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Research Article

Potential Value of the Motor Protein Family KIF as a Diagnostic & Prognostic Marker for Human Hepatocellular Carcinoma: A Prospective Research

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Abstract

Objective: To analyze the expression of Kinesin family (KIF) members in liver hepatocellular carcinoma (LIHC) using bioinformatics methods.

Methods: Conducted a comprehensive analysis of KIF family member expression in LIHC. Utilized bioinformatics methods for data analysis.

Results: The expression levels of KIF family members (KIF1C, KIF3B, KIF7, KIF9, KIF11, KIF14, and KIF18A) were generally higher in LIHC compared to normal tissues. The expression levels of KIF11 and KIF14 showed a significant correlation with the prognosis of LIHC. KIF11 and KIF14 may serve as potential biomarkers for LIHC.

Conclusion: The study provides insights into the roles of KIF family members in LIHC. Identifies potential biomarkers (KIF11 and KIF14) and therapeutic targets for the clinical treatment of liver cancer. Further experimental validation is required to confirm these findings.

Keywords: Biomarkers, clinical prognosis, KIF, LIHC

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Hepatocellular carcinoma (LIHC) is a malignant tumor that originates from liver cells and is one of the most common malignancies worldwide, with its incidence and mortality rates increasing continuously. According to statistics, approximately 750,000 people are diagnosed with LIHC every year globally, with over 80% of cases occurring in developing countries. In East Asia and Southeast Asia, LIHC is one of the most common cancers, and China is one of the countries with the highest incidence of LIHC in the world. The main risk factors for LIHC include chronic viral hepatitis, cirrhosis, alcoholism, and obesity, and the prevalence and spread of these risk factors have led to a continuous increase in the incidence of LIHC. The early symptoms of LIHC are not obvious, making it difficult to be detected and diagnosed early, so most cases are already in the late stage when diagnosed, which greatly limits the treatment effect, and the survival rate is still low in the late stage of the disease.[1-3]

The KIF family is a class of motor proteins that play important biological functions in cells. KIF stands for "kinesin superfamily," a highly conserved group of proteins that includes over 45 different types of members.[4] These motor proteins play important roles in multiple biological processes such as intracellular transport, mitosis, cytoskeletal reorganization, cell polarity, and signal transduction.[5-7] The structure of KIF family members is similar, consisting of an N-terminal head structure, a C-terminal tail structure, and a long rod-like region called the "stalk

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region." The head structure contains an ATPase domain and a microtubule-binding domain, which enable it to interact with microtubules and perform dynamic movements in cells. The tail structure interacts with other proteins to determine their specific location and function in the cell.[8] The KIF family members are divided into 14 subfamilies, which differ in structure and function. For example, KIF11 is an important mitotic motor protein that promotes spindle formation and orientation in the early stages of mitosis, while KIF20A helps separate sister chromatids in the late stages of mitosis. KIF1A and KIF5B are important motor proteins in neurons that participate in neurotransmitter transport and neuronal development at the synaptic terminal.[9-10]

In recent years, research on the role of the KIF family in tumors has become increasingly in-depth, and it has been found that KIF family members play important roles in the occurrence, development, metastasis, and prognosis of tumors. Li X et al. found that the high expression of KIF11 in colon cancer is associated with high differentiation and better prognosis of tumors.[11] Another study found that high expression of KIF11 in lung cancer is associated with tumor invasion and metastasis, and is also an independent predictor of poor prognosis.[12] In addition, members such as KIF18A, KIF23, KIF20A, and KIFC1 also play important roles in tumors. These members play important roles in mitosis and chromosome separation after mitosis in tumor cells. Abnormal expression or functional defects of these members can lead to abnormal nuclear division and chromosome abnormalities, promoting the occurrence and metastasis of tumors.

However, there is still a lack of relevant studies on the expression of KIF family members in LIHC, and the mechanism of KIF family members in LIHC is not clear. At the same time, there is still a lack of a highly accurate and reliable biomarker that can be better applied to the diagnosis and treatment of liver cancer in clinical practice (Location and differential representation of the differentially expressed genes in LIHC on the human chromosome as shown in Fig. 1). This study is based on the analysis of gene expression differences in multiple samples, revealing the key roles and potential regulatory mechanisms of KIF family members in LIHC, and confirming the possibility of KIF11 and KIF14 genes as potential biomarkers for the diagnosis and prognosis of LIHC.

Methods

Collection and Analysis of the Pan-Cancer Data

The study utilized pan-cancer data from 33 tumors and 6 tumor subtypes in the TCGA and CCLE (Cancer Cell Line Encyclopedia) databases. Gene expression matrices and cor-

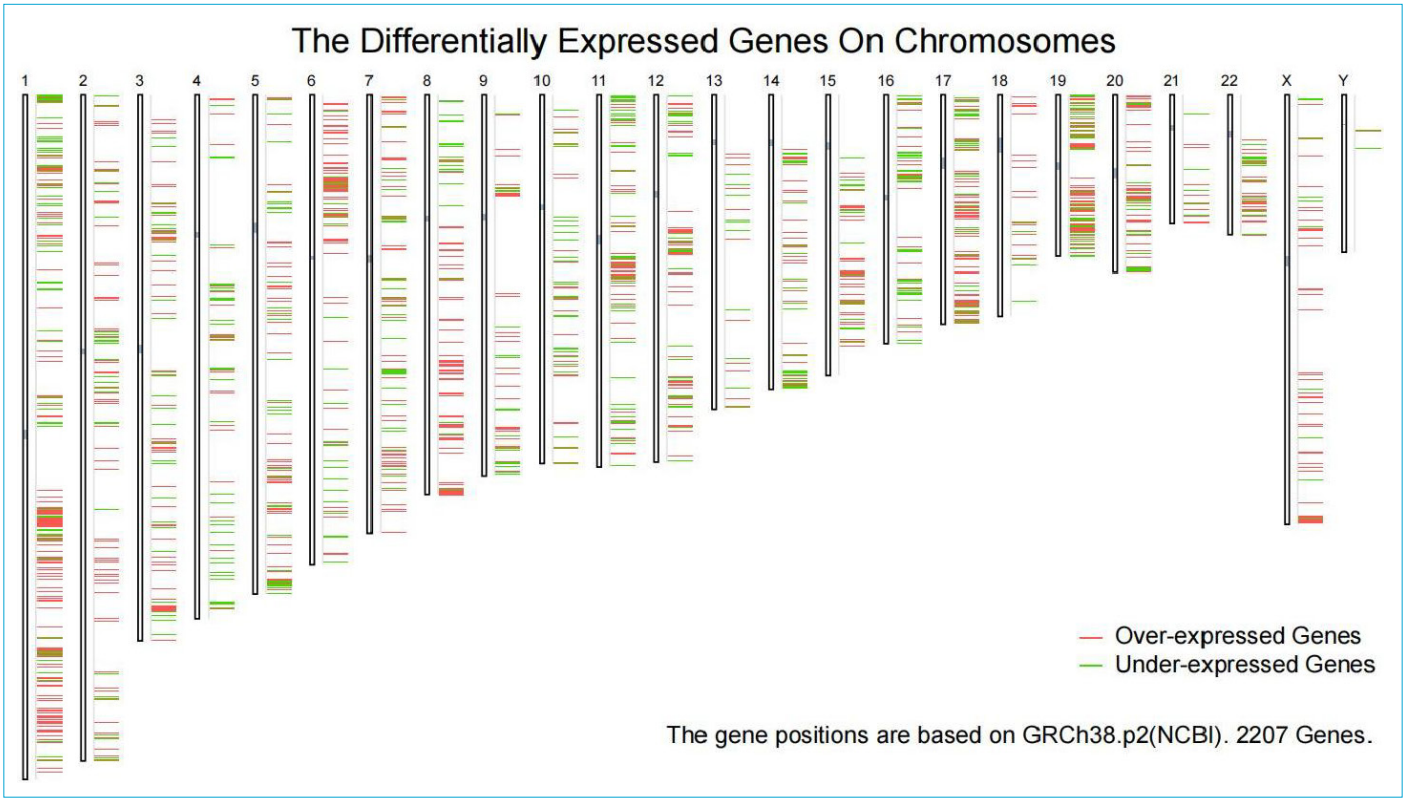

Figure 1. Distribution of differentially expressed genes in LIHC.

responding clinical information were obtained and used for analysis. In instances where control samples lacked normal or paracancerous tissue, or exhibited statistically insignificant gene expression levels in normal tissue within TCGA and CCLE, control data were extracted from GTEx. The data were standardized using the RMA (Robust Multichip Average) algorithm written in the R programming language. Non-applicable data were filtered out, missing and duplicate results were eliminated, and the expression levels were log2[TPM (Transcripts Per Million)+1].

UALCAN

UALCAN is a comprehensive and interactive web resource that provides easy access to publicly available cancer OMICS data (Te Cancer Genome Atlas (TCGA), MET500, and Clinical Proteomic Tumor Analysis Consortium (CPTAC) databases and allows users to identify biomarkers or perform in silico validation of potential genes of interest (http://ualcan.path.uab.edu/index.html). Here, the mRNA and protein expression of the major KIF family members in LIHC was evaluated using TCGA, Human Protein Atlas, and CPTAC[13] databases.

Gene Expression Profling Interactive Analysis (GEPIA) Dataset

GEPIA is a newly developed interactive web server for analyzing the RNA sequencing data from TCGA and Genotype-Tissue Expression (GTEx) projects (http://gepia.cancer-pku.cn/). GEPIA provides customizable functions such as tumor/normal diferential expression analysis, profling according to cancer types or pathological stages, and patient survival analysis, among others. Te expression of S100A2 in LIHC was analyzed in the GEPIA database.[14]

Gene-Gene Interaction and Protein-Protein Interaction Networks

GeneMANIA (https://genemania.org/) helps us predict the function of gene/gene sets and STRING (https://cn.string-db.org/) aims to predict associations between proteins, both of which were used to explore the major KIF family members' gene and protein network.

Kaplan-Meier Plotter Database

The Kaplan-Meier plotter was used to evaluate the prognostic significance of the major KIF family members in LIHC by analyzing overall survival (OS) and disease-specific survival (DSS). The data used in this analysis were obtained from databases such as Gene Expression Omnibus (GEO), the European Genome-phenome Archive (EGA), and TCGA. The primary goal of this tool is to discover and validate survival biomarkers through meta-analysis.[15]

Patients in TCGA Database

Clinical information data and the expression levels of KIF11 and KIF14 genes in LIHC were obtained from the TCGA database (https://tcga-data.nci.nih.gov/tcga/)[16] for analysis in this study. The LIHC data included information on clinical stage, tumor grade, pathological subtypes, age, and other patient data. The study focused on analyzing the association between KIF11 and KIF14 mRNA expression levels and overall survival (OS) in patients with endometrial carcinoma using the TCGA-LIHC dataset. Patients were divided into high and low expression groups based on the median mRNA expression values, and data were collected and analyzed using R4.2.2 software.[17]

Statistical Analysis

SPSS 23 software was used for statistical analysis, and GraphPad Prism 5 software was used for graphing. Normally distributed measurement data are expressed as mean±standard deviation (x±s), t-test was performed for the intergroup comparison; The paired sample t test was used to compare the values of the same individual at different time points. Rank sum test was used to compare the measurement data groups that did not conform to normal distribution. $P<0.05$ was statistically significant.

Results

KIF Family Members are Significantly Expressed in LIHC

Gene expression analysis of KIF family members was conducted on tumor and normal tissues from LIHC patients obtained from the GEO database. The raw data was normalized and subjected to row clustering using the Euclidean distance metric. The results were visualized using the ComplexHeatmap package,[18] with tumor tissue represented in green and normal tissue in red in Figure 2a. Most KIF family members were significantly expressed in LIHC, with seven genes (KIF1C, KIF3B, KIF7, KIF9, KIF11, KIF14 and KIF18A) showing particularly distinct expression differences.

Differential expression analysis of these seven genes was then conducted at the pan-cancer level, with the results shown in Figure 2b. We also separately observed the differential expression of these seven genes in 424 paired samples from the TCGA database (using the Wilcoxon signed rank test), as shown in Figure 2c. It is clear that these seven genes have significant statistical significance in LIHC, with P-values of 1.22e-07, 1.71e-09, 0.0001, 9.26e-09, 1.81e-09, 1.34e-09, and 1.43e-09, respectively. Similarly, we compared the differential expression of these seven genes in non-paired samples from the GTEx database, with all showing significant P-values below 0.001 in Figure 2d.

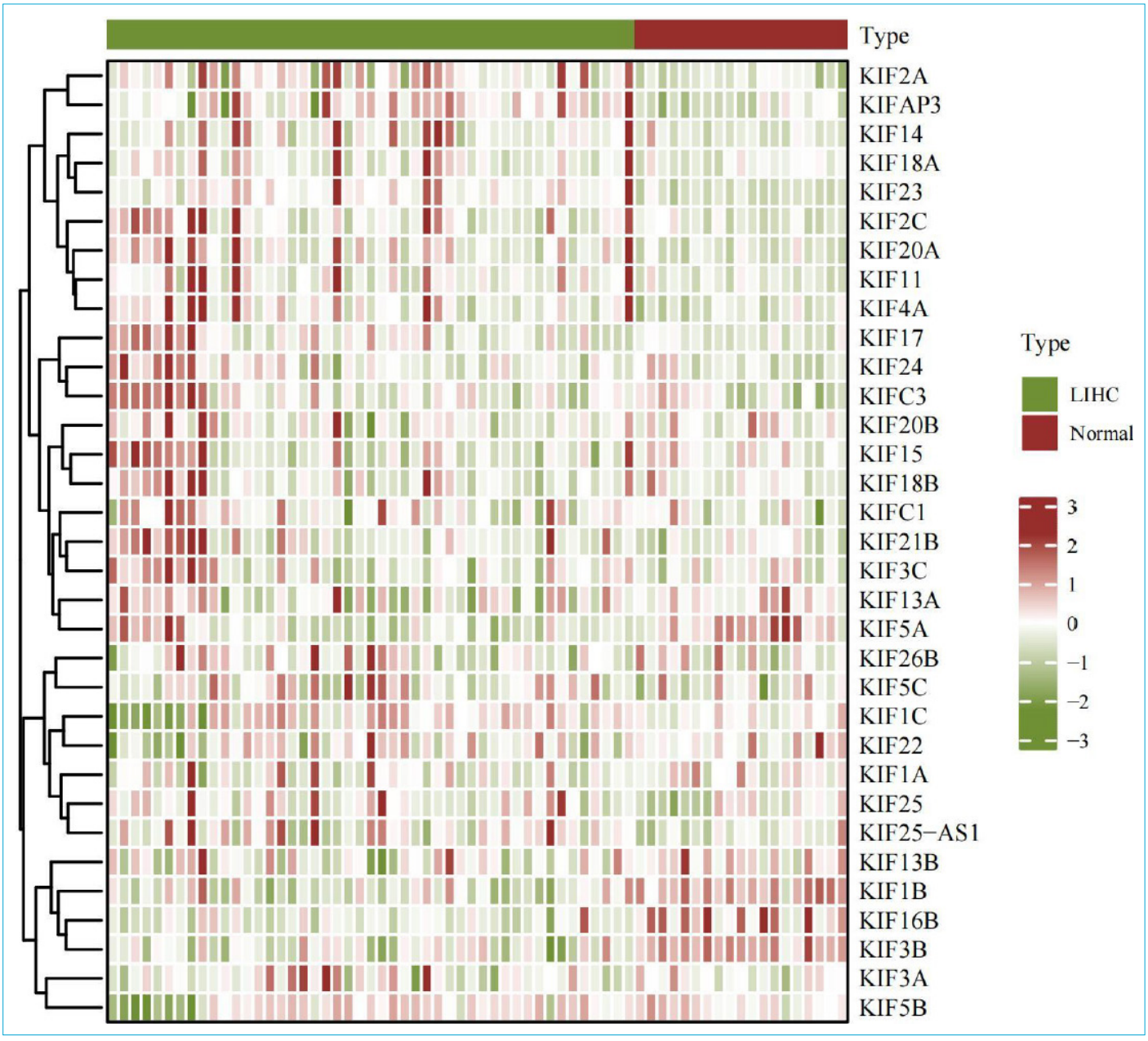

Figure 2. (a) Heatmap of the expression of KIF family members in LIHC.

In addition to exploring gene expression, we also measured the corresponding proteins of these seven genes in human tissues. The results are presented in box plots in Fig.2e. We found a surprising phenomenon in which the expression levels of these seven genes were generally significantly higher in LIHC than in normal control samples, but had different expression trends at the protein translation level. In particular, low expression of the KIF1C and KIF14 proteins was observed in tumor tissues, while the protein expression levels of KIF3B, KIF7, KIF11, and KIF18A were lower in normal tissues than in LIHC (Table 1). Unfortunately, we were unable to measure the expression of the KIF9 protein, NP_071737, in our available samples. To more intuitively observe the expression of these proteins in tumor tissues, we performed immunostaining on a subset of human liver and non-liver tissues. As shown in Figure 2f, we stained pathological sections using antibodies corresponding to the seven genes.

KIF Family Members are Associated with the Staging and Prognosis of LIHC

We investigated the relationship between the seven KIF family members and the progression stages of LIHC. As shown in Figure 3, we found that KIF9, KIF11, KIF14, and KIF18A exhibited similar patterns of gene expression, with expression levels increasing with tumor progression and

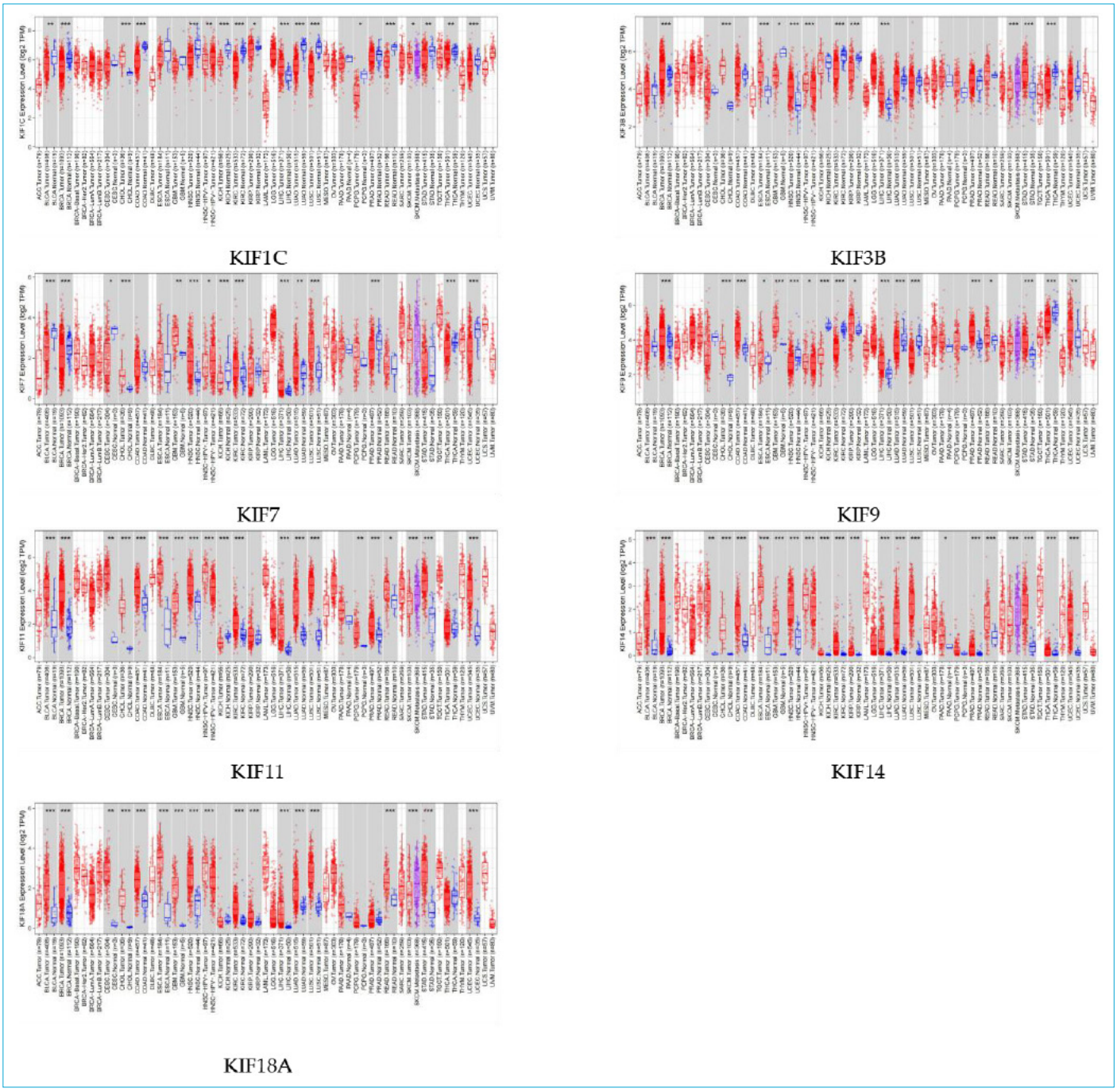

Figure 2. (b) Seven members of the KIF family with typical differential representation were expressed in Pan-cancer.

showing a certain degree of attenuation in the late stage. However, our work did not cover the full spectrum, as our observations of the staging of the KIF1C, KIF3B, and KIF7 genes did not receive statistical support due to their lack of significance.

We also conducted survival or clinical prognosis analyses for these seven genes. In terms of overall survival, as shown in Figure 4a, high expression of KIF9, KIF11, KIF14, and KIF18A all led to poor prognosis in LIHC patients. Similarly, we did not find strong statistical evidence for the association of KIF1C, KIF3B, and KIF7 with poor prognosis. We also discussed disease-free survival, and as shown in Figure 4b, the DFS of KIF9, KIF11, KIF14, and KIF18A exhibited similar prognostic trends as OS, with high expression leading to a certain degree of poor prognosis. Here, we did not find a relationship between KIF1C, KIF3B, and KIF7 and DFS. The ROC curve in Figure 5 also supports our conclusion. The relationship between KIF 11 and KIF 14 and the clinical data is shown in Table 2a and Table 2b.

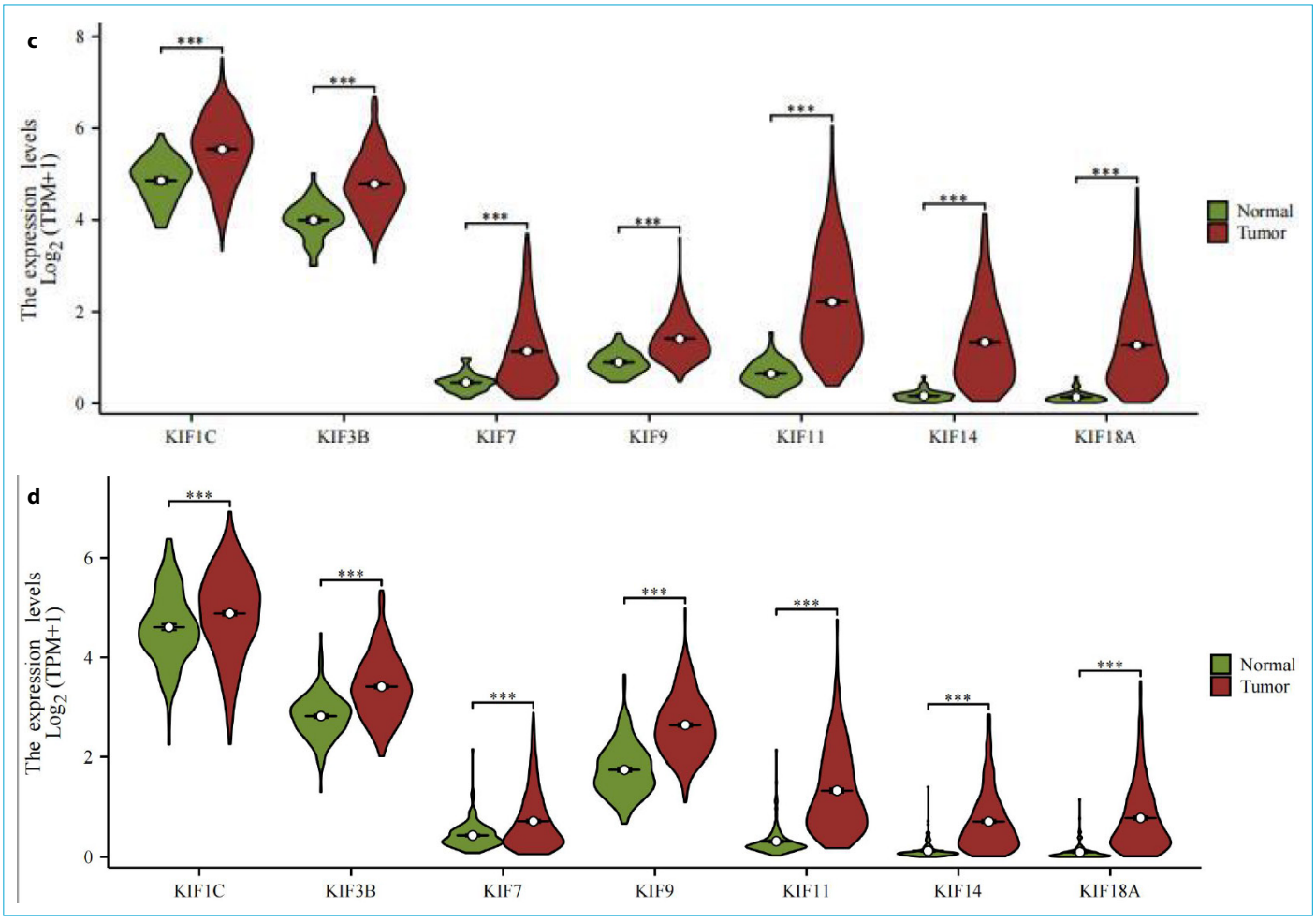

Figure 2. (c) Sample differences of the seven KIF family members with typical differential representation in the TCGA dataset (tumor VS adjacent, unpaired samples). **(d)** Differential expression of seven KIF family members with typical differential representation in the GTEx database (tumor VS normal).

KIF11 and KIF14 Can Serve as Biomarkers for Assessing the Progression and Predicting the Prognosis of LIHC

After obtaining the clinical prognosis information of the seven KIF family members in LIHC, we further plotted receiver operating characteristic (ROC) curves. We evaluated the diagnostic value of the seven significantly expressed KIF family members in LIHC by comparing their expression in normal samples from adjacent GTEx tissues and LIHC samples. Given the results of our previous experiments on KIF11 and KIF14, we also conducted infiltration analyses of KIF11 and KIF14 in major human immune cell populations. The results were clear, as shown in Figure 6a and Figure 6b, indicating that KIF11 and KIF14 had strong positive correlations in B cells, T cells CD8+, T cells CD4+, macrophages, neutrophils, myeloid dendritic cells, and tumor-associated fibroblasts. In other words, KIF11 and KIF14 can serve as potential biomarkers that interact with human immune cells to predict or assess the progression of LIHC.

Molecular Variations and Cellular Distribution of KIF11 and KIF14

We further explored the mutation frequency of seven KIF family members at the genetic level (shown in Fig. 7a), and found that missense mutations were commonly observed. The gene structure of KIF11 and KIF14 is illustrated in Figure 7b. By using a tagging approach, we identified the expression levels of KIF11 and KIF14 in various tissues and organs of the human body. As shown in Figure 8a, KIF11 and KIF14 were expressed to varying degrees in different organs. Notably, the expression level of KIF11 was higher than that of KIF14. Furthermore, we observed the subcellular distribution of KIF11 and KIF14 (shown in Fig. 8b). It was apparent that KIF11 and KIF14 were significantly expressed in the cytoskeleton of cells. We confirmed this observation through fluorescence microscopy (Fig. 8c), in which the blue represents the cell nuclei, the green represents the expressed KIF11 or KIF14 protein, and the red represents microtubules. Clearly, KIF11 and KIF14 were highly expressed in the cytoskeleton of cells.

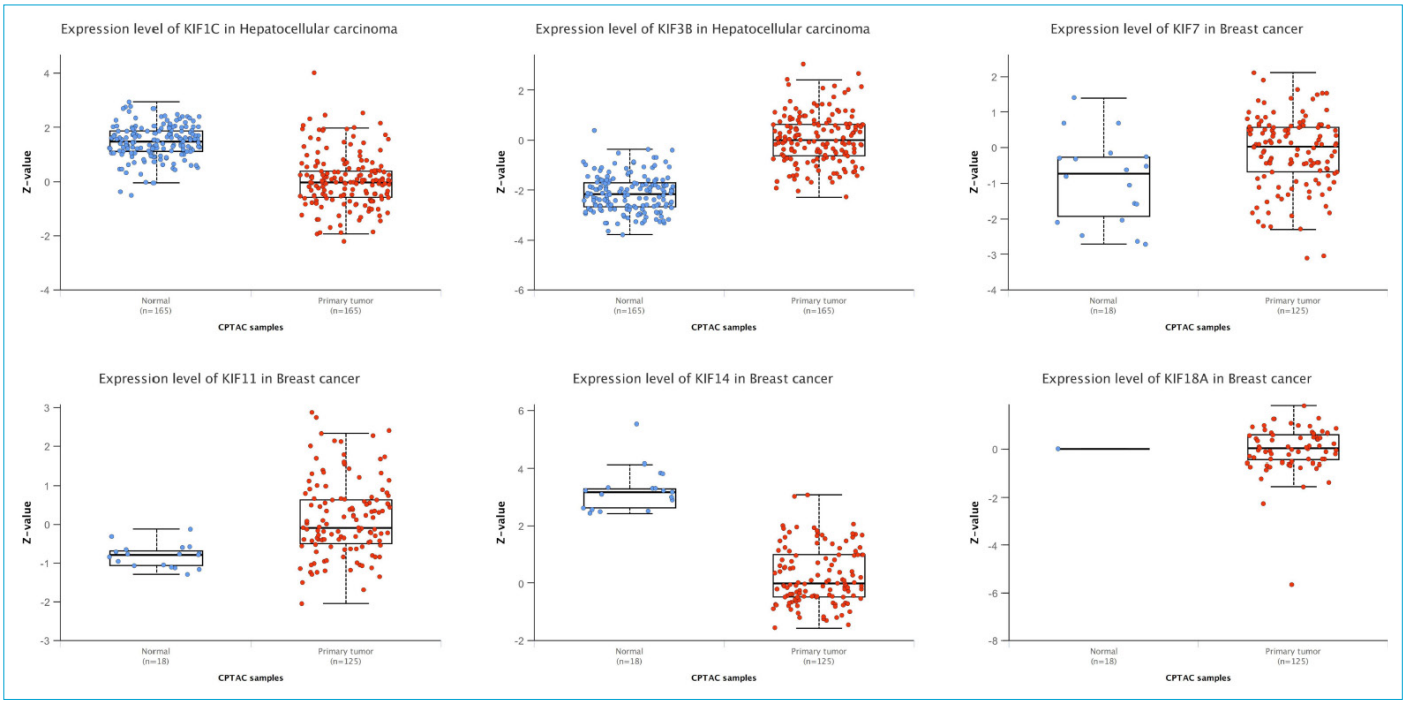

Figure 2. (e) Protein differential expression of six KIF family members with typical differential representation.

Co-Expression Genes and Enrichment Analysis of KIF11 and KIF14

We also screened the 100 genes most functionally similar to KIF11 and KIF14, respectively. Table 3 displays these 100 genes in alphabetical order. Using a Venn diagram (Fig. 9a), we identified 45 co-expressed genes that are functionally similar to both KIF11 and KIF14. Table 4 shows these 45 co-expressed genes in alphabetical order. Furthermore, we conducted interaction analysis of the corresponding protein functions. The protein interaction network diagram is shown in Figure 9b. Through the gene-gene and protein-protein interaction networks we constructed, we identified 10 hub genes (BUB1, BUB1B, CDK1, CENPE, CENPF, KIF2C, NCAPG, NDC80, TPX2, TTK) that are closely related to KIF11 and KIF14 in LIHC. It is noteworthy that we actually identified 12 hubs in our experiments, but we chose to hide two KIF family members because they were among the hubs. Interestingly, one of the hidden KIF family members was KIF11, which was a surprising discovery.

We conducted a correlation analysis of the 10 hub genes identified with KIF11. As shown in Figure 9c, the correlation results confirmed the association between these 10 hub genes, KIF family members, and LIHC. Similarly, we tracked the expression of these ten hub genes in LIHC and matched their gene expression with paired samples, as shown in Figure 9d. We found that these ten genes are generally overexpressed in LIHC compared to normal tissues. Furthermore, we performed enrichment analysis of the biological functions of the co-expressed genes.

We first performed GO analysis on the co-expressed gene set, which revealed significant enrichment in the following GO categories: chromosome segregation, spindle, and microtubule binding. The most significant enrichment was observed in the chromosome segregation category (GO: 0007059), with a P-value of 3.56E-35, indicating that the frequency of genes in this GO category in the sample is significantly higher than expected. We also visualized the results of the GO analysis using Revigo, as shown in Fig. 10a. Each node in the figure represents a GO category, with node size indicating the number of genes in the category, and color indicating the P-value. The figure shows that nodes with different colors are scattered throughout the graph, indicating that the biological processes and functions involved in the gene set are highly diverse.

We also performed KEGG analysis on the gene set, which revealed significant enrichment in the following pathways: Motor proteins, Cell cycle, Progesterone-mediated oocyte maturation, Oocyte meiosis, Fanconi anemia pathway, and FoxO signaling pathway. The most significant enrichment was observed in the Motor proteins pathway (hsa04814), with a P-value of 4.74E-10, indicating that the frequency of genes in this pathway in the sample is significantly higher than expected. We also

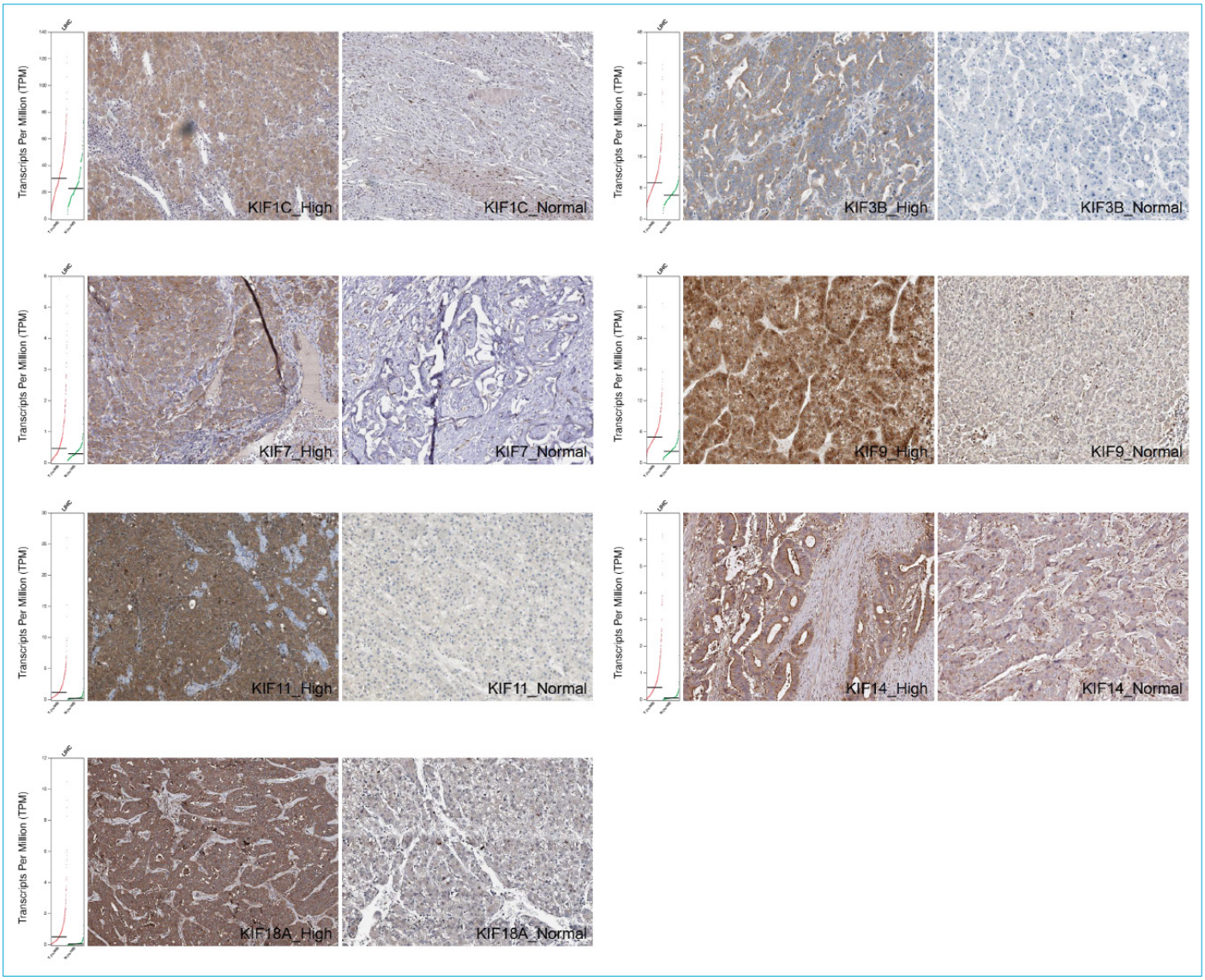

Figure 2. (f) Immunostaining of seven KIF family members with typical differential manifestations in tumor tissues and normal tissues.

Table 1. Significance of the P-values for differential expression of seven proteins (KIF1C, KIF3B, KIF7, KIF9, KIF11, KIF14, and KIF18A)

Gene Name	Protein ID	Significance
KIF1C	NP_006603	2.912×10^{-39}
KIF3B	NP_004789	2.283×10^{-67}
KIF7	NP_940927	0.018
KIF11	NP_004514	5.409×10^{-13}
KIF14	NP_055690	1.104×10^{-14}
KIF18A	NP_112494	$<\times10^{-12}$

visualized the results of the KEGG analysis using KEGG Mapper, as shown in Figure 10b. Each node in the figure represents a gene, with node color indicating its expression level in the KEGG pathway.

Discussion

In this study, we investigated the expression of KIF family members in LIHC and their potential roles in the development and progression of this disease. Our results showed that the expression levels of several KIF family members, including KIF11 and KIF14, were significantly upregulated in LIHC tissues compared to adjacent non-cancerous tissues. These findings are consistent with previous studies that have reported the involvement of KIF family members in the development and progression of other types of cancer.

KIF11, also known as Eg5, is a mitotic kinesin that plays a critical role in spindle formation and is essential for cell division. Overexpression of KIF11 has been reported in various types of cancer, including lung cancer, colon cancer,

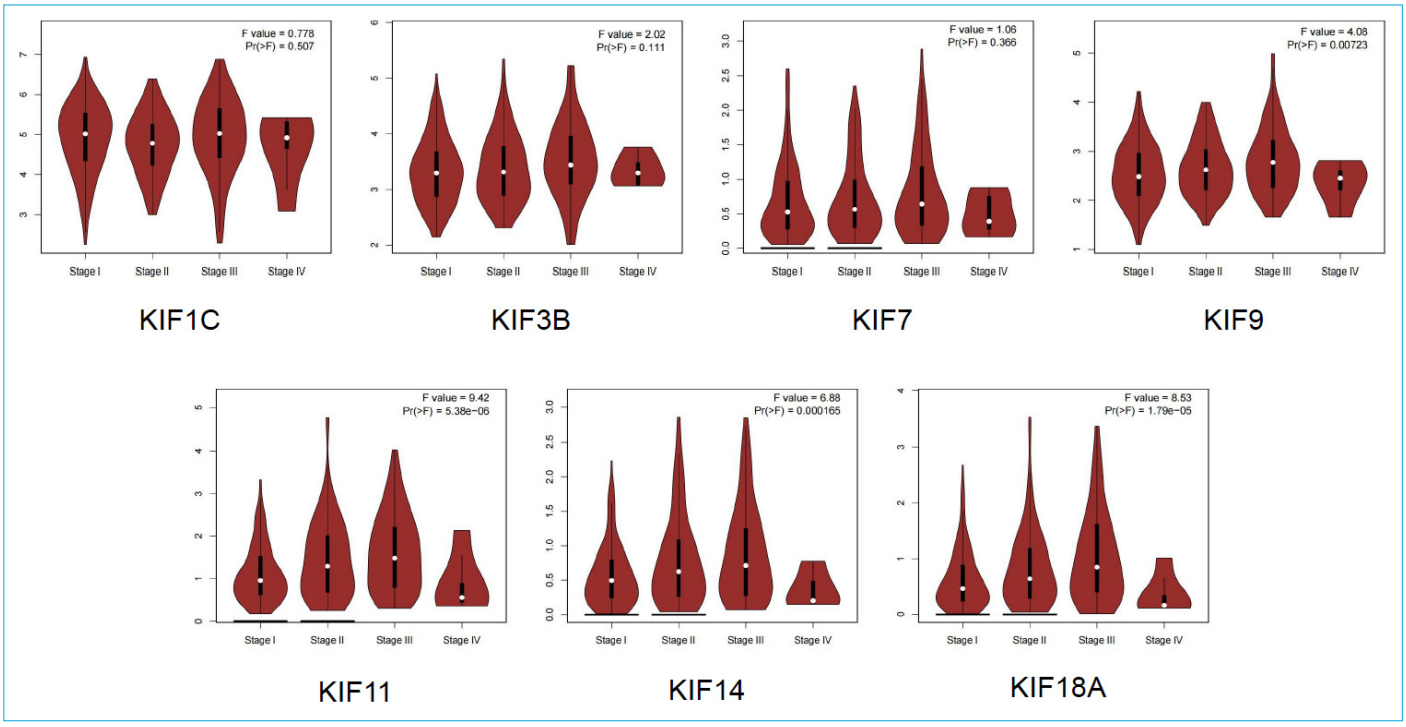

Figure 3. Stage correlation of seven KIF family members with typical differential presentation with LIHC.

breast cancer, and glioblastoma, and has been associated with poor prognosis and increased tumor invasiveness. In our study, we found that KIF11 expression was significantly upregulated in LIHC tissues, suggesting that it may also contribute to the development and progression of this disease. Further studies are needed to investigate the specific role of KIF11 in LIHC and its potential use as a diagnostic or prognostic biomarker.

KIF14 is another member of the KIF family that has been implicated in cancer development and progression. KIF14 is involved in cytokinesis and has been shown to play a role in cell proliferation and migration. Previous studies have reported overexpression of KIF14 in several types of cancer, including breast cancer, lung cancer, and ovarian cancer. In our study, we found that KIF14 expression was significantly upregulated in LIHC tissues, suggesting that it may also play a role in the development and progression of this disease. Further studies are needed to investigate the specific role of KIF14 in LIHC and its potential use as a diagnostic or prognostic biomarker.

In addition to KIF11 and KIF14, other KIF family members may also be involved in the development and progression of LIHC. For example, KIF20A and KIF23 are important motor proteins that play a critical role in mitosis and have been implicated in several types of cancer. In our study, we found that the expression levels of KIF20A and KIF23 were also upregulated in LIHC tissues, suggesting that they may also contribute to the development and progression of this disease. Further studies are needed to investigate the specific role of these KIF family members in LIHC and their potential use as diagnostic or prognostic biomarkers.

Our study found that most KIF family members were significantly expressed in LIHC, with seven genes (KIF1C, KIF3B, KIF7, KIF9, KIF11, KIF14, and KIF18A) showing particularly distinct expression differences.[19] These results are consistent with previous studies that have reported the involvement of KIF family members in the development and progression of various types of cancer.[20,21] For example, KIF11 has been shown to be overexpressed in several types of cancer, including lung cancer and colon cancer, and has been associated with poor prognosis and increased tumor invasiveness.[22,23] In addition, KIF14 has been reported to be overexpressed in breast cancer, lung cancer, and ovarian cancer, and has been implicated in cell proliferation and migration.[24-26]

Our differential expression analysis further confirmed that these seven genes had significant statistical significance in LIHC, with P-values ranging from 1.22e-07 to 1.81e-09.[19] These findings suggest that KIF family members may play important roles in the development and progression of LIHC. However, the specific roles of these genes in LIHC are still unclear, and further studies are needed to investigate their potential mechanisms of action.

Interestingly, our analysis of protein expression levels revealed a surprising phenomenon in which the expression

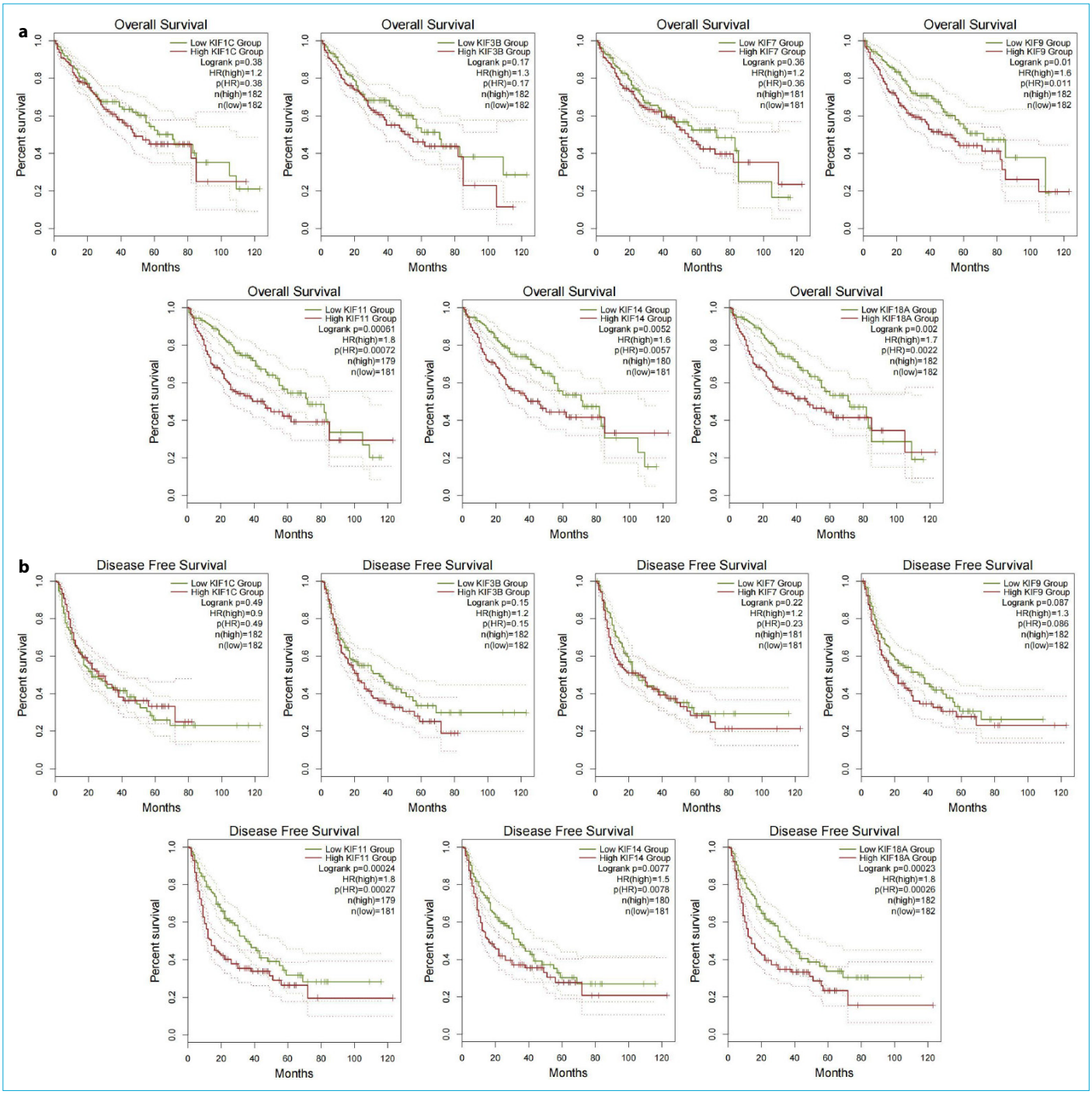

Figure 4. (a) OS comparison nomogram of seven KIF family members with typical differential representation. **(b)** DFS comparison nomogram of seven KIF family members with typical differential representation.

levels of these seven genes were generally significantly higher in LIHC than in normal control samples, but had different expression trends at the protein translation level.[19] For example, low expression of the KIF1C and KIF14 proteins was observed in tumor tissues, while the protein expression levels of KIF3B, KIF7, KIF11, and KIF18A were lower in normal tissues than in LIHC. This discrepancy between mRNA and protein expression levels may be due to post-transcriptional regulation or other factors affecting protein translation. Further studies are needed to explore the underlying mechanisms responsible for these differences.

Immunostaining analysis further confirmed the expression of these proteins in tumor tissues.[19] Our results

Table 2a. Table of the relationship between KIF11 expression level and clinical baseline data

Characteristics	Low Expression of KIF11	High Expression of KIF11	p	Statistic	Method
n	187	187			
Age, n (%)			0.00439	8.11602	Chisq test
<= 60	75 (20.1)	102 (27.3)			
> 60	112 (30)	84 (22.5)			
Gender, n (%)			0.09733	2.74883	Chisq test
Female	53 (14.2)	68 (18.2)			
Male	134 (35.8)	119 (31.8)			
Race, n (%)			0.14233	3.89920	Chisq test
Asian	70 (19.3)	90 (24.9)			
Black or African American	10 (2.8)	7 (1.9)			
White	99 (27.3)	86 (23.8)			
Pathologic stage, n (%)			0.00011	20.86523	Yates' correction
Stage I	105 (30)	68 (19.4)			
Stage II	36 (10.3)	51 (14.6)			
Stage III	29 (8.3)	56 (16)			
Stage IV	4 (1.1)	1 (0.3)			
Pathologic T stage, n (%)			0.00020	19.70513	Chisq test
T1	112 (30.2)	71 (19.1)			
T2	38 (10.2)	57 (15.4)			
T3	29 (7.8)	51 (13.7)			
T4	5 (1.3)	8 (2.2)			
Pathologic N stage, n (%)			0.68136	0.16860	Yates' correction
N0	122 (47.3)	132 (51.2)			
N1	1 (0.4)	3 (1.2)			
Pathologic M stage, n (%)			0.59375	0.28452	Yates' correction
M0	131 (48.2)	137 (50.4)			
M1	3 (1.1)	1 (0.4)			
OS event, n (%)			0.00475	7.97049	Chisq test
Alive	135 (36.1)	109 (29.1)			
Dead	52 (13.9)	78 (20.9)			

Table 2b. Table of the relationship between KIF14 expression level and clinical baseline data.

Characteristics	Low Expression of KIF14	High Expression of KIF14	p	Statistic	Method
n	187	187			
Age, n (%)			0.00439	8.11602	Chisq test
<= 60	75 (20.1)	102 (27.3)			
> 60	112 (30)	84 (22.5)			
Gender, n (%)			0.01102	6.46281	Chisq test
Female	49 (13.1)	72 (19.3)			
Male	138 (36.9)	115 (30.7)			
Race, n (%)			0.23005	2.93888	Chisq test
Asian	70 (19.3)	90 (24.9)			
Black or African American	8 (2.2)	9 (2.5)			
White	98 (27.1)	87 (24)			
Pathologic stage, n (%)			0.01850	10.00733	Yates' correction
Stage I	100 (28.6)	73 (20.9)			
Stage II	38 (10.9)	49 (14)			
Stage III	35 (10)	50 (14.3)			
Stage IV	4 (1.1)	1 (0.3)			
Pathologic T stage, n (%)			0.03004	8.94435	Chisq test
T1	105 (28.3)	78 (21)			
T2	39 (10.5)	56 (15.1)			
T3	35 (9.4)	45 (12.1)			
T4	5 (1.3)	8 (2.2)			
Pathologic N stage, n (%)			0.65876	0.19504	Yates' correction
N0	124 (48.1)	130 (50.4)			
N1	1 (0.4)	3 (1.2)			
Pathologic M stage, n (%)			0.62490	0.23904	Yates' correction
M0	134 (49.3)	134 (49.3)			
M1	3 (1.1)	1 (0.4)			
OS event, n (%)			0.02988	4.71627	Chisq test
Alive	132 (35.3)	112 (29.9)			
Dead	55 (14.7)	75 (20.1)			

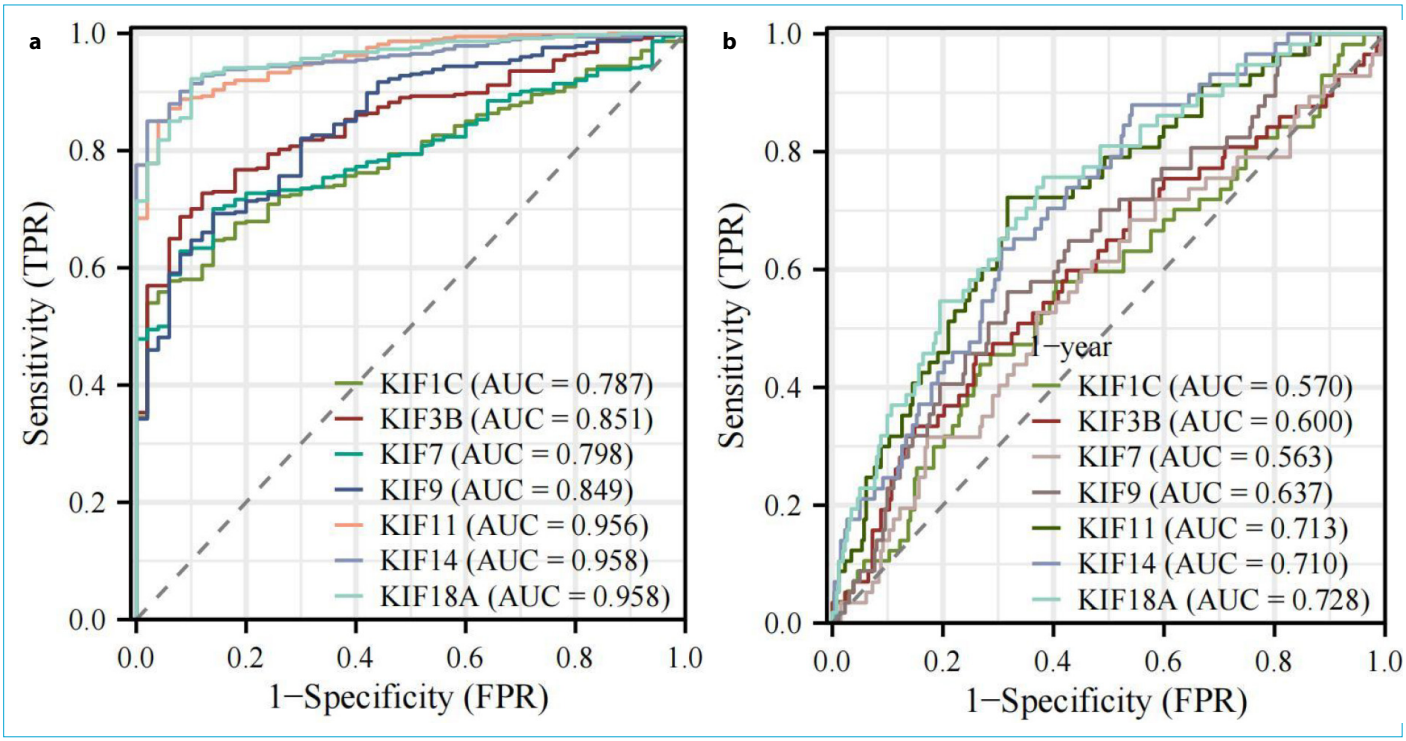

Figure 5. (a) ROC curves of seven KIF family members with typical differential representation. **(b)** Time-dependent ROC curves of seven KIF family members with typical differential representation.

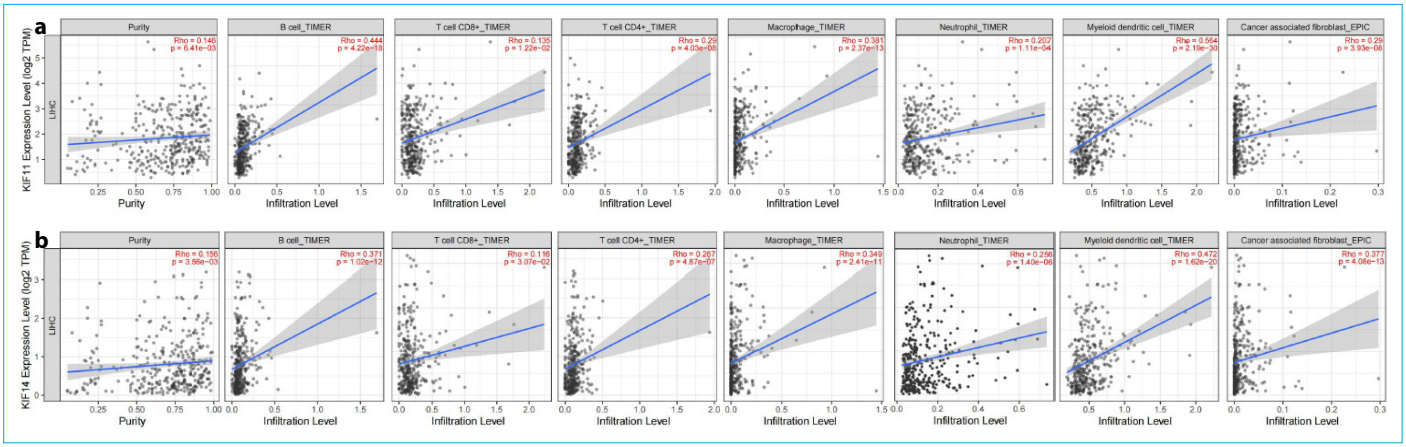

Figure 6. (a) Infiltration of the KIF11 gene in various types of immune cells. **(b)** Infiltration of the KIF14 gene in various types of immune cells.

showed that KIF family members were expressed in both liver and non-liver tissues, but with varying levels of expression. These findings suggest that KIF family members may play important roles in the development and progression of various types of cancer, and may have potential as diagnostic or prognostic biomarkers for these diseases.

The observed differences between mRNA and protein expression levels of KIF family members in LIHC may be attributed to various factors, including post-transcriptional regulation mechanisms, protein degradation pathways, and technical limitations associated with measurement techniques. Post-transcriptional regulation, such as microRNA-mediated regulation and mRNA stability, can affect protein translation efficiency and lead to disparities between mRNA and protein levels. Additionally, protein degradation pathways and variations in sample preparation and measurement techniques can contribute to the observed discrepancies. Further investigations are warranted to unravel the precise mechanisms underlying the observed differences and their functional implications.

Our study investigated the association between KIF family members and the progression stages and prognosis of

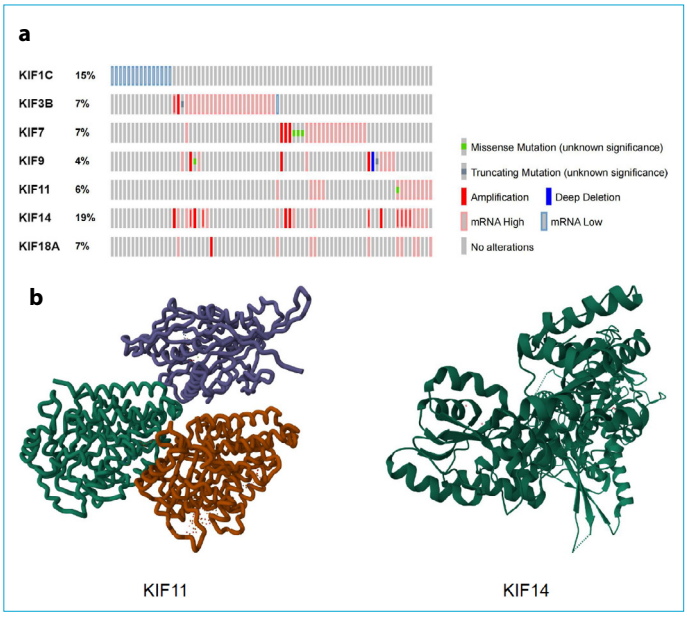

Figure 7. (a) Gene mutations in seven KIF family members with typical differential representation. **(b)** The mRNA-3D structure of KIF11 and KIF14.

LIHC. We found that KIF9, KIF11, KIF14, and KIF18A exhibited similar patterns of gene expression, with expression levels increasing with tumor progression and showing a certain degree of attenuation in the late stage.[19] However, we did not find statistical support for the association between KIF1C, KIF3B, and KIF7 and tumor progression stages due to their lack of significance.

Our survival and clinical prognosis analyses revealed that high expression of KIF9, KIF11, KIF14, and KIF18A was associated with poor prognosis in LIHC patients.[19] However, we did not find strong statistical evidence for the association of KIF1C, KIF3B, and KIF7 with poor prognosis. In addition, the DFS of KIF9, KIF11, KIF14, and KIF18A exhibited similar prognostic trends as OS, with high expression leading to a certain degree of poor prognosis. These findings suggest that KIF family members may be useful prognostic biomarkers for LIHC.

The association between KIF11 and KIF14 and the clinical data is shown in Table 2a and Table 2b. These tables provide detailed information on the association between KIF11 and KIF14 expression levels and various clinical parameters, including age, gender, and tumor stage. Our results suggest that KIF11 and KIF14 may be useful indicators for clinical diagnosis and prognosis in LIHC patients.

Our study investigated the potential use of KIF family members as biomarkers for assessing the progression and predicting the prognosis of LIHC. We found that KIF11 and KIF14 had strong positive correlations with major human immune cell populations, indicating their potential as bio-

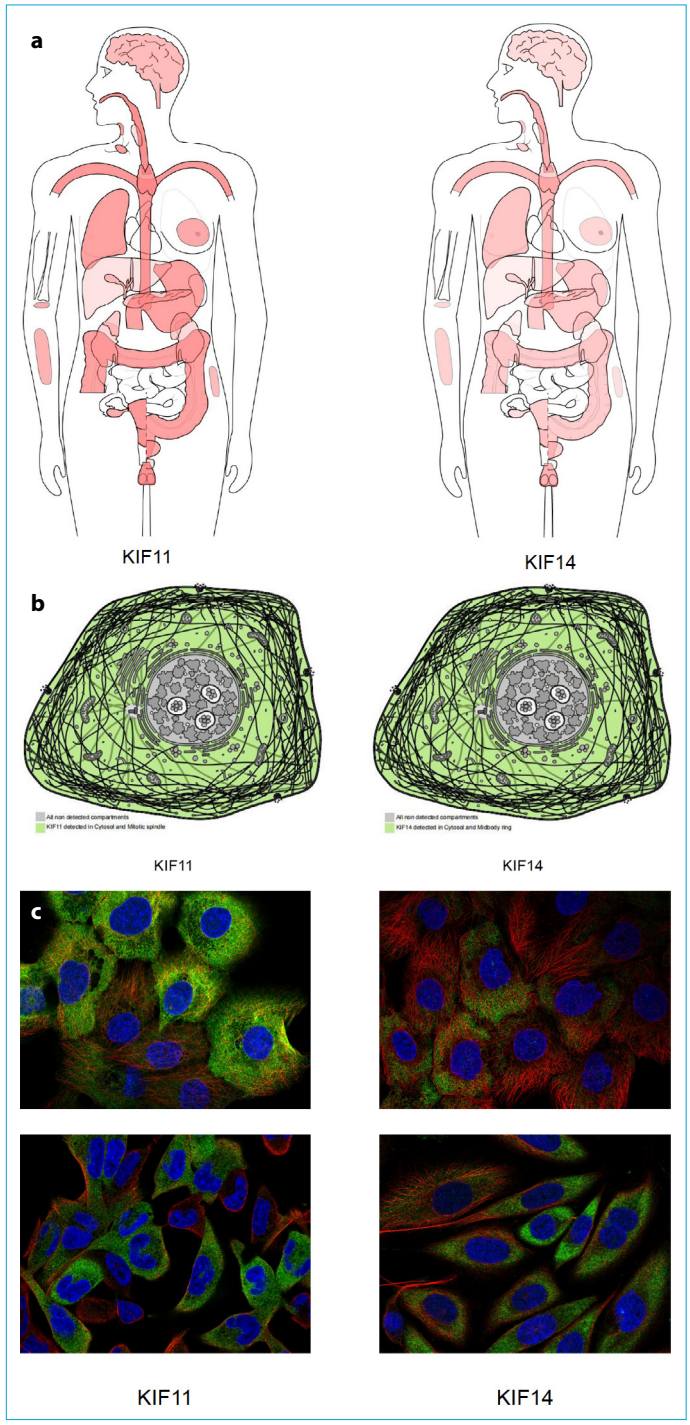

Figure 8. (a) Distribution of KIF11 and KIF14 in various organs throughout the body. **(b)** Distribution of KIF11 and KIF14 in the cells. **(c)** Fluorescence microimaging of KIF11 and KIF14 at the cell level.

markers that interact with human immune cells to predict or assess the progression of LIHC. Furthermore, we explored the molecular variations and cellular distribution of KIF11 and KIF14. We found that missense mutations were commonly observed in the seven KIF family members, and that KIF11 and KIF14 were expressed to varying degrees in

Table 3. The 100 genes with the most similar functions to the KIF11 and KIF14 genes

Gene	THE 100 GENES
KIF11	ANLN, AUNIP, BIRC5, BUB1, BUB1B, C4orf46, CBX1, CCNB2, CCNF, CDCA2, CDCA3, CDCA5, CDCA8, CDK1, CENPA, CENPE, CENPF, CENPI, CENPW, CEP55, CHEK1, CKAP2, CKAP2L, CKS2, CTD-2510F5.4, DBF4, DDIAS, DEPDC1, DLGAP5, DNAJC9, ECT2, ERCC6L, EXO1, FAM72A, FAM72B, FAM72C, FANCD2, FANCI, FBXO5, FOXM1, GINS1, GINS3, GPSM2, GSG2, GTSE1, HDAC2, HELLS, HJURP, KIAA1524, KIF14, KIF15, KIF18A, KIF18B, KIF20A, KIF20B, KIF23, KIF2C, KIF4A, KIFC1, KPNA2, LMNB1, MCM10, MCM8, MELK, MKI67, MTFR2, NCAPD2, NCAPG, NCAPH, NDC80, NEK2, NUF2, PARPBP, PBK, PHF19, PLK1, PLK4, POLQ, PRC1, PRR11, RACGAP1, RAD51AP1, RBL1, SGOL1, SGOL2, SKA1, SKA3, SPC25, TICRR, TOP2A, TPX2, TRIP13, TROAP, TTK, TUBA1B, UBE2T, UHRF1, WDR62, XRCC2, ZWINT
KIF14	ANLN, ANP32E, ARHGAP11A, ARHGAP11B, ASPM, ATAD5, BRIP1, BUB1, BUB1B, CBX1, CCNF, CDC6, CDC7, CDCA8, CDK1, CENPE, CENPF, CENPI, CENPK, CENPL, CENPQ, CHAF1B, CIT, CKAP2L, CLSPN, DEPDC1, DLGAP5, DTL, E2F8, ECT2, EXO1, FAM72A, FAM72B, FAM72D, FANCD2, GINS1, GPSM2, HJURP, HMMR, INCENP, KIAA1524, KIF11, KIF15, KIF18A, KIF18B, KIF20B, KIF23, KIF2C, KIF4A, KIFC1, LMNB1, MAD2L1, MCM10, MCM2, MCM4, MCM6, MCM8, MELK, MKI67, MSH2, MTBP, NCAPG, NCAPG2, NDC80, NEK2, NEMP1, NUF2, NUP107, NUSAP1, ORC1, PLK1, PLK4, POLA1, POLQ, PRC1, PRR11, RACGAP1, RAD51AP1, RBL1, RFC5, RRM1, SASS6, SGOL1, SGOL2, SKA3, SMC2, SMC4, SPDL1, STIL, SUZ12, TMPO, TOPBP1, TPX2, TTK, UBE2T, USP1, WDHD1, XRCC2, ZWILCH, ZWINT

Table 4. Interacting genes (intersection) with similar functional roles as KIF11 and KIF14 genes.

Genes with Similar Function
ANLN, BUB1, BUB1B, CBX1, CCNF, CDCA8, CDK1, CENPE, CENPF, CENPI, CKAP2L, DEPDC1, DLGAP5, ECT2, EXO1, FAM72A, FAM72B, FANCD2, GINS1, GPSM2, HJURP, KIAA1524, KIF15, KIF18A, KIF18B, KIF20B, KIF23, KIF2C, KIF4A, KIFC1, LMNB1, MCM10, MCM8, MELK, MKI67, NCAPG, NDC80, NEK2, NUF2, PLK1, PLK4, POLQ, PRC1, PRR11, RACGAP1, RAD51AP1, RBL1, SGOL1, SGOL2, SKA3, TPX2, TTK, UBE2T, XRCC2, ZWINT

different organs.[2] Notably, the expression level of KIF11 was higher than that of KIF14. In addition, we observed that KIF11 and KIF14 were significantly expressed in the cytoskeleton of cells, confirming their role in cell division and movement.

Our findings suggest that KIF11 and KIF14 may serve as potential biomarkers for LIHC. The strong positive correlations of KIF11 and KIF14 with major human immune cell populations indicate their potential use in predicting or assessing the progression of LIHC. Furthermore, our exploration of the molecular variations and cellular distribution of KIF11 and KIF14 provides insights into their functions and potential as therapeutic targets for LIHC. The high expression of KIF11 and KIF14 in the cytoskeleton of cells suggests their role in cell division and movement, which may contribute to the development and progression of LIHC.

Our findings revealed 45 co-expressed genes that are functionally similar to both KIF11 and KIF14, and 10 hub genes (BUB1, BUB1B, CDK1, CENPE, CENPF, KIF2C, NCAPG, NDC80, TPX2, TTK) that are closely related to KIF11 and KIF14 in LIHC. Interestingly, one of the hidden KIF family members was KIF11, which was a surprising discovery. Our correlation analysis and gene expression analysis confirmed the association between these 10 hub genes, KIF family members, and LIHC.

Our enrichment analysis of the biological functions of the co-expressed genes revealed significant enrichment in the chromosome segregation, spindle, and microtubule binding categories. This finding is consistent with previous studies that showed the involvement of KIF11 and KIF14 in the regulation of cell division and the formation of spindle fibers during mitosis. For instance, Pei YY et al.[27] reported that KIF11 contributes to the progression and prognosis of human hepatocellular carcinoma through regulating the cell cycle. Moreover, Havelange V et al.[28] identified KIF11 as a novel biomarker for acute myeloid leukemia through co-expression network analysis.

Our KEGG analysis revealed significant enrichment in pathways such as Motor proteins, Cell cycle, Progesterone-mediated oocyte maturation, Oocyte meiosis, Fanconi anemia pathway, and FoxO signaling pathway. These pathways are involved in various cellular processes, including mitosis, meiosis, and DNA repair. Several studies have reported the involvement of KIF11 and KIF14 in these pathways. For instance, Zhu Q et al.[29] reported that KIF14 promotes cell proliferation and tumorigenicity in hepatocellular carcinoma through the PI3K/AKT signaling pathway. Wang B et al.[30] also reported that KIF11 is a potential therapeutic target and is correlated with the degree of glioma malignancy.

To understand the roles of KIF11 and KIF14 in LIHC, potential mechanisms of action can be explored. Investigating their interaction partners can provide insights into their functional roles, such as identifying other motor proteins, microtubule-associated proteins, or regulators of cell cycle

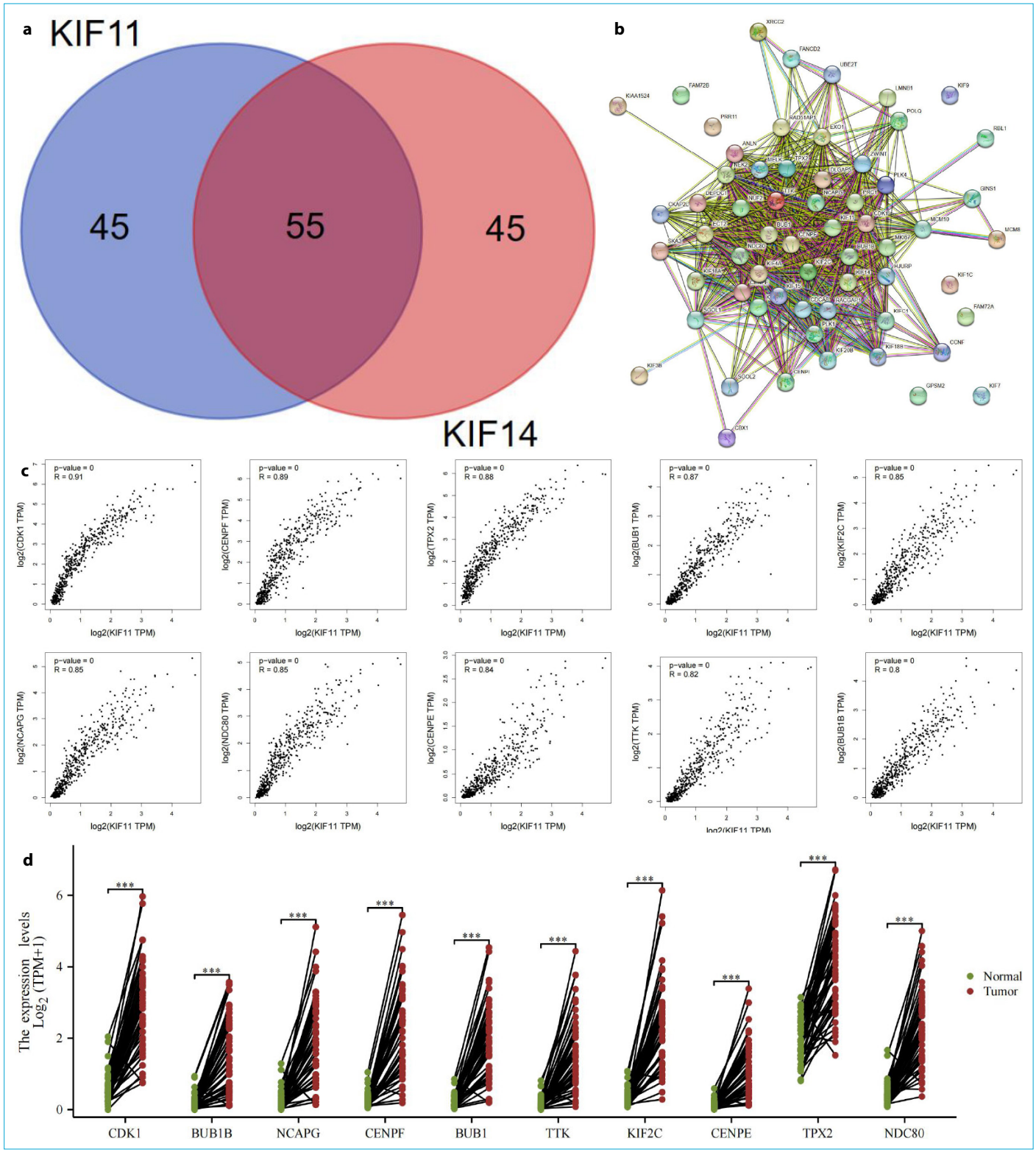

Figure 9. (a) Venn diagram of the 100 genes most closely functional to KIF11 and KIF14. **(b)** Protein interaction network of co-expressed genes between KIF 11 and KIF 14. **(c)** Correlations of the 10 hub genes with KIF11. **(d)** Paired sample variability of 10 hub in LIHC.

progression and apoptosis. Furthermore, analyzing downstream signaling pathways affected by KIF11 and KIF14 expression can uncover dysregulated pathways associated with LIHC. Functional experiments, including knockdown or overexpression studies, can directly assess their impact on cell processes like proliferation and migration. These

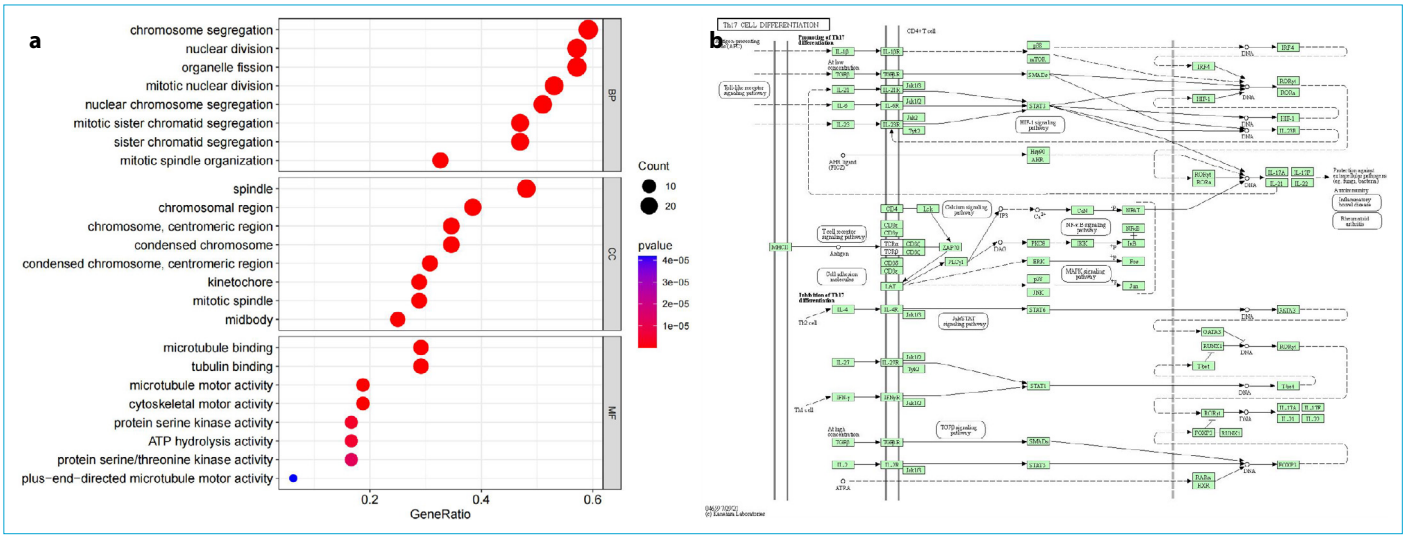

Figure 10. (a) GO pathway analysis of the co-expressed genes. **(b)** KEGG pathway analysis of the co-expressed genes.

investigations will contribute to a better understanding of the mechanisms underlying the contributions of KIF11 and KIF14 to LIHC development and progression.

In conclusion, our findings suggest that KIF11 and KIF14 play important roles in the regulation of cell division and are closely related to several hub genes and pathways in LIHC. Our results provide new insights into the potential therapeutic targets and biomarkers for LIHC. However, further experimental validation is needed to confirm our findings.

Conclusion

In this study, we conducted a comprehensive analysis of the expression of KIF family members in LIHC. We found that the expression levels of KIF family members were generally higher in LIHC, including seven genes, KIF1C, KIF3B, KIF7, KIF9, KIF11, KIF14, and KIF18A, which showed significant differential expression. At the protein translation level, the expression trends of these seven genes were different, but overall, their expression levels were generally higher in liver cancer tissues. In addition, we found that the expression levels of the KIF11 and KIF14 genes were closely related to the prognosis of LIHC, suggesting that they may be potential biomarkers for LIHC.

These findings provide important clues for further research on the role of KIF family members in LIHC. Considering the important roles of KIF family members in many biological processes, we believe that they may also play important roles in the occurrence and development of LIHC. Therefore, we suggest that in further studies, the detailed mechanisms of KIF family members in LIHC and whether they can be used as biomarkers for clinical diagnosis and treatment of LIHC can be explored.

In conclusion, this study found that the expression of KIF family members was abnormal in LIHC, and the expression levels of the KIF11 and KIF14 genes were closely related to the prognosis of LIHC. These results provide important clues for further research on the role of KIF family members in LIHC and also contribute to the identification of new biomarkers and therapeutic targets for the clinical treatment of liver cancer.

Disclosures

Ethics Committee Approval: All methods were carried out in accordance with the Declaration of Helsinki. No ethics approval was required for this work. All utilized public data sets were generated by others who obtained ethical approval.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – Z.F.; Design – Z.F.; Supervision – Y.W.; Materials – Y.L.; Data Collection and/or Processing – Y.L.; Analysis and/or Interpretation – Y.L.; Literature Search – Y.W.; Writing – Y.L.; Critical Review – Z.F.

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