Analysis of KIF2C, 4A, 10, 11, 14, 18B, 20A, and 23 in HCC and their clinical significance as new-born prognostic markers of HCC

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Abstract

Background: Hepatocellular carcinoma (HCC) is one of the most common malignant tumors. However, the molecular mechanism of its pathogenesis remains to be studied. This study aimed to identify potential KIF genes associated with HCC progression. Methods: We used bioinformatics to initially analyze the expression level and prognostic significance of KIF factors in HCC. Results: We found that compared with the normal control group, KIF2C, 4A, 10, 11, 14, 18B, 20A, and 23 mRNA expression levels increased significantly in HCC. 8 KIF factors had different levels of gene amplification, depth to delete, and missense mutation, and were closely related to the clinical-pathologic stage. Gene set enrichment analysis showed that high mRNA expression of 8 KIF factors in HCC patients were associated with cell cycle, mismatch repair, homologous recombination, and DNA replication. Conclusions: This study expands our understanding of KIF factors in HCC and can provide a theoretical basis for further basic and clinical research in HCC.

Background

Kinesins (KIFs) are proteins that belong to a group of 45 superfamily motor molecules, most of which have ATPase activity and the ability to move along microtubules [1]. KIFs are involved in a variety of physiological functions, such as mitosis, meiosis, and the transport of organelles, vesicles, and mRNA [2–4]. In addition, as a medium, KIFs can transport most proteins synthesized in the cell to where they function physiologically in order to maintain the function and morphology of the cell.

KIFs are divided into 14 subfamilies; kinesin-1 to kinesin-14. The various KIF subtypes perform different physiological functions inside the cell [5, 6]. During mitosis in eukaryotes, the abnormal expression of kinesin can cause chromosome super-condensation, abnormal spindle formation, abnormal cytokinesis, aneuploidy, and other conditions, which can result in an uneven distribution of genetic material and defects in daughter cells and may lead to the development of cancer. Many studies have shown that the abnormal expression of kinesin is related to a variety of human malignancies, including lung cancer [7–9], breast cancer [10], pancreatic cancer [11], kidney cancer [12], and colorectal cancer [13].

Hepatocellular carcinoma (HCC) is a common malignancy, and comprehensive treatment based on
surgical resection combined with chemoradiotherapy remains the most preferred option for the treatment of HCC. However, the mechanism of HCC occurrence and development encompasses various malignant aspects of tumors that are extremely complicated and hidden. The 3 years recurrence rate after resection is as high as 40–50%, and the 5 years recurrence rate is as high as 60–70% [14, 15]. Due to early diagnosis difficulties, most patients with HCC would have already developed intrahepatic metastasis or portal invasion and lost surgical indications by the time of advanced diagnosis [16, 17]. Therefore, HCC is still and important malignant tumor that threatens human health, and alternative and more effective means of treatment are of necessity.

The dysregulation of kinesin expression levels and the malignant biological process and their relationship with clinical prognosis in HCC have been reported in a few articles. With the development of microarray technology and bioinformatics, gene expression, clinical information, and molecular network relationships can be queried and analyzed. This article analyzes the expression of different KIF factors in HCC patients and screens out potential targets to provide a basis for molecular targeted therapy for HCC.

Methods
Oncomine analysis
Oncomine is the largest tumor microarray database and integrated data-mining platform, with a total of 715 datasets and 86,733 samples (https://www.oncomine.org/). We analyzed the transcription levels of KIFs in different cancers online on the Oncomine platform. The mRNA expression of KIFs in cancer and adjacent cancer specimens was analyzed using fold change and student's t-test. The cut-off p-value and fold change were set at 0.05 and 2, respectively.

Gene Expression Profiling Interactive Analysis (GEPIA) and the Kaplan-Meier plotter dataset
GEPIA (https://gepia.cancer-pku.cn/) is an interactive web server for the integrated analysis of cancer expression profile data. In this study, the GEPIA database was used to verify and analyze the correlation between screened KIF factors and HCC. Patients were divided into two groups according to the median expression values (high expression and low expression) of the samples' RNA-seq in Kaplan-Meier Plotter database. The overall survival (OS), relapse-free survival (RFS) and progress free
survival (PFS) in these patients were analyzed.

cBioPortal and the String dataset
cBioPortal provides a web page for cancer genomic research that is used to browse, visualize, and analyze cancer genome data in multiple dimensions and provide graphical information summary, network analysis, survival analysis, single case retrieval, and analysis software for multiple platform gene levels interface (https://www.cbioportal.org/). For this investigation, we selected the liver hepatocellular carcinoma (LIHC) dataset for further analysis (TCGA, Firehose Legacy). The String database is a system that searches for known proteins and predicts protein-protein interactions (https://string-db.org/). With this tool, we screened 50 genes related to the selected KIFs for functional analysis.

The Database for Annotation, Visualization, and Integrated Discovery (DAVID) dataset
DAVID is a biological information database that integrates biological data and analysis tools to provide systematic comprehensive biological function annotation information for large-scale gene or protein lists (https://david-d.ncifcrf.gov/). We analyzed 58 genes using the gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis; the cut-off p-value and FDR were set at 0.001 and 0.05, respectively. We displayed the first 10 results.

The Cancer Genome Atlas (TCGA) dataset and Gene Set Enrichment Analysis (GSEA)
TCGA is a large-scale sequencing-based genomic analysis technology for the understanding of the molecular mechanisms of cancer (https://cancergenome.nih.gov). We downloaded HCC expression profile data from TCGA and classified them into cancer tissues and adjacent tissues using the R language and then normalized them. GSEA is based on the existing knowledge of gene location, function, and biological significance and is used to build a database containing multiple functional gene sets. We divided HCC gene expression profiles into high and low expression groups according to the median values of different KIF mRNAs and used the GSEA software to enrich the KEGG pathway.

Result
Transcriptional levels of KIFs in patients with HCC
Based on the Oncomine database, we compared the transcription levels of 45 molecules in 14 KIF subfamilies in 20 cancers with those in normal samples (Fig. 1). We found that 9 KIFs were differentially expressed in HCC; 8 of them (KIF2C, 4A, 10, 11, 14, 18B, 20A, and 23) were highly expressed and 1 (KIF22) was lowly expressed. Next, we selected the 8 highly expressed KIF factors for further analysis. In Wurmbach’s HCC data set [18], the mRNA expression levels of the 8 KIFs increased significantly. In Roessler’s HCC data set [19], the fold change in the mRNA expression of KIF2C, 4A, 14, 18B, and 20A were 2.69, 2.704, 2.406, 2.093, and 3.252, respectively, compared to normal samples. In Roessler’s other HCC dataset [19], KIF2C, 4A, and 20A mRNA expressions also increased considerably. In Chen’s HCC dataset [20], the fold change in the mRNA expression of KIF11 and KIF23 were 2.690 and 2.253, respectively (Table 1).

Table 1
The significant changes in KIF expression at the transcriptional level in hepatocellular carcinoma and liver tissues (Oncomine database)

| KIF  | Fold change | P-value  | t-test   | Ref       |
|------|-------------|----------|----------|-----------|
| KIF2C| 3.462       | 4.20E-7  | 5.958    | Wurmbach [18] |
|      | 2.169       | 3.95E-48 | 17.795   | Roessler [19] |
|      | 2.867       | 5.84E-7  | 6.561    | Roessler [19] |
| KIF4A| 4.704       | 1.88E-9  | 7.769    | Wurmbach [18] |
|      | 2.704       | 3.54E-62 | 22.006   | Roessler [19] |
|      | 2.665       | 4.05E-8  | 7.415    | Roessler [19] |
| KIF10| 3.123       | 4.48E-8  | 6.758    | Wurmbach [18] |
| KIF11| 3.846       | 1.84E-8  | 7.052    | Wurmbach [18] |
|      | 2.690       | 4.05E-12 | 7.504    | Chen [20]     |
| KIF14| 5.344       | 9.34E-14 | 10.605   | Wurmbach [18] |
|      | 2.406       | 2.19E-8  | 7.893    | Roessler [19] |
| KIF18B| 2.093      | 3.22E-8  | 7.182    | Roessler [19] |
|      | 2.680       | 6.23E-6  | 5.081    | Wurmbach [18] |
| KIF20A| 6.336      | 8.38E-11 | 8.766    | Wurmbach [18] |
|      | 3.252       | 2.47E-68 | 24.329   | Roessler [19] |
|      | 2.711       | 5.62E-8  | 7.398    | Roessler [19] |
| KIF23| 2.253       | 3.93E-17 | 9.286    | Chen [20]     |
|      | 2.143       | 2.17E-5  | 4.633    | Wurmbach [18] |

The relationship between the mRNA levels of 8 KIFs and clinicopathological parameters in HCC

Based on the GEPIA dataset, we compared the mRNA expression of 8 KIFs between HCC and healthy liver tissues and determined the correlation between these levels and the tumor stage. Our results showed that the expression levels of the 8 KIFs in HCC were higher than those in normal liver tissues (Fig. 2A, B), and there were significant differences in tumor staging (Fig. 2C).

Increased mRNA expression of 8 KIFs is associated with poor prognosis in HCC patients

We used Kaplan-Meier to further explore the prognostic significance of the 8 KIFs in HCC patients. Correlations between the mRNA levels of KIFs and OS, RFS, and PFS in 364, 316, and 370 cases of HCC, respectively, were
analyzed. The Kaplan-Meier curve and log-rank test analysis showed that the elevated mRNA levels of the 8 KIFs were closely related to poor OS, poor RFS, and poor PFS (Fig. 3).

Function prediction and pathway information of 8 KIFs and their closely related neighboring genes in HCC

We used the cBioPortal online tool to analyze the interaction network relationship between 8 KIFs and HCC. As shown in Fig. 4A, KIFs changed in 107 (29.72%) of the 360 HCC patient samples, with two or more changes detected in almost half of the changed samples (53 samples) (Fig. 4A). We also analyzed the mRNA expression of KIFs (RNA Seq V2 RSEM) using the cBioPortal online tool and determined intercorrelations among the 8 KIFs using Pearson’s correction coefficient. The results showed significant positive correlations between the 8 KIFs (Fig. 4B). Next, we filtered the 50 neighboring genes most relevant to the 8 KIFs through the String database and constructed a network map. Per our findings, cell cycle-related genes, including CDK1, RACGAP1, PLK1, ECT2, CCNB1, CCNB2, CDC20, CCNA2, and CDC5L, were closely linked to changes in the 8 KIFs (Fig. 4C).

We then used DAVID and KEGG to predict GO enrichment analysis, including biological processes, cellular components, and molecular functions, and to determine the KEGG pathway map of these 58 genes. We found that GO: 000706 (mitosis), GO: 0000087 (M phase of the mitotic cell cycle), and GO: 0022402 (cell cycle process) were significantly regulated by changes in KIFs (Fig. 5A). GO: 0005819 (spindle), GO: 0015630 (microtubule cytoskeleton), GO: 0000793 (condensed chromosome), GO: 0003777 (microtubule motor activity), and GO: 0005524 (ATP binding) were also significantly controlled by these alterations in KIFs (Fig. 5B and 5C). KEGG analysis revealed 13 pathways related to changes in KIFs (Fig. 5D). Among these pathways, hsa04110: cell cycle, hsa04115: P53 signaling pathway, hsa04068: FoxO signaling pathway, hsa05203: viral carcinogenesis, hsa05161: Hepatitis B, hsa04390: Hippo signaling pathway, and hsa04152: AMPK signaling pathway reportedly participate in HCC occurrence and pathogenesis.

Finally, we used GSEA to analyze the pathway enrichment of the 8 KIFs in HCC to verify our results. Consistent with our earlier finding, the high expression of the 8 evaluated KIFs was most relevant to the cell cycle pathway in HCC. In addition, the high expression of these KIFS is involved in DNA replication, base excision repair, P53 signaling pathway, Notch signaling pathway, and more (Fig. 6).

Discussion

The KIF family is a highly conserved family of proteins found in all eukaryotes [21]. The overexpression of KIFs
disrupts the balance associated with normal spindle assembly and function, leading to spindle defects, genetic instability, and tumor development. KIF disorders have been reported in many cancers [7, 22–25], and the mechanism by which KIFs play a pro-cancer role in different cancers has been partially confirmed. However, how KIFs impact HCC has been studied less. Before this research, Hu demonstrated that FOXM1 regulates KIF4A expression positively and interacts with BS3 (5'-AGATGGAGT-3') in the KIF4A promoter binding site, directly mediating HCC cell proliferation [26]. Here, we used bioinformatics to analyze the mRNA expression and prognosis values of different KIF factors in HCC. We hope that our findings will help guide further research on KIFs in HCC and provide biomarkers of prognostic value.

Among KIF factors, the KIF2C protein is required for centromere-microtubule attachment during cell mitosis and spindle formation [27]. KIF2C dysfunction can cause chromosome misconvergence and instability, as well as abnormal cell signaling and transport, which can induce cancer [28]. The overexpression of KIF2C is associated with the growth and malignant invasion of colorectal cancer, gastric cancer, and breast cancer, leading to poor prognosis for patients [29–31]. So far, the correlation between KIF2C and HCC has not been reported. In this article, data analysis shows that KIF2C is highly expressed in HCC and is related to clinicopathological features and prognosis. Based on the network signal diagram, we suggest that KIF2C may play an important role in the repair of chromosomal abnormalities and mismatches in HCC cells.

KIF4A is a cancer-related KIF factor that has been studied more recently. The imbalance of KIF4A induces abnormal spindle separation and leads to aneuploidy formation [32, 33]. KIF4A is highly expressed in breast cancer, colorectal cancer, lung cancer, and liver cancer and reportedly participates in regulating the M phase of the cell cycle and control of cell proliferation by activating spindle assembly checkpoints [26, 34, 35]. Here, we confirm that KIF4A is linked to the progression and poor prognosis of HCC, and also show, with the analysis of biological processes, that KIF factors has a significant correlation with the M phase of the cell cycle, strengthening the reliability of our results.

KIF10 is also known as centrosome-associated protein E (CENPE). Deleting CENPE expression inhibits chromosome alignment during mitosis, leading to checkpoint activation and cell mitotic arrest [36, 37]. In human tissues, CENPE mRNA expression is closely associated with cell proliferation. CENPE is upregulated abnormally in many types of cancers, such as epithelial ovarian cancer, prostate cancer, and triple-negative breast cancer.
[38–40], and is linked to the promotion of cell cycle progression and tumor cell growth. In this research, we discussed the expression profile of CENPE mRNA and its prognostic value in HCC, providing a theoretical basis for the further study of CENPE.

KIF11 belongs to the kinesin 5 subfamily and is involved primarily in chromosomal localization, the formation of bipolar spindles during cell mitosis, and the transport of protein complexes between cells [41–43]. KIF11 is closely associated with many cancers, such as kidney cancer, pancreatic cancer, and lung cancer [44–47]. Reportedly, endogenous KIF11 promotes the self-renewal ability of breast cancer cells and enhances the characteristics of breast cancer stem cells by participating in the activation of the Wnt/β-catenin signaling pathway [48]. However, KIF-specific inhibitors allegedly prevent the growth and self-renewal of glioblastoma tumor stem cells [49]. Additionally, KIF11 is highly expressed in HCC and is associated with liver cirrhosis and TNM staging and can be used as a poor prognostic biomarker [50]. We demonstrate here that the high expression of KIF11 in HCC tissues is linked to negative OS, negative RFS, and negative PFS.

The KIF14 protein is located in the central region of the spindle and centrosome and is a microtubular motor. KIF14 mRNA expression is a prognostic indicator for breast, lung, and gastric cancer patients [51–53]. Previously, Xu et al. showed that KIF14 is a molecular target and oncogenic mechanism of overexpression in liver cancer [54], suggesting that targeting KIF14 could be a new treatment strategy for HCC.

KIF18B belongs to the kinesin 8 subfamily and is involved in the separation of chromosomes and spindle localization during mitosis [55]. In recent years, investigations have shown that KIF18B participates in the growth and development of a variety of cancers [11, 56]. Per Li et al., KIF18B expression is regulated in a cell cycle-dependent manner and, therefore, could be involved in the regulation of cell cycle and cell proliferation [11]. Our analysis results revealed that KIF18B is an important regulator of HCC prognosis and is closely connected to cell cycle and DNA replication-related pathways, which provides ideas and strategies for our next focus on the role of KIF18B in HCC.

Both KIF20A and KIF23 belong to the kinesin 6 subfamily. KIF20A participates in the assembly of mitotic spindles, while KIF23 is required for the formation of mitotic centrosomes and cytokinesis [57]. The abnormal expression of both KIF20A and KIF23 can lead to the inhibition of tumor cell proliferation or accelerated apoptosis [58–60]. In our study, GSEA results showed that elevated KIF20A and KIF23 levels in HCC patients were closely linked to
nucleotide splicing repair and homologous recombination genes.

Conclusions
In summary, our results suggest that the expression levels of KIF2C, 4A, 10, 11, 14, 18B, 20A, and 23 are significantly associated with poor prognosis of HCC. The underlying mechanisms involve not only cell cycle, DNA replication, and mismatch repair but also P53, Notch, AMPK, and Hippo signaling pathways. We predict that KIF2C, 4A, 10, 11, 14, 18B, 20A, and 23 will become novel biomarkers and could serve as targets for the treatment of HCC.

Abbreviations
HCC: hepatocellular carcinoma; KIFs: kinesins; GEPIA: gene expression profiling interactive analysis; OS: overall survival; RFS: relapse-free survival; PFS: progression free survival; LiHC: hepatocellular carcinoma; DAVID: database for annotation, visualization, and integrated discovery; GO: gene ontology; KEGG: kyoto encyclopedia of genes and genomes; TCGA: the cancer genome atlas; GSEA: gene set enrichment analysis; CENPE: centrosome-associated protein E.

Declarations

Ethics approval and consent to participate
Not applicable

Consent for publication
The manuscript is approved by all authors for publication

Availability of data and materials
The raw data are available upon request on the following e-mail address: d201981842@hust.edu.cn.

Competing Interests
The authors report no conflicts of interest.

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Authors Contributions
QMJ conceived and designed the manuscript. WQS and HXX analyzed the data. LQT and JX contributed the materials/analysis tools for the study. XZF and YSL wrote the paper. All authors read and approved the manuscript.

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Figures
The transcription levels of KIFs in different types of cancers. Different expressions of the KIF14 subfamilies in 20 cancers; KIF2C, 4A, 10, 11, 14, 18B, 20A, and 23 were significantly overexpressed in liver cancer (p<0.05). A. Different expressions of KIF1, 2, 5, 6, 7, 10 in 20 cancers. B. Different expressions of KIF3, 4 in 20 cancers. C. Different expressions of KIF8, 9, 11, 12, 13 in 20 cancers. D. Different expressions of KIF14 in 20 cancers.
B

C

Transcripts Per L

KIF2C  KIF4A  KIF10  KIF11  KIF14  KIF18B  KIF20A  KIF23

LHC
(num(T)=300; num(N)=100)

KIF2C
KIF4A
KIF10
KIF11
KIF14
KIF18B
KIF20A
KIF23

F value = 15.1
P value = 8.3e-05

F value = 5.22
P value = 8.3e-05

F value = 3.34
P value = 4.8e-03

F value = 3.42
P value = 5.3e-03
The expression of 8 KIFs and the correlation between them and the tumor stage in LIHC. A. The transcripts per million of KIF2C, 4A, 10, 11, 14, 18B, 20A, and 23 were compared between the control and LIHC. B. The mRNA expression level analysis of 8 KIFs in liver cancer patient specimens (n = 369) and non-tumor liver tissues (n = 160). The KIF2C, 4A, 20A, and 23 groups varied significantly, whereas the KIF10, 11, 14, and 18B groups did not differ considerably. C. The expression levels of KIF2C, 4A, 10, 11, 14, 18B, 20A, and 23 correlated significantly with the tumor stage.
The prognostic values of the mRNA levels of 8 KIFs in HCC patients. High mRNA expression of KIF2C, 4A, 10, 11, 14, 18B, 20A, and 23 was significantly associated with poor overall survival and poor relapse-free survival of patients with HCC, as well as with the poor disease-free progression in these patients.
8 KIF expression and mutation analysis in HCC. Genetic alterations of KIF2C, 4A, 10, 11, 14, 18B, 20A, and 23. The oncoprints of 8 KIFs were identified. A. The column represents HCC patients, and the row represents gene alterations, including missense mutation, truncating mutation, amplification, deep deletion, and high mRNA. B. Pearson’s test revealed that there was a significant positive intercorrelation between the 8 KIF factors. C. The String database retrieved the first 50 genes and networks that were most relevant to the 8 KIF factors.
The functions of 8 KIFs and genes significantly associated with KIF alterations were predicted with the analysis of GO and KEGG using the DAVID database. GO enrichment analysis predicted the functional roles of target host genes based on three aspects: biological processes (A), cellular components (B), and molecular functions (C), and the correlation involving each predicted pathway is represented by a circular ratio (D).
Figure 6

GSEA analysis of 8 KIFs in HCC. Pathways of the cell cycle were most significantly increased in the high KIF2C, 4A, 10, 11, 14, 18B, 20A, and 23 groups. The 8 KIFs also participated in the homologous recombination, DNA replication, mismatch repair, and base excision repair pathways to varying degrees. A. KIF2C mainly participated in the cell cycle and mismatch repair. B. KIF4A mainly participated in the cell cycle and P53 signaling pathway. C. KIF10 mainly participated in the cell cycle and oocyte meiosis. D. KIF11 mainly participated in the cell cycle and base excision repair. E. KIF14 mainly participated in the cell cycle and notch signaling pathway. F. KIF18B mainly participated in the cell cycle and DNA replication. G. KIF20A mainly participated in the cell cycle and nucleotide excision repair. H. KIF23 mainly participated in the cell cycle and homologous recombination.