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Overexpression of NEK2 is correlated with poor prognosis in human clear cell renal cell carcinoma

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Abstract

Objectives: Never in mitosis gene A-related kinase 2 (NEK2) has been implicated in tumorigenesis in various tissues, but its function in clear cell renal cell carcinoma (ccRCC) tumorigenesis is unclear. We evaluated the correlation between NEK2 expression and ccRCC. Methods: Immunohistochemistry analysis of NEK2 protein was done on high-density multi-organ Human Cancer tissue microarray derived from the patient samples from clear cell renal cell carcinoma. We used multiple clinical cohorts to analyze the NEK2 immunohistochemical staining expression across human cancers. The cancer genome atlas (TCGA) data analysis of NEK2 was done through UALCAN web servers. Association of NEK2 and Kaplan–Meier survival analysis was done on both of our clinical database and available TCGA datasets. Results: Using the UALCAN cancer transcriptional data analysis website, we found that NEK2 is overexpressed in ccRCC, and its expression was associated with overall survival. According to the analyses of our own clinical database and immunohistochemical staining, protein levels of NEK2 were elevated in renal carcinoma compared to adjacent normal tissues. Kaplan–Meier survival analysis of both UALCAN and our database showed that high expression of NEK2 was associated with a poor prognosis. Multivariate and univariate analyses showed that NEK2 expression was closely related to a poor prognosis. The findings suggest that NEK2 is associated with ccRCC. Conclusion: These studies show that NEK2 is over-expressed in clear cell renal cell carcinoma and plays an essential role in cancer cell survival, as such NEK2 could serve as a novel potential target for therapeutic intervention in ccRCC.

Keywords

clear cell renal cell carcinoma, NEK2, prognosis

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Introduction

Renal cell carcinoma (RCC) is the most common fatal malignancy originating from tubular epithelioid cells, and its incidence is increasing annually.[1] Clear cell renal cell carcinoma (ccRCC) is the main pathological subtype of RCC.[2]

Never in mitosis gene A-related kinase 2 (NEK2) is a cell cycle-associated protein, as are aurora kinases and polo-like kinases.[3,4] In recent years, the oncogenic roles of NEK2 have attracted considerable attention. Plenty of

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studies have reported that NEK2 is highly expressed in various cancers and usually predicts poor overall survival.[5] Two studies on the role of NEK2 in chromosomal instability, tumorigenesis, progression, and drug resistance have been published.[6,7] However, the potential link between NEK2 and ccRCC has not been explored. We systematically investigated the prognostic utility of the NEK2 expression level in patients with ccRCC using The Cancer Genome Atlas (TCGA) database and our hospital's database.

Materials and methods

Analysis of NEK2 expression

The expression of NEK2 was analyzed using UALCAN (http://ualcan.path.uab.edu), a user-friendly, interactive website for analyzing cancer transcriptome data (TCGA and MET500 transcriptome sequencing data) (Chandrashekar et al., 2017). UALCAN allows analysis of relative gene expression levels in tumor and normal tissue samples according to age, sex, tumor stage, and clinicopathological characteristics.

Patients and specimens

This study was retrospective in nature. The inclusion criteria for patient enrollment are as follows: We obtained 181 samples from 97 patients (82 pairs of tumor tissues and adjacent tissues and 17 single tumor tissues) diagnosed with ccRCC by clinical, radiological, and histopathological assessment. All patients underwent nephrectomy in the People's Liberation Army (PLA) General Hospital from April 2013 to November 2017. They had no other tumor history and did not undergo radiotherapy and chemotherapy before nephrectomy. The exclusion criteria were (1) subjects with infection, rheumatic immune disease, renal failure, and mental disease; (2) other types of tumors; (3) chronic renal diseases; (4) severe immunosuppression; and (5) incomplete information. Histological diagnosis was made using sections stained with hematoxylin and eosin according to World Health Organization guidelines. Clinical information, including histopathological diagnosis and tumor grade, was extracted from the medical records. Recruited subjects were followed up through telephone and outpatient review. This study was approved by the Human Subject Protection Committee of the General Hospital of the Chinese PLA (internal registration no. S2013-065-01). The patients provided written informed consent, which conformed to standards for the use of human subjects. The specimens were stored in the hospital repository.

Immunohistochemistry. Immunohistochemical (IHC) analysis was conducted using tissue microarray (TMA) of ccRCC and adjacent normal tissue specimens according to the protocol of the Two-Step IHC Kit (ZSGB-BIO, Beijing, China) using an anti-NEK2 antibody (ab227958, 1:200; Abcam, Cambridge, UK). The specimens of TMA were reviewed by one uropathologist and the most representative areas of renal tumor cells and adjacent tumor stroma were selected for the donor block. The TMAs were made by using a tissue-arraying instrument (Manual Tissue Microarrayer Quick-Ray ®). Cores with 2 mm diameter from per-donor block were diverted into a recipient block microarray.

The StrataQuest software (TissueGnostics, Vienna, Austria) was used to scan and analyze the images to quantify the tumor tissues, para-carcinoma tissues and NEK2 positive cells in the TMA. We multiplied the quantitative value of positive staining intensity (negative = 1, weak = 2, moderate = 3, strong = 4) by percentages of positive staining (0% = 0, ≤25% = 1, 26%–50% = 2, 51%–75% = 3, ≥76% = 4), and the data obtained was weighted and set into four levels (I, II, III, IV). All results were verified by three experienced independent investigators.

Statistical analysis

Data were analyzed using SPSS 21.0 (IBM Corp., Chicago, IL, USA) and Prism 8.0 (GraphPad Software, Inc., San Diego, CA, USA) software. Normally distributed data are presented as means ± SD and were analyzed by Student's *t*-test. Multiple comparisons were performed by one-way analysis of variance. Categorical data were analyzed by the chi-squared test or Fisher's exact test. Correlations of gene expression were analyzed by Pearson's coefficient. Univariate and multivariate logistic regression analyses were performed. Overall survival (OS) and progression-free survival (PFS) was analyzed by the Kaplan–Meier and log-rank tests. For two-sided hypotheses, $p < 0.05$ was considered indicative of statistical significance.

Results

NEK2 is upregulated in clear cell renal cell carcinoma

UALCAN was used to assess NEK2 expression in ccRCC (Figure 1(a)). The mRNA level of NEK2 in tumor tissues was significantly higher than in normal tissues (Figure 1(b)). TCGA analysis showed that NEK2 was highly expressed in ccRCC. The expression level of NEK2 was significantly correlated with tumor stage. Furthermore, high expression of NEK2 was associated with a worse OS in patients with ccRCC (Figure 1(c)).

Immunohistochemical analysis of NEK2

Immunohistochemical staining, and representative images of ccRCC tissue, adjacent normal renal tissue and negative control are shown in Figure 2, Supplementary Figure S2.

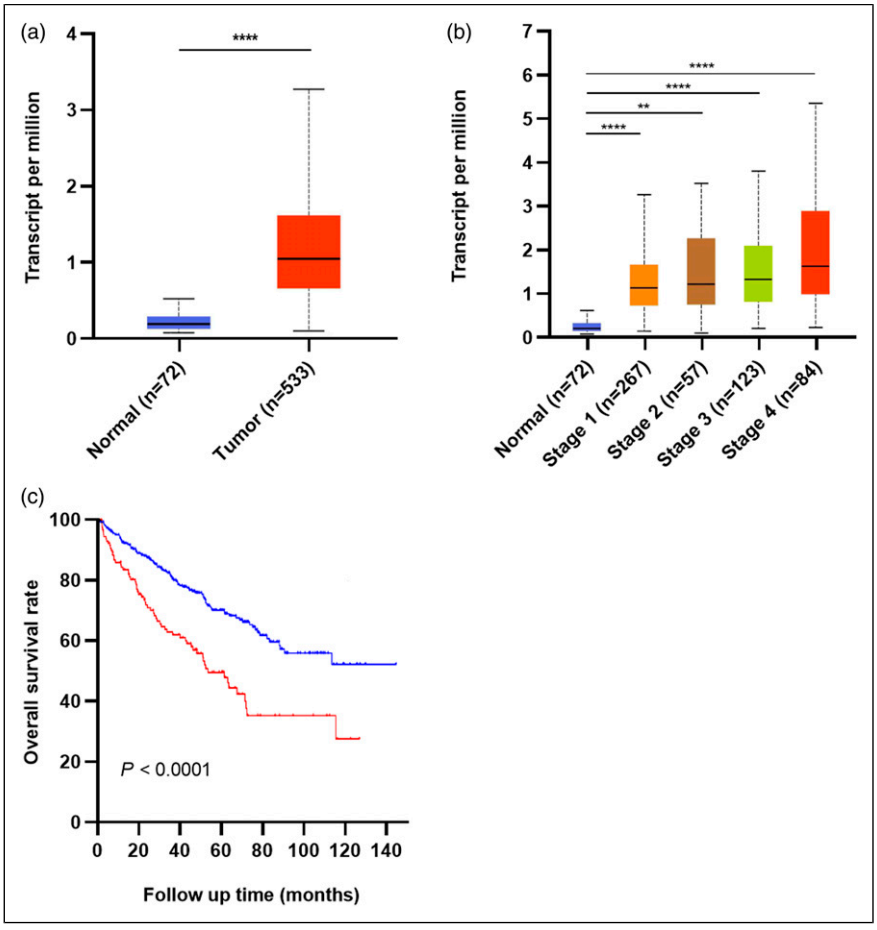

Figure 1. UALCAN analysis. (a) mRNA levels of NEK2 in ccRCC tissues and adjacent normal renal tissues. Data are mean ± SE. ****$p < 0.0001$. (b) NEK2 expression in normal tissues and ccRCC tissues differing in tumor stage. Data are mean ± SE. **$p < 0.01$; ****$p < 0.0001$. (c) Kaplan–Meier survival curves of patients with ccRCC. The overall survival rate is classified as low or high. ccRCC: clear cell renal cell carcinoma.

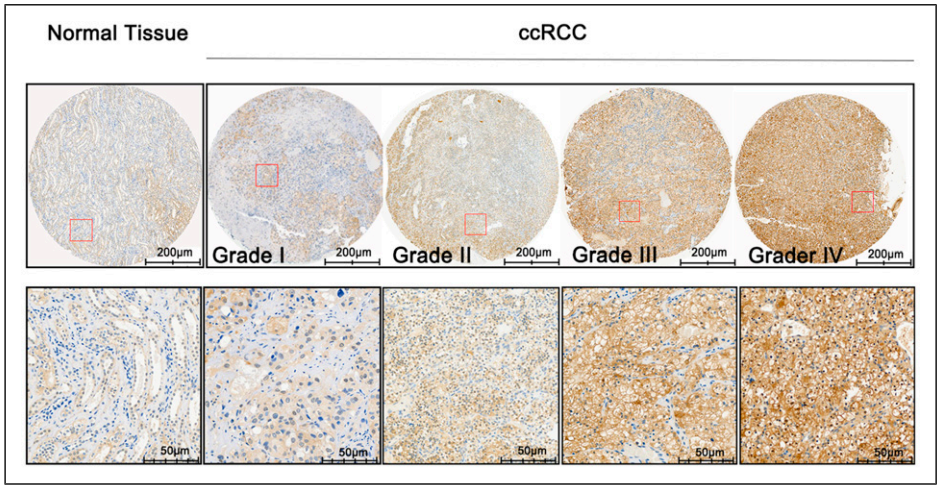

Figure 2. Tissue microarray immunohistochemical staining of NEK2 in clear cell renal cell carcinoma and adjacent normal tissues.

The IHC scores of 82 paired ccRCC samples revealed higher expression of NEK2 in tumor tissues compared to adjacent normal tissues. The mean NEK2 IHC-P (Immunohistochemistry-paraffin) scores for ccRCC tissues and adjacent normal renal tissues were 3 and 2, respectively (Figure 3(a)). The frequency distributions of the IHC-P scores are shown in Figure 3(b).

Upregulation of NEK2 correlates with a poor prognosis of clear cell renal cell carcinoma

The 97 patients with ccRCC underwent radical or partial nephrectomy. We evaluated the associations of NEK2 IHC score with age, body mass index (BMI), gender, overall TNM stage, Fuhrman grade, and tumor diameter. The clinicopathological parameters and NEK2 IHC scores of patients with ccRCC are shown in Table 1. The NEK2 IHC score was lower in the Fuhrman grade III versus grade IV patients (32 vs. 65, $p < 0.05$), and in the T2N0M0 than T3N0M0 patients (42 vs 55, $p < 0.01$). No significant differences were observed in subgroup analyses by age ($p = 0.418$), BMI ($p = 0.898$), gender ($p = 0.985$), or tumor diameter ($p = 0.145$). Furthermore, the NEK2 IHC score significantly increased with a more advanced T-stage (Figure 3(c)).

NEK2 has potential for predicting the prognosis of patients with ccRCC. Our patients with high NEK2 expression had a decreased PFS and OS compared to the other patients, as indicated by separated PFS (Figure 3(d)) and OS (Supplementary Figure S1) curves. To evaluate whether NEK2 can be used as an independent prognostic marker in patients with ccRCC, we performed univariate and multivariate Cox regression analyses of our clinical

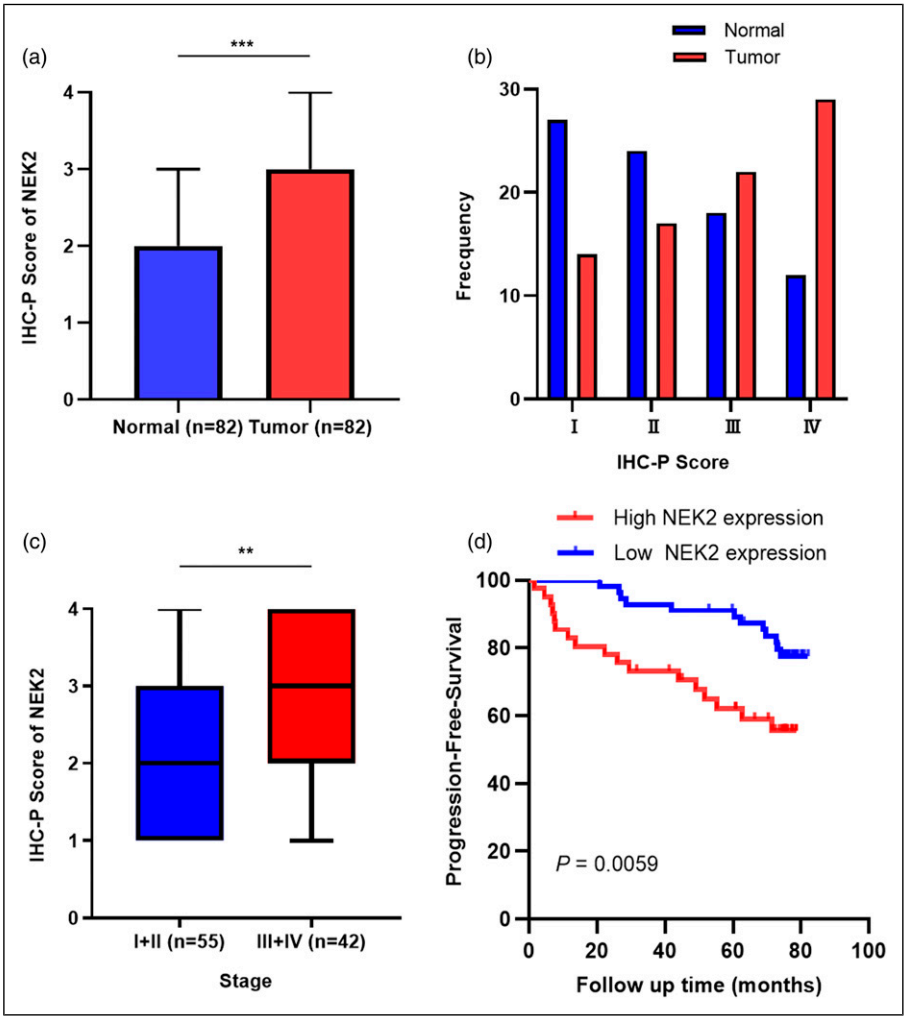

Figure 3. (a) (b) Immunohistochemical analysis of the NEK2 protein level in renal cell carcinoma and adjacent normal renal tissue. The average IHC staining score and the frequency distribution of protein levels are shown; $p < 0.001$. (c) The NEK2 staining score increased with more advanced T stage; $^{**}p < 0.01$. (d) Kaplan–Meier survival curves based on our database. IHC: immunohistochemical.

database, including initial pathologic diagnosis, gender, tumor size (longest dimension), Fuhrman score, and NEK2 expression level. In univariate Cox regression analyses, high NEK2 expression, tumor size, pathologic T score, Fuhrman score, and BMI were associated with a worse OS in patients with ccRCC (Table 2). Multivariate Cox regression analysis indicated that high NEK2 expression, pathologic T score, and Fuhrman score were independent prognostic factors for OS (Table 2).

Discussion

NEK family, a group of crucial proteins, executes vital processes in cells; they participate in the differentiation of cells and maintenance of cellular homeostasis. Their involvement in cellular mechanisms related to the formation and progression of cancers, including lung, breast, prostate, ovarian, colorectal, pancreatic, hepatic, and gastric neoplasias, is also remarkable and may be considered for future therapeutic approaches.[8] NEK2 was initially identified as a serine/threonine kinase with roles in cell cycle and mitosis regulation. Aberrant expression of NEK2 has been observed in several types of human cancers. In diffuse large B-cell lymphoma, NEK2 participate in regulating glycolysis and overexpression of it predicted a worse prognosis.[9] NEK2 knockdown inhibited the occurrence and development of non-small cell lung cancer, M2 polarization of macrophages, and

Table 1. Association between clear cell renal cell carcinoma and NEK2 expression and clinicopathological features.

Characteristics	NO (n = 97)	NEK2IHC score M (Q1, Q3)*	p value
Age (year)			0.418
<60	67	2 (1, 3)	
≥60	30	3 (1, 4)	
BMI (kg/m^2)			0.898
<25	35	2 (2, 4)	
≥25	62	2.5 (1, 3.25)	
Gender			0.985
Male	77	3 (1, 3)	
Female	20	2 (2, 4)	
Overall TNM staging			0.005
Stage I + II	55	2 (1, 3)	
Stage III + IV	42	3 (3, 4)	
Fuhrman grade			0.032
≤2	65	2 (1, 3)	
>2	32	3 (2, 4)	
Diameter (cm)			0.145
≤7	76	2 (1, 3)	
>7	21	3 (2, 4)	

* M: Median, Q1: Quartile 1, Q3: Quartile 3; BMI: body mass index.

Table 2. Univariate analysis and multivariate analysis of overall survival in our clinical database.

	Univariate analysis		Multivariate analysis	
Variable	HR (95% CI)	p value	HR (95% CI)	p value
NEK2 level (high vs low)	3.0215 (1.317, 6.929)	0.009	2.985 (1.234, 7.218)	0.015
BMI	0.849 (0.743, 0.969)	0.016		
Age at initial pathologic diagnosis (60 vs. < 60)	1.406 (0.615, 3.214)	0.419		
Gender (male vs female)	1.276 (0.506, 3.213)	0.605		
Size (longest dimension) (4.6 cm vs. < 4.6 cm)	5.345 (2.364, 12.086)	<0.001		
Pathologic T	6.901 (1.840, 25.886)	0.004	8.383 (2.195, 32.015)	0.002
Pathologic (T2 vs.T1)	4.617 (1.320, 16.151)	0.017	2.219 (1.466, 8.501)	0.226
Pathologic (T4 vs.T1)	13.478 (4.567, 39.775)	<0.001	12.629 (4.136, 38.564)	<0.001
Fuhrman scores (3 + 4 vs 1 + 2)	4.435 (1.938, 10.153)	<0.001	3.5301 (1.466, 8.501)	0.007

BMI: body mass index.

angiogenesis.[10] Inactivating AKT by NEK2 silencing decreases aerobic glycolysis and promotes autophagic cell death, which eventually inhibits the growth of gastric cancer cell.[11] To our knowledge, this is the first study of the role of NEK2 in ccRCC. According to UALCAN database, NEK2 was highly expressed in ccRCC, which was confirmed at the protein level by IHC. Moreover, NEK2 expression was strongly correlated with clinical outcomes in patients with ccRCC, particularly the Fuhrman score and T stage, suggesting that NEK2 has potential as a diagnostic biomarker for ccRCC. Besides, Kaplan-Meier and Cox regression analyses performed on a cohort of patients from our hospital revealed that high expression of NEK2 had poor prognosis and short survival time. This study is subject to several limitations. Future mechanistic studies are needed to elucidate the exact mechanism underlying the trends observed. Another limitation of this study is the relatively short follow-up time and small sample size. A larger sample size and longer follow-up are needed for further investigation.

Conclusion

In summary, the NEK2 protein level was significantly increased in ccRCC, which provides insight into its diagnosis, prognosis, and potential therapeutic targets. The mechanisms by which NEK2 impacts ccRCC are unclear and further studies are needed.

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Declaration of conflicting interests

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Ethics approval

Ethical approval for this study was obtained from the Ethics Committee of the Chinese PLA General Hospital (internal registration no. S2013-065-01).

Informed consent

Written informed consent was obtained from all subjects before the study.

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Supplemental material

Supplemental material for this article is available online.

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