Involvement of NEK2 and its interaction with NDC80 and CEP250 in hepatocellular carcinoma

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Abstract

Background: NEK2 has an established involvement in hepatocellular carcinoma (HCC) but the roles of NEK2 and its interacting proteins in HCC have not been systematically explored.

Methods: This study examined NEK2 and its interacting proteins in HCC based on multiple databases.

Results: NEK2 mRNA was highly expressed in HCC tissues compared with normal liver tissues. The survival of HCC patients with high NEK2 mRNA expression was shorter than those with low expression. MAD1L1, CEP250, MAPK1, NDC80, PPP1CA, PPP1R2 and NEK11 were the interacting proteins of NEK2. Among them, NDC80 and CEP250 were the key interacting proteins of NEK2. Mitotic prometaphase may be the key pathway that NEK2 and its interacting proteins contributed to HCC pathogenesis. NEK2, NDC80 and CEP250 mRNAs were highly expressed in HCC tissues compared with normal liver tissues. The mRNA levels of NEK2 were positively correlated with those of NDC80 or CEP250. Univariate regression showed that NEK2, NDC80 and CEP250 mRNA expressions were significantly associated with HCC patients’ survival. Multivariate regression showed that NDC80 mRNA expression was an independent predictor for HCC patients’ survival. Methylation and genetic alterations of NEK2, NDC80 and CEP250 were observed in HCC samples. The alterations of NEK2, NDC80 and CEP250 genes were co-occurrence. Patients with high mRNA expression and genetic alterations of NEK2, NDC80 and CEP250 had poor prognosis.

Conclusions: NEK2 and its interacting proteins NDC80 and CEP250 play important roles in HCC development and progression and thus may be potentially used as biomarkers and therapeutic targets of HCC.

Keywords: NEK2, NDC80, CEP250, Hepatocellular carcinoma, Biomarker

Background

Hepatocellular carcinoma (HCC) has high morbidity and mortality and is the third leading cause of cancer-associated deaths worldwide [1]. Characteristics of HCC include aggressive, high malignancy, early metastasis and poor prognosis. With the application of alpha-fetoprotein (AFP) and improvement of imaging techniques such as ultrasound and computerized tomography and the progress of surgical and interventional therapy for HCC, the early diagnostic rate of HCC and the prognosis of HCC patients appear to have been increased and improved. However, many patients were diagnosed at the advanced stage of HCC and had poor prognosis. The 5-year survival rate in patients with early stage HCC is more than 70%, but it is less than 5% in patients with advanced HCC [2]. Therefore, the exploration of biomarkers for the early...
NEK2 belongs to serine/threonine kinase and widely exists in the centrosome of cells [3]. NEK2 plays important roles in regulating the mitosis process, including chromatin separation, spindle assembly, centrosome division and tumor drug resistance [4–6]. Studies have shown that the high mRNA expression of NEK2 was related to the incidence, differentiation, metastasis and prognosis of multiple myeloma [7], pancreatic ductal adenocarcinoma [8], ovarian cancer [9], colorectal cancer [10] and breast cancer [11]. Studies also showed the involvement of NEK2 in the development and progression of HCC [12] and overexpression of NEK2 was indicated to be associated with a poor survival in HCC patients [13]. However, most of the previous studies were conducted in small sample sizes of patients and the possible network proteins of NEK2 remain to be investigated. Therefore, this study systematically examined the role of NEK2 in HCC, mined the NEK2 interacting proteins and explored the underlying mechanisms by the analyses of multiple databases to provide evidence for the potential of NEK2 and its interacting proteins in the diagnosis and therapy of HCC.

**Methods**

Multiple databases were used to systematically investigate the potential involvement of NEK2 and its interacting proteins in HCC and explored the possible mechanisms. First, the mRNA expression of NEK2 in HCC and the association of NEK2 mRNA expression levels with prognosis of HCC patients were analyzed by UALCAN. Second, the upstream or downstream interacting proteins of NEK2 were mined using GeneSense and the relationships between NEK2 and its interacting proteins and the key interacting proteins were verified and identified by STRING (Search Tool for the Retrieval of Interacting Genes/Proteins). Third, the functional enrichment pathways of NEK2 and its interacting proteins were explored by DAVID (the Database for Annotation, Visualization and Integrated Discovery) and KOBAS (KEGG Orthology Based Annotation System) and the pathways of NEK2 and its interacting proteins were verified using Reactome. Fourth, the effects of NEK2 and its key interacting proteins on HCC patients’ survival were examined using data downloaded from TCGA (The Cancer Genome Atlas) and the mRNA expression of NEK2, NDC80 and CEP250 in HCC were analyzed by Oncomine. Lastly, the genetic alterations of NEK2 and the genes of NEK2 key interacting proteins in HCC, the relationship of the genetic alterations between the genes, and the influence of the genetic alterations on HCC patients’ survival were analyzed by cBioPortal. The flow chart of the study was shown in Additional file 1: Figure S1.

**UALCAN database**

UALCAN (https://ualcan.path.uab.edu/analysis.html) is a user-friendly and interactive website, which provides easy access to publicly available cancer transcriptome data (TCGA and MET500 transcriptome sequencing). Plots depicting gene expression and patient survival information based on different gene expression can be obtained by UALCAN. This study explored the mRNA expression of NEK2 in HCC in comparison with normal liver, and plotted the survival curve of HCC patients with different mRNA expression levels of NEK2 by UALCAN.

**GeneSense database**

GeneSense (https://www.biomedsense.org/genesense.php) is a web-based platform that allows users to construct, visualize, manipulate and analyze gene information. It aims to assist researchers to find the optimal gene regulatory factors by associated networks. GeneSense adopts a number of methods to annotate protein–protein interaction (PPI). The PPI network which GeneSense can construct has three styles, namely nodenet, leafnet and loopnet. Nodenet supports basic queries of PPI network for upstream or downstream protein analysis. Leafnet model visualizes the complexity of the queried protein and its upstream or downstream proteins. Loopnet visualizes the upstream and downstream target of the queried protein. This study used loopnet style to visualize the possible upstream and downstream interacting proteins of NEK2 and both the upstream and downstream proteins were all considered to be NEK2 interacting proteins.

**STRING: Protein–protein interaction network construction**

STRING: functional protein association networks (https://string-db.org/), is a website which can predict PPIs. We used STRING to verify PPI network of NEK2 and its interacting proteins obtained from GeneSense. An interaction score > 0.9 was regarded as high confidence PPIs and the related interacting protein was considered to be a key interacting protein of NEK2.

**DAVID and KOBAS functional enrichment analyses**

The functional and signaling pathway analyses of NEK2 and its interacting proteins were performed on two public database platforms, DAVID (https://david.ncifcrf.gov/), and KOBAS 3.0 (https://kobas.cbi.pku.edu.cn/index.php). DAVID database was used to acquire the Gene Ontology (GO) terms that include three categories, namely molecular function (MF), cellular component (CC) and biological process (BP). KOBAS 3.0 database was used to obtain the main Kyoto Encyclopedia of
Genes and Genomes (KEGG) pathways. The enriched GO terms and KEGG pathways of NEK2 and its interacting proteins were visualized by R software and Cytoscape 3.6.1.

**Reactome database**

Reactome database (https://reactome.org/what-is-reactome) provides intuitive bioinformatics tools for the visualization, interpretation and analysis of pathway knowledge, and can present data which describe possible pathways if annotated proteins and small molecules were present in a cell. The possible pathways that interesting proteins can be obtained and the details of the pathways can be visualized. This study used the ReactomeFIPrugin in Cytoscape software to analyze the pathways that NEK2 and its interacting proteins may involve in and to acquire the pathway diagram.

**TCGA database**

The Cancer Genome Atlas (TCGA) database (https://cancergenome.nih.gov/), which integrates gene expression data and clinical data, was used to verify and analyze the prognostic value of NEK2 and its interacting proteins in HCC patients. R “survival” package [14] was used to visualize the results. The clinical covariates of 373 HCC patients from TCGA database were included in Cox proportional hazard model to identify the independent predictor for HCC patients’ survival. The correlations of mRNA expression between NEK2 and NDC80 or CEP250 were analyzed.

**Oncomine database**

Oncomine database (https://www.oncomine.org/resource/login.html), an online microarray database, was used to explore and confirm the mRNA expression levels of NEK2 and the genes of NEK2 key interacting proteins in HCC. The conditions for filter setting were as follows: Gene: “NEK2 or its key interacting proteins”, Analysis Type: “Cancer vs. Normal Analysis”, Cancer Type: “Hepatocellular Carcinoma”, Data Type: “mRNA”, Sample Type: “Clinical Specimen”. The studies incorporating mRNA expression data of NEK2 and the genes of NEK2 key interacting proteins were obtained, and ordered by “Over-expression: Gene Rank”. The different studies could be compared by conducting meta-analysis to demonstrate the reliability of these data.

**cbioPortal database**

cBioPortal database (https://www.cbioportal.org/) provides visualization tools for research and analysis of cancer gene data. The integrated genomic data types include somatic cell mutation, DNA copy number changes, mRNA and microRNA expression, DNA methylation, protein abundance and phosphoprotein abundance. The genetic alterations, relationship between genetic alterations, methylation, co-expression and the survival of HCC patients in relation to NEK2 and its key interacting proteins were explored through the database. “Liver Hepatocellular Carcinoma (TCGA, Provisional)” study was selected for analyzing, and there were 366 patients/samples with mutation and copy number alteration (CNA) data which comprise “mutation, putative copy-number alterations” from GISTIC, and mRNA expression z-Scores (RNA Seq V2 RSEM)” information. The mRNA expression z-Score threshold was ±2.0.

**Statistical analysis**

Statistical analyses were carried out by SPSS statistical software (Version 22.0; Inc., IBM, NY, USA) and Graphpad Prism software (Version 6.0; Inc., Graghpad, San Diego, CA, USA). The mRNA expression was expressed as mean ± standard deviation (SD), and the expression differences between groups were compared by nonparametric test. Cox regression model was used to identify independent predictors for HCC patients’ survival. Survival of HCC patients was estimated using the Kaplan–Meier method and compared by the log-rank test. The mRNA expression correlation between NEK2 and other genes were evaluated by Spearman correlation analyses. A p value of < 0.05 was considered statistically significant.

**Results**

**mRNA expression of NEK2 in normal and HCC tissues and its effect on HCC patient survival by UALCAN**

Based on UALCAN analyses [15], the mRNA expression of NEK2 was elevated in HCC tissues (n = 371) compared with normal liver tissues (n = 50, p = 1.625 × 10^-12, Fig. 1a). Survival analysis of patients with different levels of NEK2 mRNA expression demonstrated that the survival time of patients with high NEK2 mRNA expression (n = 89) was shorter than those with low expression (n = 276, p = 0.012, Fig. 1b).

**The upstream and downstream interacting proteins of NEK2 by Genesense**

The upstream and downstream interacting proteins of NEK2 were mined by Genesense website [16]. MAD1L1 [MAD1 mitotic arrest deficient-1 (yeast)] [17] was found to be the upstream protein of NEK2 while CEP250 (centrosomal protein 250 kDa) [18], MAPK1 (mitogen-activated protein kinase 1) [19], NDC80 (nuclear division cycle 80) [20], PPP1R2 [protein phosphatase 1, regulatory (inhibitor) subunit 2] [21] and PPP1CA (protein phosphatase 1 catalytic subunit alpha) were the downstream proteins of NEK2. NEK11 [NIMA (never in mitosis gene
a)-related kinase 11] [22] was indicated to be both the upstream and downstream protein of NEK2 (Fig. 2a).

**Protein–protein interaction by STRING database**

The PPI network was constructed by STRING database [23] to verify the association between NEK2 and its interacting proteins. The results showed that NEK2 had strong interaction with its downstream and upstream proteins. The PPI network's enrichment p-value was 1.23e−07. Among these proteins, NDC80 [24, 25] and CEP250 [26] had the highest interaction scores (Fig. 2b). The interaction scores between NEK2 and NDC80, and NEK2 and CEP250 were 0.994 and 0.995, respectively, indicating that NDC80 and CEP250 were NEK2 key interacting proteins. The main GO and KEGG pathways involving NEK2 and its interacting proteins analyzed by STRING database were downloaded (Table 1). Cell cycle [false discovery rate (FDR) = 2.78E−05], regulation of mitotic cell cycle phase transition (FDR = 4.01E−05) and mitotic cell cycle process (FDR = 0.0002) were the main GO biological progress pathways. Phosphatase binding (FDR = 0.0026), protein serine/threonine
kinase activity (FDR = 0.0219) and protein phosphatase binding (FDR = 0.0265) were the main GO molecular function pathways. Microtubule organizing center (FDR = 0.00037), centrosome (FDR = 0.00059) and condensed chromosome kinetochore (FDR = 0.00059) were the main GO cellular component pathways. Long-term potentiation (FDR = 0.0337), progesterone-mediated oocyte maturation (FDR = 0.0356) and cGMP-PKG signaling pathway (FDR = 0.0358) were the main KEGG pathways. The proteins enriched in each pathways were displayed in Table 1.

**Functional enrichment by DAVID and KOBAS 3.0 databases**
In order to further analyze the functional enrichment of NEK2 and its interacting proteins (MAD1L1, CEP250, MAPK1, NDC80, NEK11, PPP1CA and PPP1R2) in HCC, GO and KEGG pathways were investigated based on DAVID [27] and KOBAS 3.0 [28] databases. The details of the main GO terms and KEGG pathways were shown in Table 2, and the main GO terms and KEGG pathways were demonstrated in Fig. 3a and Fig. 3b, respectively. Phosphorus metabolic process (p = 0.001496693), phosphate metabolic process (p = 0.001496693), and cell cycle (p = 0.005071148) were significantly enriched in the category of GO biological process. Protein serine/threonine kinase activity (p = 0.005262684) was the mainly enriched pathway in the category of GO molecular function. There were no pathways enriched in GO cellular component. Long-term potentiation (p = 0.000150473), oocyte meiosis (p = 0.000484062), vascular smooth muscle contraction (p = 0.000508059), platelet activation (p = 0.000524374) and insulin signaling pathway (p = 0.000663998) were found to be the significantly enriched KEGG pathways (Table 2). MAPK1 and

### Table 1 The main GO and KEGG pathways involving NEK2 and its interacting proteins downloaded from STRING database

| Categories                  | Pathway ID | Pathway description                                      | Gene count | FDR       | Genes                  |
|-----------------------------|------------|----------------------------------------------------------|------------|-----------|------------------------|
| Biological process (GO)     | GO:0007049 | Cell cycle                                               | 7          | 2.78E-05  | CEP250, MAD1L1, MAPK1, NDC80, NEK1, NEK2, PPP1CA |
| GO:1901990                  | Regulatio - | n of mitotic cell cycle phase transition                 | 5          | 4.01E-05  | CEP250, MAD1L1, NDC80, NEK11, NEK2 |
| GO:1903047                  | Mitotic cell cycle process                            | 5          | 0.00021   | CEP250, MAD1L1, NDC80, NEK11, NEK2 |
| Molecular function (GO)     | GO:0019902 | Phosphatase binding                                      | 3          | 0.0026    | MAPK1, NEK2, PPP1CA   |
| GO:0004674                  | Protein serine/threonine kinase activity              | 3          | 0.0219    | MAPK1, NEK11, NEK2   |
| GO:0019903                  | Protein phosphatase binding                           | 2          | 0.0265    | NEK2, PPP1CA       |
| Cellular component (GO)     | GO:0005815 | Microtubule organizing center                            | 5          | 0.00037   | CEP250, MAD1L1, MAPK1, NDC80, NEK2 |
| GO:0005813                  | Centrosome                                           | 4          | 0.00059   | CEP250, MAD1L1, NDC80, NEK2 |
| GO:0000777                  | Condensed chromosome kinetochore                      | 3          | 0.00059   | MAD1L1, NDC80, NEK2  |
| KEGG pathways               | hsa04720    | Long-term potentiation                                   | 2          | 0.0337    | MAPK1, PPP1CA         |
| hsa04914                    | Progesterone-mediated oocyte maturation               | 2          | 0.0356    | MAD1L1, MAPK1      |
| hsa04022                    | cGMP-PKG signaling pathway                           | 2          | 0.0358    | MAPK1, PPP1CA      |

**Table 2 The main GO and KEGG pathways associated with NEK2 and its interacting proteins by DAVID and KOBAS 3.0 databases analyses**

| Categories                  | Pathway ID | Pathway description                                      | Gene count | p-value  | Genes                  |
|-----------------------------|------------|----------------------------------------------------------|------------|----------|------------------------|
| Biological process (GO)     | GO:0006793 | Phosphorus metabolic process                             | 4          | 0.0015   | MAPK1, PPP1CA, NEK2, NEK11 |
| GO:0006796                  | Phosphate metabolic process                           | 4          | 0.0015   | MAPK1, PPP1CA, NEK2, NEK11 |
| GO:0007049                  | Cell Cycle                                           | 3          | 0.00507  | MAPK1, PPP1CA, NEK2   |
| Molecular function (GO)     | GO:0004674 | Protein serine/threonine kinase activity                 | 3          | 0.00526  | MAPK1, NEK2, NEK11    |
| KEGG pathways               | hsa04720    | Long-term potentiation                                   | 2          | 0.00015  | MAPK1, PPP1CA         |
| hsa04114                    | Oocyte meiosis                                       | 2          | 0.00048  | MAPK1, PPP1CA       |
| hsa04270                    | Vascular smooth muscle contraction                    | 2          | 0.00051  | MAPK1, PPP1CA       |
| hsa04611                    | Platelet activation                                  | 2          | 0.00052  | MAPK1, PPP1CA       |
| hsa04910                    | Insulin signaling pathway                             | 2          | 0.00066  | MAPK1, PPP1CA       |
PPP1CA were the major proteins involved in the KEGG pathways (Fig. 3b).

Reactome analyses
Reactome database [29] was used to verify the pathways that NEK2 and its interacting proteins were likely to participate in. The Analyze Data tool of Reactome was used to perform the analyses. NEK2 and its interacting proteins list were inputs. Human was chosen as species. Results showed that there were 122 significant pathways which NEK2 and its interacting proteins involved in ($p < 0.05$) (Additional file 2: Figure S2). The top 5 pathways were displayed in Table 3. Among the results, mitotic prometaphase ($p = 2.63E−06$), M phase ($p = 2.24E−05$), and cell cycle, mitotic ($p = 9.11E−05$) were the three pathways including the most queried genes. The details of mitotic prometaphase pathway were shown in Additional file 3: Figure S3. The main biological processes that NEK2 and its interacting proteins involved in included recruitment of NuMA (nuclear mitotic apparatus protein) to mitotic centrosomes, kinetochore capture of astral microtubules, resolution of sister chromatid cohesion and condensation of prometaphase chromosomes.

Survival analyses by TCGA database
Clinical data of HCC patients were downloaded from TCGA database [30] and the effects of different mRNA expression levels of NEK2 and the genes of NEK2 interacting proteins on prognosis of HCC patients were investigated. A total of 373 HCC patients' clinical data from TCGA database were downloaded, and the patients were divided into high mRNA expression group (n = 187) and low mRNA expression group (n = 186) based on the median mRNA expression value of interesting genes. Only NEK2, NDC80 and CEP250 were found to be associated with the survival of HCC patients ($P = 6 × 10^{-5}$, $P = 0.00122$ and $P = 0.00073$, respectively, Fig. 4). The

![Fig. 3](image)

**Fig. 3** The main GO terms that NEK2 and its interacting proteins enriched and the main KEGG pathways that NEK2 and its interacting proteins enriched. a The main GO terms that NEK2 and its interacting proteins enriched. The shorter the bar graph and the darker the color of each term, the greater the statistical difference is. b The main KEGG pathways that NEK2 and its interacting proteins enriched. MAPK1 and PPP1CA enriched the most KEGG pathways

| Pathway description                  | Gene count | $p$ value       | FDR   | Genes                      |
|-------------------------------------|------------|-----------------|-------|---------------------------|
| Mitotic prometaphase                | 4          | $2.63E−06$      | 3.52E-04 | CEP250, NEK2, MAD11, NDC80 |
| M phase                             | 4          | $2.24E−05$      | 1.50E-03 | CEP250, NEK2, MAD11, NDC80 |
| Cell cycle, mitotic                 | 4          | $9.11E−05$      | 4.01E-03 | CEP250, NEK2, MAD11, NDC80 |
| RHO GTPase effectors                | 3          | $3.20E−04$      | 0.0106 | MAPK1, MAD11, NDC80        |
| Opioid signaling                    | 2          | $7.85E−04$      | 0.0123 | MAPK1, PPP1CA              |

FDR false discovery rate

Table 3 The top 5 pathways associated with NEK2 and its interacting proteins by Reactome analyses
detailed information of 373 HCC patients from TCGA database were displayed in Additional file 4: Table S1. The NEK2, NDC80 and CEP250 mRNA expressions were different in relation to age, weight, recurrence status, histological grade, family HCC history, pathologic stage, tumor size, and survival time. Cox models incorporating clinical covariates of the 373 HCC patients from TCGA database were set up. Clinical covariates and mRNA expression values of the three genes (NEK2, NDC80, CEP250) were fitted into Cox regression models (Table 4). In univariate Cox regression analysis, HCC recurrence, pathologic stage, tumor size, and NEK2, NDC80 and CEP250 mRNA expression were all significantly associated with HCC patients’ survival. In multivariate Cox regression models (the covariates with \( p < 0.2 \) in univariate analysis were included), age, pathologic stage, and NDC80 mRNA expression were independent predictors for HCC patients’ survival. The correlation between NDC80, CEP250 and NEK2 mRNA expression were analyzed. Reactome analysis revealed that CCNB1, CCNB2, and CDK1 were involved in mitotic prometaphase pathway (Additional file 3: Figure S3). Therefore, CCNB1, CCNB2, and CDK1 were also included in correlation analysis. The mRNA expressions of all the five genes (NDC80, CEP250, CCNB1, CCNB2 and CDK1) were positively correlated with NEK2 mRNA expression (Additional file 5: Figure S4).

The mRNA expression of NEK2 and the genes of NEK2 key interacting proteins (NDC80 and CEP250) by Oncomine database

The mRNA expression of NEK2 and the genes of NEK2 key interacting proteins (NDC80 and CEP250) in HCC was analyzed by Oncomine database [31]. There were five eligible HCC studies: Chen Liver [32], Mas Liver [33], Roessler Liver [34], Roessler Liver 2 [34] and Wurmbach Liver [35]. The detailed information was Chen Liver: 99 HCC samples and 77 normal samples, Mas Liver: 38 HCC samples and 19 normal liver samples, Roessler Liver: 22 HCC samples and 21 normal samples, Roessler Liver 2: 225 HCC samples and 220 normal samples, and Wurmbach Liver: 35 HCC samples and 10 normal samples. Meta-analysis of the mRNA expression level of the three genes in the five studies showed that NEK2 \( (p = 2.95 \times 10^{-67}) \), NDC80 \( (p = 8.31 \times 10^{-57}) \) and CEP250 \( (p = 0.003) \) were highly expressed in HCC (Fig. 5). NEK2 was obviously over-expression in 4 studies, especially in Wurmbach Liver study. NDC80 was also obviously over-expression in 4 studies, especially in Roessler Liver and Wurmbach Liver studies. CEP250 was only obviously over-expression in Chen Liver study. None of the three genes showed low mRNA expression in the studies.

Generally alteration and mutual exclusivity of NEK2, NDC80 and CEP250, and the effects on the survival of HCC patients by cBioPortal database

Oncoprint feature of the cBioPortal database [36] was used to determine the genetic alteration frequency and epigenetic alteration level of NEK2, NDC80 and CEP250 in HCC. The queried genes (NEK2, NDC80, CEP250) were altered in 111/366 (30%) of queried samples. The genetic alteration frequencies of NEK2, NDC80 and CEP250 were 19%, 7% and 14%, respectively. Among these, NEK2 has the highest frequencies of gene mutation and copy-number alterations. The detailed genetic alteration types included amplification, missense mutation, truncating mutation and mRNA high, although missense mutation and truncating mutation remained unknown significance (Fig. 6a). For epigenetic alterations, the methylation level of NEK2 and CEP250 were 19%, 7% and 14%, respectively. Among these, NEK2 has the highest frequencies of gene mutation and copy-number alterations. The detailed genetic alteration types included amplification, missense mutation, truncating mutation and mRNA high, although missense mutation and truncating mutation remained unknown significance (Fig. 6a). For epigenetic alterations, the methylation level of NEK2 and CEP250 were 19%, 7% and 14%, respectively. Among these, NEK2 has the highest frequencies of gene mutation and copy-number alterations. The detailed genetic alteration types included amplification, missense mutation, truncating mutation and mRNA high, although missense mutation and truncating mutation remained unknown significance (Fig. 6a). For epigenetic alterations, the methylation level of NEK2 and CEP250 were 19%, 7% and 14%, respectively. Among these, NEK2 has the highest frequencies of gene mutation and copy-number alterations.
determine whether there were any relationship between the genetic alterations of \textit{NEK2}, \textit{NDC80} and \textit{CEP250}. The results showed that the relationships between the alterations of the three genes were all co-occurrence (Table 5). The correlation coefficient between \textit{NEK2} and \textit{NDC80} or \textit{CEP250} mRNA expression were 0.87 and 0.59, respectively (Additional file 5: Figure S5). The overall survival and disease/progression-free survival rates for HCC patients with \textit{NEK2}, \textit{NDC80} and \textit{CEP250} genetic alterations were significantly lower than those without the alterations of the three gene ($p = 0.0426$ and 0.0341, respectively, Fig. 7).

### Discussion

Identification of the differences and mechanisms of genes involved in HCC are of great significance for biomarkers and therapeutic targets. \textit{NEK2} is a microtubule-binding protein that regulates spindles in human cells. Overexpression of \textit{NEK2} mRNA can lead to chromosomal instability, abnormal cell proliferation and drug resistance [13]. Studies showed that \textit{NEK2} mRNA
expression was abnormal in HCC. A study in 63 cases of HCC and matched adjacent non-tumorous liver tissues showed that the mRNA expression of NEK2 was increased, and compared to patients with low mRNA expression, patients with high NEK2 mRNA expression had a poor prognosis. Possible mechanism was that NEK2 influenced the invasion and metastasis of HCC by activating AKT signaling pathway and promoting the expression of matrix metalloproteinases-2 (MMP-2) protein [37]. Another study also demonstrated that the prognosis of HCC patients with high mRNA expression of NEK2 were poorer than patients with low expression [38]. However, study has shown that the mRNA expression of NEK2 in HCC tissues was lower than adjacent normal tissues, and was associated with larger tumor diameter, higher alpha-fetoprotein (AFP) concentration, higher tumor stage, worse prognosis and shorter survival time [39]. The differences between the studies need to be clarified by more studies.

In the present study, the mRNA expression of NEK2 in HCC was systematically examined by multiple databases. Results showed that NEK2 mRNA was highly expressed in HCC, and HCC patients with high NEK2 mRNA expression had poor prognosis. MAD1L1, CEP250, MAPK1, NDC80, PPP1CA, PPP1R2 and NEK11 were found to be the possible upstream or downstream interacting proteins of NEK2. Among these proteins, MAD1L1 was the upstream protein and CEP250, MAPK1, NDC80, NEK11, PPP1CA and PPP1R2 were the downstream proteins of NEK2. NEK11 was both the upstream and downstream protein of NEK2. PPI network confirmed the relationship between NEK2 and its interacting proteins, and NDC80 and CEP250 were found to be NEK2 key interacting proteins. STRING database displayed some GO terms and KEGG pathways that NEK2 and its interacting proteins participated in. Functional pathways by DAVID and KOBAS 3.0 databases also showed that NEK2 and its interacting proteins were

### Table 5: Relationships between the genetic alterations of NEK2, NDC80 and CEP250

| Gene A | Gene B | Log odds ratio | p value | Adjusted p value | Tendency |
|--------|--------|----------------|---------|------------------|----------|
| NEK2   | CEP250 | 1.267          | <0.001  | <0.001           | Co-occurrence |
| NEK2   | NDC80  | 1.182          | 0.006   | 0.017            | Co-occurrence |
| NDC80  | CEP250 | 1.27           | 0.006   | 0.018            | Co-occurrence |

![Fig. 6](image_url) The genetic alterations and methylation level of NEK2, NDC80 and CEP250 in HCC. 

a) The genetic alterations of NEK2, NDC80 and CEP250 were altered in 111/366 (30%) of queried samples. The genetic alteration frequencies of NEK2, NDC80 and CEP250 were 19%, 7% and 14%, respectively. The detailed genetic alteration types included Amplification, Missense Mutation, Truncating Mutation and mRNA High.

b) The methylation level of NEK2, NDC80 and CEP250 in HCC. Red represents high methylation level, and blue represents low methylation level.
involved in important biological processes. Of note, the analysis results of the databases had some differences. To increase the reliability of the results, Reactome database was chosen for further verification and the results showed that NEK2, NDC80, CEP250, MAPK1 and PPP1CA all participated in important biological pathways. Cell cycle was consistently presented in all the three pathway analyzing tools and mitotic prometaphase, the subset of cell cycle, was the most significant pathway which NEK2 and its interacting proteins involved in. Further analysis indicated that mRNA expressions of the three genes (CCNB1, CCNB2 and CDK1) involved in mitotic prometaphase all had high correlation coefficient with NEK2 mRNA expression. We infer that there may be some interrelations between NEK2 and CCNB1, CCNB2 or CDK1, indirectly suggesting the role of NEK2 in mitotic prometaphase pathway. Kaplan–Meier survival analyses of NEK2 and its interacting proteins in HCC patients’ survival. In univariate Cox regression analyses, NEK2, NDC80 and CEP250 mRNA expression were all significantly related to HCC patients’ survival. In multivariate Cox regression model, only NDC80 was the independent predictor of HCC prognosis. These outcomes indicated the critical role of NDC80 in HCC. cBioPortal database revealed the alterations of the three genes were co-occurrence, and clinical correlation analysis also showed the mRNA expression of NDC80, CEP250 and NEK2 were positively correlated. Genensense demonstrated that NDC80 and CEP250 were the downstream protein of NEK2. Therefore, NEK2 may play its role in HCC through interaction with NDC80 and CEP250, especially NDC80. Of course, studies are required to confirm this hypothesis.

NDC80 acts as a component of the essential kinetochore-associated NDC80 complex, plays a role in chromosome congression and is essential for the end-on attachment of the kinetochores to spindle microtubules. NDC80 mRNA expression levels in HCC tissues were found to be significantly higher than those in the adjacent tissues, and NDC80 was believed to contribute to HCC progression by reducing apoptosis and overcoming cell cycle arrest [40]. NDC80 silencing was shown to significantly reduce Hep3 B cell proliferation and colony formation [41]. CEP250 encodes a core centrosomal protein contributed to the centrosome cohesion, centriole biogenesis, and centrosome duplication processes at

![Fig. 7](image-url) Effects of NEK2, NDC80 and CEP250 genetic alterations on overall survival (a) and disease/progression-free survival (b) of HCC patients by cBioPortal database analyses.
different cell cycle stages. Phosphorylation of the C-terminal region of CEP250 by NEK2A is essential for promoting centrosome duplication. The phosphorylation of CEP250 induces the release of rootletin from the distal end of the centriole, which in turn leads to the centrosome disjunction [42]. Studies have confirmed that NDC80 and CEP250, in connection with NEK2, participated in the mechanism of some cancers. For example, NEK2 regulated G2/M phases through phosphorylation of HEC1 [20], and HEC1 was crucial for faithful chromosome segregation and high-expression in most cancer cells. HEC1 is a homologous to NDC80 protein located at the budding yeast kinetochore during mitosis [20]. C-Nap1 (also known as CEP250) was demonstrated to be very important at the onset of mitosis and have a dynamic association with NEK2 [43]. It is suggested that NEK2 can phosphorylate multiple sites of C-Nap1 on the C-terminal domain (CTD), rather than a specific site, changing the overall charge of this domain and perturbing oligomerization and centrosome localization. These studies showed the strong connection between NDC80, CEP250 and NEK2.

MAD1L1, a component of the mitotic spindle-assembly checkpoint, belongs to MAD (mitotic arrest deficiency) family. MAD1L1 may play a pathogenic role in various types of human cancer [44] including HCC [45]. MAD1 interacts with NEK2A via a leucine zipper-containing domain located at the C terminus of MAD1 in vitro and in vivo [17]. MAPK1 encodes a member of the MAP kinase family. MAP kinases, also known as extracellular signal-regulated kinases (ERKs), act as an integration point for multiple biochemical signals, and are involved in a wide variety of cellular processes such as proliferation, differentiation, transcription regulation and development. Studies have demonstrated that MAPK signaling pathway plays an important role in HCC [46, 47]. NEK2 may regulate proliferation, apoptosis, and other biological behaviors of HCC via MAPK signal pathway [48]. PPP1CA encodes protein phosphatase 1 (PP1) that is essential for cell division and participates in the regulation of glycogen metabolism, muscle contractility and protein synthesis. Few studies have presented its role in HCC currently. However, PP1 alpha was identified as a likely physiological antagonist of NEK2 [49]. Therefore, PP1 encoded by PPP1CA may influence the function of NEK2. The results from DAVID and KOBAS indicated that MAPK1 and PPP1CA were enriched in most pathways whereas NEK2 did not enriched in most pathways. This may be related to the fact that data about the roles of NEK2 in these pathways are still rare at present. STRING database verified that there was weak interaction between MAPK1, PPP1CA and NEK2. NEK2 might play its roles through interacting with MAPK1 and PPP1CA and result in alterations in biological pathways although further studies are needed to clarify this possibility. PPP1R2 belongs to PPP1R (protein phosphatase 1 regulatory) family, which participates in the inhibition of protein serine/threonine phosphatase. Evidence pertaining the role of PPP1R2 in HCC is scarce, but PPP1R42 (protein phosphatase 1, regulatory subunit 42) depletion may reduce the activity of PP1, leading to activation of NEK2 [50]. Therefore, PPP1R1 family may assist NEK2 in its role in HCC. NEK11 is a member of the never in mitosis gene A family of kinases, appears to play roles in DNA replication and response to genotoxic stress, and may function with NEK2A in the S-phase checkpoint [22]. Its role in HCC has not been clarified. To sum up, MAD1L1, MAPK1, PPP1CA, PPP1R2, and NEK11 all have something to do with NEK2. Certainly, the detailed roles of these proteins in HCC need to be verified.

In this study, there were some contradictory results. For example, the molecular function of GO terms in STRING database was enriched by the interesting proteins, but the DAVID database did not show similar result. The number of enriched proteins in the pathways of STRING database was higher than in those of DAVID and KOBAS 3.0 databases. Also, MAPK1 and PPP1CA were enriched in most KEGG pathways, but the interesting proteins (NEK2, NDC80 and CEP250) did not enriched in KEGG pathways. The inconsistencies of pathways among different analyses in our study might be related to the differences of data sources and analyzing focus in different databases. STRING and Reactome databases contain more data from different sources than DAVID and KOBASE 3.0 databases. Reactome database focuses on combining signaling, metabolic molecules and biological pathways into a vivid map. The core unit of the Reactome data model is the reaction. The functions of DAVID and KOBASE 3.0 databases concentrate on annotation and functional gene set enrichment. Differences between databases may lead to different results. However, there was a consistency between the different databases. Namely, cell cycle pathway was presented in all the analyses. Therefore, NEK2 and its interacting proteins might be implicated in the pathogenesis of HCC through cell cycle pathway, especially mitotic prometaphase pathway, a subset of cell cycle. NEK2, NDC80, CEP250 and MAD1L1 were the proteins enriched in mitotic prometaphase pathway. These results support that NEK2, NDC80 and CEP250 may coordinate play important roles in influencing HCC development. In univariate Cox regression model, NEK2, NDC80, and CEP250 were all significantly associated with HCC patient’s survival. In multivariate Cox regression model, NDC80 was an independent predictor for
HCC patients’ survival, but NEK2 and CEP250 were not independent predictors. We speculate that the function of NEK2 was influenced by NDC80. In univariate Cox regression model, the interrelationship exited between NEK2, NDC80 and CEP250, suggesting the influential role of NEK2 and CEP250 on the survival of HCC patients. In multivariate Cox regression model, the interrelationship between NEK2, NDC80 and CEP250 was diminished, and the influence of NEK2 and CEP250 on HCC patients’ survival disappeared. We speculate that NEK2 alone may play a weak role in HCC. Genesense analysis showed that NDC80 was the downstream protein of NEK2. Therefore, NEK2 might exert its effect on HCC mainly by influencing NDC80. CCNB1, CCNB2 and CDK1 were all proteins that participate in mitotic prometaphase pathway, and the mRNA expression of the three genes have been shown to have strong positive correlation with NEK2 mRNA expression. Genesense website did not show the upstream and downstream relationship between CCNB1, CCNB2, CDK1 and NEK2, but the high correlation coefficient suggested that there must be some relationship between these proteins. NEK2, NDC80, and CEP250 may play some roles together with CCNB1, CCNB2 and CDK1 in mitotic prometaphase pathway. The survival analysis outcome in our study was similar with the study by Li et al. [13]. The clinical covariates in the study of Li et al. could not be incorporated in our analysis because our study used the median value of NEK2 mRNA expression while Li et al. used the cut-off point of NEK2 mRNA expression defined by the receiver operating characteristic curve to categorize the HCC patients to high or low expression groups. However, the conclusions of the two studies reach a consensus, that is, the prognosis of HCC patients with high NEK2 mRNA expression was poorer than those with low expression.

Small molecule therapies targeting NEK2 and NDC80 in different tumors have been reported. NEK2 siRNA was found to inhibit tumor growth in a subcutaneous xenograft mouse model of pancreatic cancer, prolong the survival time in an intraperitoneal xenograft mouse model and efficiently prevent the progression of liver metastasis [51]. Combination of NEK2 siRNA and chemotherapeutic agents may be effective treatment for colorectal cancer [52]. NDC80 might be a good targeting option in suppressing breast cancer tumor growth, and dual targeting of NEK2 and NDC80 might improve the prognosis [53]. Currently, there have been no studies on molecular therapy by targeting CEP250. Whether targeting NEK2, NDC80 or CEP250 alone or in various combinations may be therapeutically feasible for HCC still needs experimental studies in future.

Conclusions

In summary, the systematic bioinformatics analyses in this study showed that NEK2 and its interacting proteins (MAD1L1, CEP250, MAPK1, NDC80, PPP1CA, PPP1R2, NEK11), especially NEK2 key interacting proteins (NDC80 and CEP250), are significantly involved in HCC. Mitotic prometaphase pathway, a subset of cell cycle pathway, might be the crucial pathway that NEK2, NDC80 and CEP250 are involved in the pathogenesis of HCC. There are strong interactions between NEK2, NDC80 and CEP250. The mRNA expressions of NEK2, NDC80 and CEP250 were significantly associated with HCC patients’ survival. Patients with high mRNA expression and genetic alterations of NEK2, NDC80 and CEP250 had poor prognosis. These findings provide evidence for the potential application of NEK2 as well as NDC80 and CEP250 as biomarkers for HCC diagnosis and HCC patient prognosis and as targets for HCC therapy.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12920-020-00812-y.

Additional file 1. Figure S1: Flow chart of the study and the sample numbers for each data mining part.

Additional file 2. Figure S2: The pathways that NEK2 and its interacting proteins participate in by Reactome database analysis. The red lines in the figure fireworks present the pathways that NEK2 and its interacting proteins may be involved in, and the blue lines present the pathways that NEK2 and its interacting proteins may not be involved in.

Additional file 3. Figure S3: The details of mitotic prometaphase pathway. The purple boxes represent the main biological processes that NEK2 and its interacting proteins may involve in.

Additional file 4. Figure S4: The correlation of mRNA expression between NDC80, CEP250, CCNB1, CCNB2, CDK1 and NEK2 from TCGA database. A. NEK2 vs. NDC80; B. NEK2 vs. CEP250; C. NEK2 vs. CCNB1; D. NEK2 vs. CCNB2; E. NEK2 vs. CDK1.

Additional file 5. Figure S5: The correlation of mRNA expression from cbioPortal database. A. NEK2 vs. NDC80; B. NEK2 vs. CEP250.

Additional file 6. Table S1: The characteristics of 373 HCC patients from TCGA database.

Abbreviations

AFP: Alpha-fetoprotein; BP: Biological process; CC: Cellular component; CE: Centrosomal protein 250 kDa; CNA: Copy number alteration; CT: C-terminal domain; DAVID: The Database for Annotation, Visualization and Integrated Discovery; ERK: Extracellular signal-regulated kinase; FDR: False discovery rate; GISTIC: Genomic identification of significant targets in cancer; GO: Gene Ontology; HCC: Hepatocellular carcinoma; KEGG: Kyoto Encyclopedia of Genes and Genomes; KOBAS: KEGG Ontology Based Annotation System; MAD: Mitotic arrest deficiency; MAD1L1: MAD1 mitotic arrest deficient-like 1 (yeast); MAPK1: Mitogen-activated protein kinase 1; MF: Molecular function; MMP-2: Matrix metalloproteinases-2; NDC80: Nuclear division cycle 80; NEK2: Never in mitosis gene A-related kinase 2; NEK11: NIMA (never in mitosis gene a)-related kinase 11; NuMA: Nuclear mitotic apparatus protein; PPP1R2: Protein phosphatase 1, regulatory subunit alpha; PPP1R: Protein phosphatase 1 regulatory; PPP1R2: Protein phosphatase 1, regulatory subunit 2; PPP1R2: Protein phosphatase 1, regulatory subunit 42; SD: Standard deviation; STRING: Search
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