloma, lung cancer, prostate cancer breast cancer, glioma and pancreatic ductal cancer [15-22]. Moreover, it was reported that elevated *NEK2* expression could contribute to drug resistance in multiple myeloma and triple-negative breast cancer [21, 23]. These findings indicate that *NEK2* is a vital oncogene and may be a potential therapeutic target for cancer treatment.

Recent reports have suggested that *NEK2* expression is related to survival in HCC patients; however, the evidence remains inconclusive. Some studies have suggested that *NEK2* is overexpressed in HCC tissues compared with adjacent normal tissues and is associated with poor survival [24-31]. In addition, there is evidence showing that elevated *NEK2* expression could enhance stem-like properties, drug resistance, and the progression of HCC [27, 28]. However, a study by Fu et al. reported that HCC patients with high *NEK2* expression had better survival [32].

To date, the prognostic and clinicopathological significance of *NEK2* in HCC remains controversial. Thus, we conducted a two-part study that comprising a retrospective cohort study and a meta-analysis to further explore the prognostic and clinicopathological significance of *NEK2* in HCC patients.

Materials and Methods

Study population

This study was approved by the institutional review board of the Third Affiliated Hospital of Sun Yat-sen University, and written informed consent was obtained from the 206 patients who underwent hepatectomy for HCC between January 2009 and December 2012. These patients were divided into high *NEK2* expression and low *NEK2* expression groups by median immunohistochemical scores. The diagnosis of hepatocellular carcinoma was histologically confirmed. Patients were excluded if they had preoperative extrahepatic metastasis, other malignancies, or received preoperative anti-cancer therapy. Surgery was performed by several surgeons in accordance with the standard criteria for liver resection in our hospital. Clinical information related to sociodemographic data diagnostic procedures, tumor features, and treatment regimen were collected from medical records. Follow-up information was collected annually from hospital records or direct telephone interviews conducted by trained monitors using structured questionnaires.

Histopathological assessment

Histopathological evaluation of all surgical specimens was fulfilled based on standard pathological procedures, and a hepatic pathologist reviewed the hematoxylin and eosin (H&E)-stained histological sections of all patients without any knowledge of the clinical outcomes. The 2010 UICC TNM classification was used to stage the tumors.

Immunohistochemistry (IHC) and IHC evaluation

IHC was performed to assess the expression of *NEK2* in cancerous tissues and matched adjacent noncancerous tissues according to the standard process. Briefly, all surgical samples were formalin-fixed, paraffin embedded, and cut into 4-μm-thick sections. The sections were deparaffinized, rehydrated, and boiled in a microwave oven with 0.01 mol/L sodium citrate buffer (pH 6.0) for 10 min for antigen retrieval. Endogenous peroxidase activity was blocked by hydrogen peroxide (3%) in PBS and non-specific staining was blocked by BSA. Sections were incubated separately with the primary antibody against *NEK2* (ab55550, 1:1, 000 dilution; Abcam, Cambridge, MA, USA) at 4 °C overnight. After washing, sections were incubated with the secondary antibody (Envision™ Detection kit; Gene Tech, Shanghai, China) at 37 °C for 30 min. Finally, immunohistochemical staining was carried out using an EliVision Plus kit (Maixin-Bio, Fuzhou, China); positive staining was visualized with diaminobenzidine (DAB) reagent and counterstained with hematoxylin. IHC was scored in a double-blinded manner by two authors. The German semi-quantitative scoring system, which takes into consideration the staining intensity and percentage of stained cancer cells, was applied to score *NEK2* expression [33]. Staining levels were scored as 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining), according to the staining intensity in the tumor cells. The percentage of stained cancer cells in each section was calculated and the sections were scored accordingly (<10% = 0; 10–25% = 1; 26–50% = 2; 51–75% = 3; 76–100% = 4). The final immunostaining score for each cancerous tissue section was obtained by multiplying the intensity scores with the scores of positively stained cancerous cells, with the scores ranging from 0 to 12.

Statistical analysis

The prognostic significance of *NEK2* was evaluated by analysis in SPSS 20.0 statistical software (SPSS Inc., Chicago, USA). The relationship between *NEK2* expression and patient characteristics was investigated via Pearson's $\chi 2$ test and Spearman's correlation coefficient. The differences in overall survival (OS) and recurrence-free survival (RFS) between the two groups were analyzed using the Kaplan-Meier method with the log-rank test. Univariate analysis was used to establish the potential prognostic factors for OS and DFS, and multivariate analysis for significant factors was performed by Cox proportional hazard regression models. A p-value of < 0.05 indicated a statistically significant difference.

Meta-analysis

This meta-analysis was performed according to the PRISMA statement issued in 2009 [34].

Literature search strategy

A comprehensive literature search was performed using PubMed, EMBASE, Web of Science, and CNKI for potential eligible articles assessing the prognostic effects of *NEK2* in HCC published up until August 11, 2018. The keywords used to perform the searches were the follow: ("HCC" or "hepatocellular carcinoma" or "liver cancer" or "liver primary cancer" or "liver primary tumor" or "liver carcinoma"), and ("*NEK2*" or "*NIMA- related kinase 2*"). There were no language or regional restrictions in this meta-analysis.

Selection criteria

All potential articles were screened and selected by two independent authors with the following inclusion criteria: 1) pathologically confirmed HCC; 2) prospective or retrospective cohort study design; and 3) reported on the association of *NEK2* with survival outcomes or clinicopathological parameters in HCC. The exclusion criteria were as followings: 1) reviews, letters, case reports, meeting abstracts, and meta-analyses; 2) studies investigating other cancers; 3) studies without extractable data without accessible full text of articles; and 4) patients not classified into two groups of high *NEK2* and low *NEK2* expression.

Data collection and quality assessment

Two investigators independently performed full-text review of eligible articles and extracted the data. The collected data included the name of the first author, publication year, study type, study region, the number of patients in high/low *NEK2* expression groups, sex, recruitment period, tumor-node-metastasis (TNM) stage, detection method, cut-off value, overall survival (OS), disease-free survival (DFS), recurrence-free survival (RFS), and clinicopathological characteristics. Moreover, we treated OS as the primary endpoint because most of the included studies reported the OS. If the hazard ratio (HR) and its 95% confidence interval (CI) of univariate and multivariate analyses were both reported, only the latter was used since it considers the confounding factors and provides more accuracy. If the HR of survival outcomes was only described using Kaplan-Meier curves, Engauge Digitizer version 4.1 (http://digitizer.sourceforge.net) was used to extract the survival data, and Tierney's method [35] was used to calculate the estimated HRs and corresponding 95% CIs. The quality of eligible articles was evaluated by two investigators using the Newcastle-Ottawa Quality Assessment Scale (NOS) [36]. Among the NOS system scores ranging from 0 to 9, we considered values of 6 and over to represent high-quality articles in this meta-analysis.

Statistical analysis

STATA version 12.0 (Stata Corporation, College Station, TX, USA) was used to perform the meta-analysis. Synthesized HRs with 95% CIs were used to describe the association between *NEK2* expression and survival in HCC patients, while synthesized odds ratios (ORs) with 95% CIs were used to assess the association between *NEK2* expression and the clinicopathological features of HCC. HR > 1 indicated that *NEK2* expression was a risk factor for shorter survival in HCC if the corresponding 95% CI did not contain 1. Cochran's Q and Higgins I^2 statistics were used to evaluate the heterogeneity across the studies. We considered $P < 0.05$ and $I^2 > 50\%$ to indicate significant heterogeneity, under which conditions the random effects model was applied to synthesize the data. In turn, if there was significant heterogeneity, the fixed effects model was used for pooling the data. To validate the stability of the synthesized results, a sensitivity analysis was carried out by sequentially removing each individual study. Begg's funnel plots and Egger's linear regression tests [37, 38] were used to assess publication bias and if the P value was < 0.05 and the funnel plot was asymmetric, we considered that statistically significant publication bias existed.

Results

Increased expression of NEK2 in HCC tissues

NEK2 expression in 206 resected HCC tissue specimens and adjacent non-tumorous liver tissues was analyzed using an IHC staining technique. Immunohistochemical analysis showed that, in all cases, the adjacent non-tumorous liver tissues did not express *NEK2*. The patients were divided into two arms according to the median values of staining scores: a *NEK2*-positive expression group (n = 103) and a *NEK2*-negative expression group (n =

103) (Table 1). Furthermore, it was observed from IHC analysis that *NEK2* was mainly expressed in the nuclei (Fig. 1).

Correlations between NEK2 expression and clinicopathological parameters

For a better understanding of the clinical value of *NEK2* expression in HCC, we further investigated the clinicopathological characteristics among the 206 HCC cases. The expression of *NEK2* was significantly higher in cases with large tumors (P < 0.001), and in patients with poor differentiation (P = 0.002) (Table 1). Nevertheless, there were no associations between the expression of *NEK2* and the other clinicopathological parameters, including age, sex, tumor number, portal vein thrombosis, TNM stage, HBs antigen, α-fetoprotein (AFP) level, and cirrhosis (P > 0.05) (Table 1).

Table 1. Correlation between NIMA-related kinase 2 (NEK2) expression and clinicopathological features. NEK2, NIMA-related kinase 2; HBs, antigen, hepatitis B surface antigen; AFP, α-fetoprotein

Clinical parameters	Number	NEK2 expression		P value
		Low (N=103)	High (N=103)	
Age (years)				
< 55	124	58	66	0.255
> 55	82	45	37	
Gender				
Male	180	93	87	0.208
Female	26	10	16	
Tumor size				
≤5cm	76	55	21	<0.001
> 5cm	130	48	82	
Tumor number				
Single	145	69	76	0.285
Multiple	61	34	27	
Differentiation				
Well/moderate	155	87	68	0.002
Poorly	51	16	35	
Portal vein thrombosis				
Absence	162	89	73	0.144
Presence	44	14	20	
TNM stage				0.669
I	135	64	61	
II/III	71	39	42	
HBs antigen				
Negative	24	10	14	0.385
Positive	182	93	89	
Cirrhosis				
Absence	40	18	12	0.236
Presence	176	85	91	
AFP(ng/ml)				
≤400	90	47	43	0.574
>400	116	56	60	

Correlations between NEK2 expression and prognosis

To evaluate the prognostic value of *NEK2* expression, we analyzed Kaplan-Meier curves for OS and RFS rates. The 5-year OS and 5-year RFS rates were 46.5% and 24.7% for the patients with high *NEK2* expression, respectively, and 67.3% and 54.3% for patients with no *NEK2* expression, respectively. The Kaplan-Meier curves indicated there were significant differences among OS and RFS rates between patients with *NKE2*-positive expression and those with *NEK2*-negative expression (Fig. 2). Furthermore, we performed univariate Cox regression analysis to assess the impact of *NEK2* expression levels and other common factors on OS and RFS in HCC patients. *NEK2* expression (P < 0.001), differentiation (P < 0.001), portal vein thrombosis (P < 0.001), tumor size (P < 0.001), tumor number (P = 0.037), HBs antigen (P = 0.004), cirrhosis (P = 0.034) and AFP level (P = 0.004) were determined as crucial risk factors for OS (Table 2). Nevertheless, after multivariate Cox analysis, only *NEK2* expression, tumor size, and portal vein thrombosis were found to be independent predictors of OS in patients with

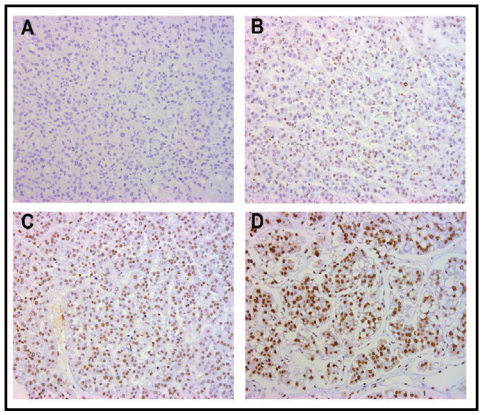

Fig. 1. Immunohistochemical staining of NEK2 in HCC specimens and adjacent noncancerous tissues at 200 x magnification. Negative (-) (A) or weak (+) (B) staining was defined as negative expression; moderate (++) (C) or strong (+++) (D) staining was defined as positive expression.

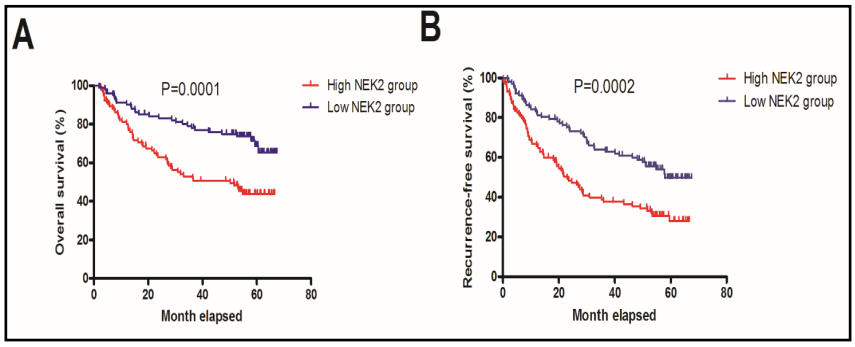

Fig. 2. Kaplan-Meier curves for overall survival (OS) and recurrence-free survival (RFS) in patients with hepatocellular carcinoma (HCC) by NEK2 expression. Patients with high NEK2 expression exhibited a shorter OS (A. p<0.01) and RFS (B. p<0.01) compared with low NEK2 expression.

HCC (Table 2). With respect to RFS, univariate Cox regression analysis indicated that *NEK2* expression (P < 0.001), tumor size (P < 0.001), tumor number (P = 0.037), degree of differentiation (P = 0.003), portal vein thrombosis (P = 0.001), and HBs antigen (P = 0.026) were closely associated with RFS (Table 3). However, multivariate Cox analysis suggested that only *NEK2* expression and tumor size were independent predictors of RFS in patients with HCC (Table 3).

Table 2. Univariate and multivariate Cox regression analysis for OS (HR hazard ratio, CI confidence interval). OS, overall survival; NEK2, NIMA-related kinase 2; HBs, antigen, hepatitis B surface antigen; AFP, α-fetoprotein; HR, hazard ratio; CI, confidence interval; RFS, recurrence-free survival

	OS			
	Univariate analysis		Multivariate analysis	
Variables	HR (95% CI)	P-value	HR (95% CI)	P-value
Age (years)	0.989(0.968-1.009)	0.282		
Gender	1.781(0.821-3.868)	0.144		
Tumor size	2.278(1.695-3.061)	<0.001	1.663(1.077-2.567)	0.022
Tumor number	1.672(1.031-2.711)	0.037		
Differentiation	2.628(1.646-4.193)	<0.001		
Portal vein thrombosis	2.009(1.497-2.695)	<0.001	2.395(1.175-4.879)	0.016
TNM stage	1.068(0.688-1.658)	0.769		
HBs antigen	1.910(1.233-2.958)	0.004		
Cirrhosis	8.411(1.170-60.462)	0.034		
AFP	1.906(1.224-2.966)	0.004		
NEK2 expression	2.356(1.493-3.718)	<0.001	1.763(1.060-2.935)	0.029

Table 3. Univariate and multivariate Cox regression analysis for RFS (HR hazard ratio, CI confidence interval). RFS, recurrence-free survival; NEK2, NIMA-related kinase 2; HBs, antigen, hepatitis B surface antigen; AFP, α-fetoprotein; HR, hazard ratio; CI, confidence interval; RFS, recurrence-free survival

	RFS			
	Univariate analysis		Multivariate analysis	
Variables	HR (95% CI)	P-value	HR (95% CI)	P-value
Age (years)	0.994(0.976-1.011)	0.461		
Gender	1.627(0.714-3.625)	0.212		
Tumor size	1.796(1.416-2.278)	<0.001	1.747(1.233-2.475)	0.002
Tumor number	1.710(1.366-2.141)	<0.001		
Differentiation	1.765(1.219-2.555)	0.003		
Portal vein thrombosis	1.490(1.166-1.903)	0.001		
TNM stage	1.270(0.881-1.830)	0.200		
HBs antigen	2.170(1.099-4.286)	0.026		
Cirrhosis	2.445(0.998-5.991)	0.051		
AFP	1.387(0.962-1.999)	0.080		
NEK2 expression	1.994(1.374-2.893)	<0.001	1.687(1.102-2.584)	0.016

Study search and study characteristics in meta-analysis

The primary search found 92 potential articles in PubMed, EMBASE, Web of Science, and CNKI. After excluding 7 duplicates, two independent investigators screened the titles and abstracts of the remaining 85 articles, during which 71 articles were excluded as reviews or abstracts, unrelated topics, or nonclinical studies. In the full-text review of the remaining 14 articles, 4 studies were further excluded since the articles did not provide extractable data. Finally, 10 previously published articles and our current cohort study were included in the meta-analysis [25, 30, 32, 39-45]. The details of the literature selection process are presented in Fig. 3.

The basic characteristics of the eligible articles are shown in Table 4. All reports were published between 2016 and 2018. In addition to our cohort study, 9 of the previous studies were conducted in China [25, 32, 39-45], and 1 in Japan [30]. The case numbers in each study ranged between

40 and 359. IHC was the most common method used to detect the expression of *NEK2*, and all patients in the studies included were classified into two groups, i.e., either high or low *NEK2* expression. In addition to our cohort study, the correlation between *NEK2* expression and OS was investigated in 7 studies [30, 32, 39-41, 43, 45], DFS was reported in 1 study [39], and RFS was reported in 3 [30, 41, 45]. In addition to our cohort study, a total of 8 articles analyzed the relationship between *NEK2* expression and the clinicopathological features, with 7 referring to tumor size [25, 32, 39, 41, 42, 44, 45], 6 referring to tumor differentiation [25, 30, 32, 39, 41, 45], 5 referring to tumor number [30, 32, 41, 42, 45], 3 referring to portal vein thrombosis [25, 27, 30], 7 referring to AFP level [24, 25, 28, 30-32, 45], 5 referring to HBV status [30, 32, 39, 42, 45], and 5 referring to liver cirrhosis [32, 41, 42, 44, 45]. The NOS scores of the studies included ranged from 5 to 7, indicating that the quality of these studies was moderate to high.

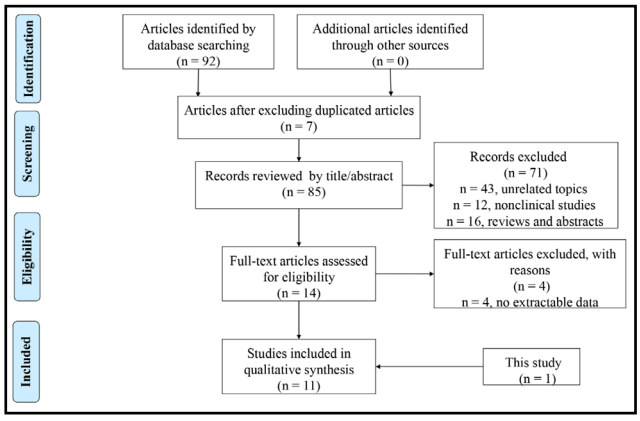

Fig. 3. Flow chart of study selection process.

Meta-analysis of NEK2 expression and OS in HCC patients

There were 8 studies that included 1, 542 patients and analyzed the link between *NEK2* expression and OS, including 7 previously published studies [30, 32, 39-41, 43, 45] and our cohort study. The fixed model effect was used to synthesize the data, since no significant heterogeneity existed among these studies (I^2 = 28.3%, P = 0.202). The pooled result showed that high *NEK2* expression correlated closely with worse OS in HCC patients (HR = 1.47; 95% CI, 1.21 to 1.80; P < 0.01) (Fig. 4A).

Meta-analysis of NEK2 expression and DFS/RFS in HCC patients

Among the eligible studies, 4 referred to the correlation between NEK2 expression and DFS/RFS, including 3 previously published studies [24, 27, 30] and our cohort study. Because of the similarity between DFS and RFS, we merged them for a pooled analysis. Considering that there was no evidence of significant heterogeneity among the 4 studies (I^2 = 0%, p = 0.393), we used the fixed effects model to conduct the meta-analysis. We found that HCC patients with high *NEK2* expression had shorter DFS/RFS (HR = 1.92; 95% CI, 1.41 to 2.63; P < 0.01). (Fig. 4B).

Meta-analysis of NEK2 expression and clinicopathological features in HCC patients

To comprehensively explore the roles of *NEK2* in HCC, we further investigated the relationships between high expression of *NEK2* and the clinicopathological features. The results indicated that the proportion of patients with high expression of *NEK2* was greater in the large tumor size group (> 5 cm) than the group with smaller tumors (≤ 5 cm) (OR = 2.02; 95% CI, 1.13–3.64; P < 0.01) (Fig. 5). However, there were no significant differences in the proportion of patients with high expression of *NEK2* in a comparison of serum AFP concentrations (> 400 ng/mL vs ≤ 400 ng/mL) (OR= 1.45; 95% CI, 0.95–2.20; P < 0.01) (Fig. 6A), tumor differentiation (well/moderately vs poorly differentiated) (OR = 1.37; 95% CI, 0.76–2.47; P = 0.12) (Fig. 6B), tumor number (multiple vs single) (OR = 1.05; 95% CI, 0.78–1.42; P = 0.14) (Fig. 6C), portal vein thrombosis (present vs absent) (OR = 1.88; 95% CI, 0.78–4.51; P = 0.12) (Fig. 6D), liver cirrhosis (present vs absent) (OR = 1.16; 95% CI, 0.87–1.54; P = 0.07) (Fig. 6E), or HBV status (positive vs negative) (OR = 0.85; 95% CI, 0.59–1.21; P = 0.11) (Fig. 6F).

Table 4. Basic characteristics of the included studies in meta-analysis. Abbreviation: OS: overall survival; RFS: recurrence survival; DFS: disease-free survival; NR: not reported; immunohistochemistry (IHC)

Study	Country	Study design	Recruitment period	Case Number	Detection method of NEK2 expression	Evaluation method	Cut-off value of NEK2	Survival analysis	Quality score
Fu, L., et al. (2017).	China	Retrospective	2008-2015	310	IHC	Multiplying the staining intensity score (0, 1 or 3) with score for the proportion of stained tumor cells (0, 1, 2 or 4)	0 to 6 were defined as NEK2-low 7 or more were defined as NEK2-high	OS	7
Fu, S. J., et al. (2017).	China	Retrospective	2008-2013	100	IHC	The proportion of stained tumor cells	0 to 1 were defined as NEK2-low 2 to 3 were defined as NEK2-high 0 to 10% were defined as NEK2-low	OS, DFS	6
Lai, X. B., et al. (2017).	China	Retrospective	2010-2013	52	IHC	The proportion of stained tumor cells	10% or more were defined as NEK2-high	NR	6
Li, G., et al. (2017).	China	Retrospective	NR	359	RNASeqV2 data	NR	NR	OS	5
Lin, S., et al. (2016).	China	Retrospective	2006-2010	104	IHC	The proportion of stained tumor cells	0 to 1 were defined as NEK2-low 2 to 3 were defined as NEK2-high	OS, RFS	7
Wen, S., et al. (2016).	China	Retrospective	2011-2012	64	IHC	NR	NR	NR	6
Wu, S. M., et al. (2017).	China	Retrospective	2005-2010	154	qRT-PCR	mRNA level	NR Below the median value was defined as NEK2-low	OS	6
Wubetu, G. Y., et al. (2016).	Japan	Retrospective	NR	50	qRT-PCR	mRNA level	Above the median value was defined as NEK2-low	OS, RFS	6
Zhang, M. X., et al. (2016).	China	Retrospective	2013-2014	40	IHC	NR	NR	NR	5
Zhang, Y, et al. (2018)	China	Retrospective	2006-2009	259	IHC	Multiplying the staining intensity score (0, 1 or 3) with score for the proportion of stained tumor cells (0, 1, 2 or 4)	0 to 4 were defined as NEK2-low 5 or more were defined as NEK2-high Below the median score was defined as NEK2-low	OS	7
The current cohort study	China	Retrospective	2009-2012	206	IHC	Multiplying the staining intensity score (0, 1 or 3) with score for the proportion of stained tumor cells (0, 1, 2 or 4)	Above the median score was defined as NEK2-low	OS, RFS	7

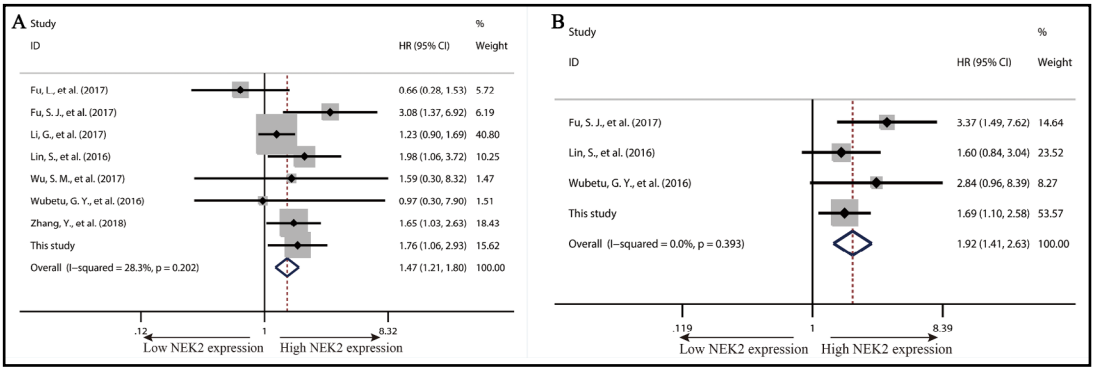

Fig. 4. Forest plots for pooled HR with 95% CI for the associations between NEK2 expression with overall survival and disease-free survival.

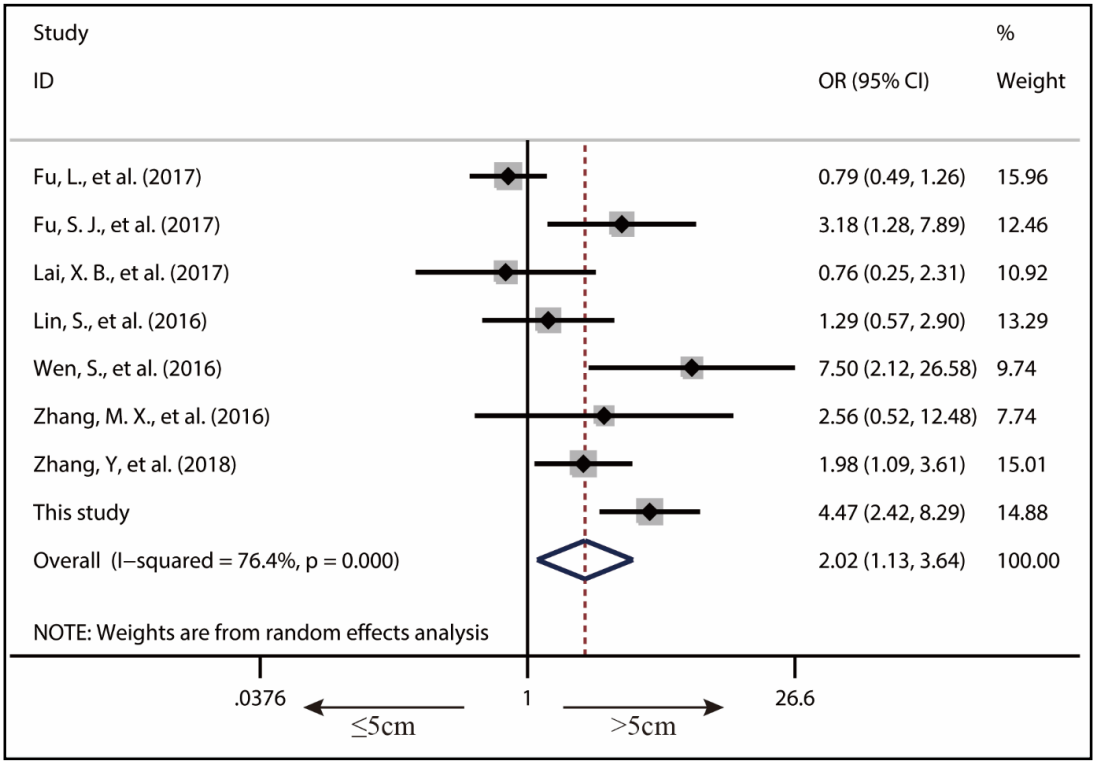

Fig. 5. Forest plots for pooled OR with 95% CI for the proportion of HCC patients with high NEK2 expression and the comparison of tumor size.

Sensitivity analysis

To evaluate the stability of the synthesized HR for OS, a sensitivity analysis was performed by sequentially removing each individual study. The result showed that the synthesized HR did not alter substantially with the removal of any individual study, indicating that the pooled HR of OS was stable and reliable (Fig. 7A).

Publication bias

Begg's funnel plots and Egger's linear regression tests were used to assess publication bias of the synthesized HR for OS. The results showed that Begg's funnel plot was symmetrical (Fig. 7B), and the p value for Egger's regression asymmetry test was 0.784, suggesting that there was no significant publication bias.

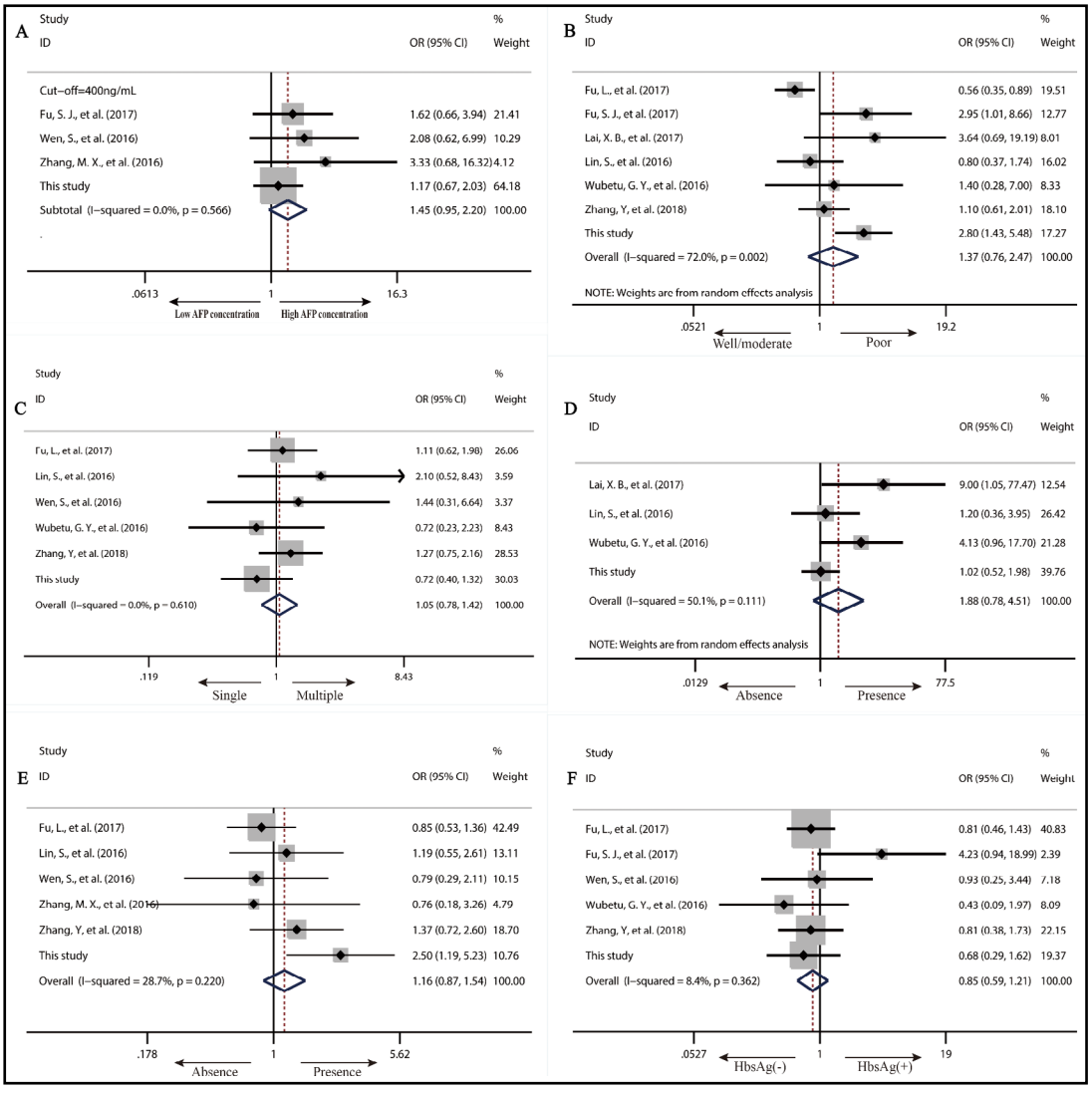

Fig. 6. Forest plots for pooled OR with 95% CI for the proportion of HCC patients with high NEK2 expression in comparisons of serum AFP concentration (>400 ng/mL vs ≤400 ng/mL) (A), tumor differentiation (well/moderately vs poorly differentiated) (B), tumor number (multiple vs single) (C), portal vein thrombosis (present vs absent) (D), liver cirrhosis (present vs absent) (E), and HBV status (positive vs negative).

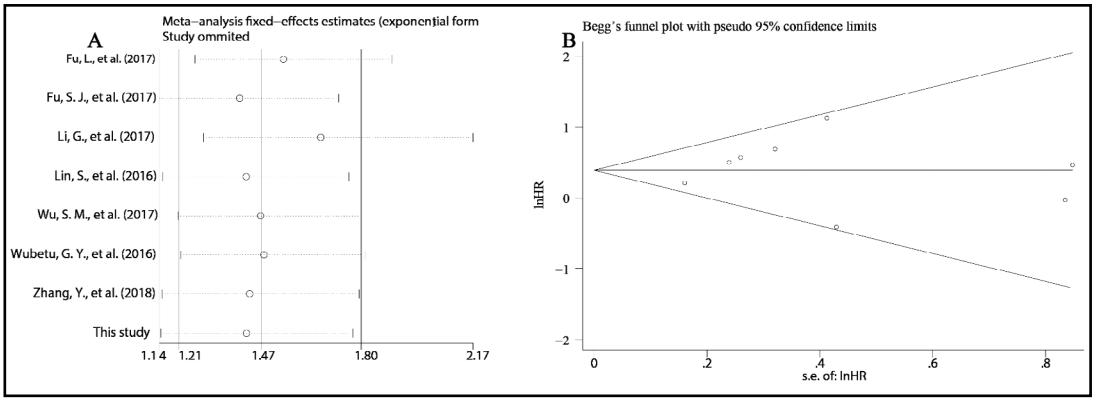

Fig. 7. Sensitivity analysis of pooled HR for overall survival (OS) (A); funnel plots of Begg's test estimating publication bias of pooled HR for OS (B). s.e., standard error.

Discussion

Comprehensive analyses of the molecular mechanisms underlying the initiation and progression of HCC play a role in improving the rates of early diagnosis and individualized therapy. This two-step study focused on thoroughly exploring the prognostic and clinicopathological significance of *NEK2* expression and reviewed its relevant molecular mechanisms in HCC. Consistent with the majority of previous studies [24-31], our current cohort study also showed that *NEK2* was overexpressed in HCC tissues compared with adjacent normal tissues, and high *NEK2* expression correlated positively with larger tumor size, portal vein invasion, and poor tumor differentiation. However, the meta-analysis showed that high *NEK2* expression was only related to larger tumor size and not to the other clinicopathological features. With respect to survival outcomes, both our current cohort study and the meta-analysis confirmed that there were close correlations between high *NEK2* expression and shorter OS and DFS/RFS.

Numerous researchers have explored the underlying mechanisms of the prognostic significance of *NEK2* in HCC patients. A study by Zhang et al., which investigated the association between *NEK2* expression and HCC initiation and progression for the first time, revealed that *NEK2* overexpression could promote the proliferation and survival of HepG2 cells by activating the mitogen activated protein kinase (MAPK) signaling pathway. Aberrant activation of Wnt/β-catenin signaling pathway plays an important role in sustaining cancer cell stemness [46]. Consistently, Lin et al. reported that the overexpression of *NEK2* could enhance self-renewal properties in HCC by activating the Wnt/β-catenin pathway [27]. Moreover, a study by Lai et al. suggested that the overexpression of NEK2 could induce cell cycle progression and promote proliferation via the activation of Wnt/β-catenin signaling pathway [25]. *ABCG2*, a member of ABC transporter family, is a vital element of multi-drug resistance system in cancer [47] and Lin et al. demonstrated that *NEK2* plays a crucial role in chemotherapeutic resistance by regulating the expression of *ABCG2* in HCC cells [27]. Similarly, a study by Wu et al. demonstrated that *NKE2* overexpression can induce the resistance of hepatoma J7 or Hep3B cells to chemotherapy via upregulation of *ABCC10* and the expression of Twist [29]. Additionally, abnormal activation of the p-AKT/NF-κB signaling pathway and matrix metalloproteinase-2 was found to be involved in *NEK2* overexpression-induced migration, invasion, and angiogenesis in HCC [26, 29].

To our knowledge, this two-part study is the first meta-analysis to thoroughly evaluate the prognostic and clinicopathological significance of *NEK2* expression in HCC. However, it did have several limitations. First, all eligible studies in the meta-analysis were of a retrospective nature, which might have introduced a degree of bias. Second, although no statistically significant heterogeneity was found during the meta-analysis of OS and DFS/RFS, inconsistencies among the treatments, methods of testing for *NEK2* expression, cut-off values for high *NEK2* expression, and recruitment period might also have introduced a degree of bias and heterogeneity and thus might have influenced the robustness of our pooled results. Third, in some studies, the HRs for OS were calculated by univariate analysis or manually calculated from the Kaplan-Meier curve, which did not exclude the impact of key confounding factors on survival, and thus bias and heterogeneity might have occurred to a degree. Fourth, among the 11 studies included in the meta-analysis, 10 were from China and only 1 study was from Japan, but none focused on Caucasian and African populations. Therefore, future work is required to explore the prognostic value of *NEK2* expression in Caucasian and African populations with HCC.

Conclusion

Our study demonstrated that high expression of *NEK2* is a risk factor for poor survival in HCC patients. More prospective, homogeneous, and multi-ethnic studies are required to validate our current findings.

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Disclosure Statement

The authors declare that there were no conflicts of interest.

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