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Original Article

Comprehensive bioinformatics analysis of KIF20A as a prognosis biomarker for clear cell renal cell carcinoma

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Abstract

It has been shown that kinesin family member 20A (KIF20A) is involved in the development of several cancers. However, research on clear cell renal cell carcinoma (ccRCC) and KIF20A is still exploratory. The current research was carried out to determine whether KIF20A expression has any prognosis value in ccRCC. Data were downloaded from The Cancer Genome Atlas (TCGA) database to validate the KIF20A mRNA expression and to perform clinicopathological analysis. Receiver operating characteristic (ROC) curves were used in evaluating KIF20A's diagnostic performance for ccRCC. The prognostic value of KIF20A in ccRCC was estimated by the Kaplan–Meier survival curve and Cox regression analysis. Gene set enrichment analysis (GSEA), functional annotations, and immune infiltration analysis were used to determine the potential mechanism of KIF20A's role in ccRCC. The increase in KIF20A mRNA expression was associated with sex, clinical stage, histologic grade, and TNM stage. ROC curve indicated that KIF20A could distinguish ccRCC from normal kidney samples. Survival study showed that high KIF20A expression predicted poor ccRCC prognosis. Thus, KIF20A expression could be used as an independent overall survival (OS) risk factor for ccRCC patients. Co-expression analysis identified TPX2 as a strong, positively correlated factor with KIF20A in ccRCC. Functional enrichment analyses and GSEA showed that KIF20A and TPX2 participated in various tumor-related pathways. Moreover, KIF20A and TPX2 expression were significantly associated with the level of immune infiltration into ccRCC. KIF20A may be a therapeutic target and a prognostic biomarker for ccRCC.

Keywords: The Cancer Genome Atlas (TCGA) database, Kinesin family member 20A (KIF20A), Targeting protein for Xenopus kinesin-like protein 2 (TPX2), Clear cell renal cell carcinoma (ccRCC), Biomarker.

1. Introduction

Renal cell carcinoma (RCC) is the most commonly occurring adult kidney tumor, with an incidence of around 3% of all malignant tumors in adults [1]. Clear cell renal cell carcinoma (ccRCC) is derived from kidney tubular epithelial cells and is the most common type of histology in RCC, accounting for approximately 75% of all RCC cases [2]. The malignant degree of ccRCC is relatively low; however, the incidence and mortality of ccRCC are rapidly increasing worldwide [3]. Most ccRCC patients are not timely diagnosed owing to the lack of obvious early onset of clinical symptoms. Approximately one-third of the patients have developed distant metastasis at the time of initial diagnosis [4,5]. Although significant progress has been achieved in ccRCC diagnostic technologies and targeted therapies in recent decades, the prognosis of ccRCC patients is still not optimistic, primarily because of distant metastases and recurrence of the disease. There is no effective treatment for advanced ccRCC [6-8]. Therefore, discovering novel potential biomarkers for early diagnostic and prognostic prediction for ccRCC is highly urgent.

Next-generation sequencing technologies have been widely applied in screening for genetic alterations in oncogenesis and identifying potential biomarkers for prognosis. Many genes have been determined to be involved in tumorigenesis and the development of ccRCC [9]. Kinesins are microtubule-dependent molecular motors that participate in vital cell functions such as intracellular protein transport and cell division [10]. Studies have shown that kinesins play vital roles in the tumorigenesis and progression of different types of malignancy [11-14]. KIF20A, also named RAB6KIFL and MKLP2, is a kinesin family member primarily localized in the central area of the mitotic spindle and participates in cell division [15]. A significant increase in KIF20A expression was observed in many tumors, such as breast, liver, and gastric cancers [16-18], and was reported to be associated with poor prognosis [19-23]. Moreover, several studies have demonstrated the potential of KIF20A for immunotherapy. Imai et al demonstrated that HLA-A2-restricted cytotoxic T lymphocytes (CTLs) could be induced in HLA-A2 transgenic mice by several KIF20A peptides [24]. Both *in vitro* and *in vivo,* the induction of KIF20A long peptide was found in both tumor-specific T-helper type 1 (TH1) cells and CTLs [25]. The above studies suggest that KIF20A is a promising target for developing anticancer drugs and

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cancer vaccines. To date, the prognostic significance and specific mechanisms of KIF20A in ccRCC remain to be further explored. Xenopus kinesin proteins, such as targeting protein for Xenopus kinesin-like protein 2 (TPX2), are required for the assembly of the mitotic spindle. High expression of TPX2 have been reported in patients with non-small cell lung cancer, cervical cancer, and oral cancer [26-28], suggesting the possible involvement of TPX2 in the development of a variety of tumors. Elevated expression of KIF20A and TPX2 have been simultaneously detected by bioinformatics analysis in many malignancies [29-32], indicating that these two factors may function in similar mechanisms and collaboratively promote the development and progression of various tumors.

In this study, we investigated the involvement and mechanism of KIF20A in ccRCC. Comprehensive bioinformatics analyses, including protein-protein interaction (PPI) network construction, Gene Ontology (GO) enrichment, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment, and gene set enrichment, were conducted using several databases and various online resources. In summary, these data demonstrated that KIF20A can serve as an important candidate for therapeutic target and prognostic biomarkers in ccRCC.

2. Materials and Methods

2.1. ONCOMINE database and UALCAN database analysis

ONCOMINE is a cancer microarray database and web-based data-mining platform (https://www.oncomine.org) [33]. In this study, we used the database from ONCOMINE to analyze KIF20A mRNA expression levels in various cancer samples in comparison to the normal controls using a Student's *t*-test. We set the cut-off threshold for the P value and fold-change as 0.001 and 2, respectively. UALCAN is an authoritative, comprehensive, and widely known web resource for cancer OMICS data analysis. Multiple gene expression analysis resources also could be found at UALCAN, including cancer OMICS data (e.g., TCGA, CPTAC, and MET500) [34]. In this work, we explored KIF20A expression in ccRCC using the UALCAN CPTAC-KIRC dataset.

2.2. TCGA database

RNA-sequencing data and corresponding clinical information of ccRCC patients were downloaded from The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/) database [35]. Relevant clinicopathological information, such as age, sex, histological grade, clinical stage, and TMN stage, is listed in Table 1. Patients with unavailable or unknown clinical data were excluded. Finally, we collected 530 clinical information and mRNA expression of KIF20A data from ccRCC patients in the analyses.

2.3. PPI network construction, GO enrichment, and KEGG pathway analysis

The Retrieval of Interacting Genes Search Tool (STRING; http://string-db.org) [36]. We used the data-

Table 1. Clinical features of ccRCC patients from the TCGA database.

Clinical feature	Variable	Number (total n = 530)	Percentage (%)
Age	≤60	264	49.81
	>60	266	50.19
Gender	female	186	35.09
	male	344	64.91
Histological grade	G1	14	2.64
	G2	227	42.83
	G3	206	38.87
	G4	75	14.15
	GX	5	0.94
	Unknown	3	0.57
Clinical stage	Stage I	265	50
	Stage II	57	10.74
	Stage III	123	23.21
	Stage IV	82	15.48
	Unknown	3	0.57
T classification	T1	271	51.13
	T2	69	13.02
	T3	179	33.77
	T4	11	2.08
M classification	M0	420	79.25
	M1	78	14.72
	MX	30	5.66
	Unknown	2	0.37
N classification	N0	239	45.09
	N1	16	3.02
	NX	275	51.89

Abbreviations: ccRCC, clear cell renal cell carcinoma; TCGA, The Cancer Genome Atlas.

base to search for the KIF20A gene network to build a PPI network. We regarded an interaction with >0.4 combined score as statistically significant. To further understand the ability and potential mechanisms of KIF20A in ccRCC, we used the cluster Profiler package (http://www.bioconductor.org/packages/release/bioc/html/clusterProfiler.html) for R software to conduct GO and KEGG pathway enrichment analysis. clusterProfiler can automatically perform biological terminology classification and gene cluster enrichment analysis [37]; P and false discovery rate (FDR) <0.05 served as a cut-off for statistical significance.

2.4. Co-expression analysis

The cBioPortal (https://www.cbioportal.org/) is an open-resource web tool for searching, inferring, and analyzing data for TCGA genomics and clinical cases [38]. Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn), an interactive tool for cancer gene profiling and expression analysis, was used for expression profile plotting, relationship analysis, survival analysis, similar gene detection, and dimensionality reduction analysis [39]. In this study, we used the cBioPortal database to identify genes co-expressed with KIF20A and served as interacting partners in the PPI network, which were further validated in the GEPIA database.

2.5. Gene set enrichment analysis (GSEA)

To understand the potential KIF20A and TPX2 signaling pathways in ccRCC, we collected transcriptomic data from the TCGA database using GSEA. According to the KIF20A and TPX2 median expression levels, we divided the 530 ccRCC patients into high-expression and low-expression groups. We used GSEA 3.0 software to analyze the *c2.cp.kegg.v7.2.symbols.gmt* gene sets from the Molecular Signatures Database (MSigDB) and carried out enrichment analysis using the default weighted enrichment statistical method. We set the number of permutations for each analysis to 1,000 iterations. Finally, we used normalized enrichment score (NES) to determine the significance of signaling pathways enriched in KIF20A- and TPX2-high expression groups. $P < 0.05$ and FDR of <0.25 were set as cut-off criteria.

2.6. TIMER database analysis

The Tumor Immunity Resource (TIMER, https://cistrome.shinyapps.io/timer/) database, a curated tool for a systematic immune infiltration analysis, was used in various cancer types (40). For this study, the correlation between KIF20A and TPX2 expression and immune cell infiltration, including $CD4^+$ T cells, B cells, $CD8^+$ T cells, dendritic cells, neutrophils, and macrophages, was evaluated using Spearman's correlation analysis. The relationship between KIF20A and TPX2 expression and tumor purity was also investigated. $P < 0.05$ represents statistical significance.

2.7. Statistical analysis

We used R 3.6.1 software to perform all statistical analyses, and $P < 0.05$ was considered statistically significant. We also used the Wilcoxon signed-rank test to compare the KIF20A mRNA expression in ccRCC and paracancerous normal kidney tissues, and utilized the receiver operating characteristic (ROC) curve to evaluate the diagnostic ability of KIF20A for ccRCC. We used the logistic regression and Wilcoxon signed-rank test to analyze the relationship between KIF20A expression and clinicopathological characteristics of patients with ccRCC. The 530 ccRCC patients were divided into high- and low-expression groups according to median KIF20A and TPX2 expression. Moreover, we used Kaplan-Meier survival curves and log-rank test to assess the overall survival (OS), and the log-rank test was used to assess the difference in total survival rate between the two groups. We used one-way and multi-way Cox regression analyses to determine the individual variables associated with OS in ccRCC patients.

3. Results

3.1. Clinical correlation and prognosis analysis

We assessed the relationship between KIF20A expression and pathological characteristics of the patients. Our result showed that sex was significantly associated with the upregulation of KIF20A ($P < 0.001$) (Figure 1A), clinical stage ($P < 0.0001$) (Figure 1B), histological grade ($P < 0.0001$) (Figure 1C), T classification ($P < 0.0001$) (Figure 1D), N classification (number of metastatic lymph nodes) ($P < 0.001$) (Figure 1E), and M classification (distant metastasis) ($P < 0.0001$) (Figure 1F). Furthermore, logistic regression analysis also confirmed that elevated KIF20A expression in ccRCC was remarkably associated with gender (OR = 2.024 for male vs. female, P = 0.0001), histological grade (OR = 8.529 for G4 vs. G1, P = 1.001), clinical stage (OR = 3.011 for stage III vs. stage I, P <0.001; OR = 5.560 for stage IV vs. stage I, P <0.001), T classification (OR = 3.735 for T3 vs. T1, P <0.001; OR = 16.569 for T4 vs. T1, P = 0.008), N classification (OR = 17.009 for N1 vs. N0, P = 0.006), and M classification (OR = 4.074 for M1 vs. M0, P <0.001) (Table 2). These results showed that KIF20A may be effective in promoting the malignant phenotype and high KIF20A expression in ccRCC patients predicts a poor clinical outcome.

Kaplan-Meier survival analysis indicated a lower OS in ccRCC patients with high KIF20 expression in comparison with those with low KIF20 expression ($P < 0.0001$) (Figure 2A). We drew ROC curves to assess the value of KIF20A in ccRCC prognosis. As shown in Figure 2B, the AUC of KIF20A expression for predicting one-year, three-year, and five-year survival was 0.672, 0.623, and 0.627, respectively. Figure 2C shows the distribution of KIF20A

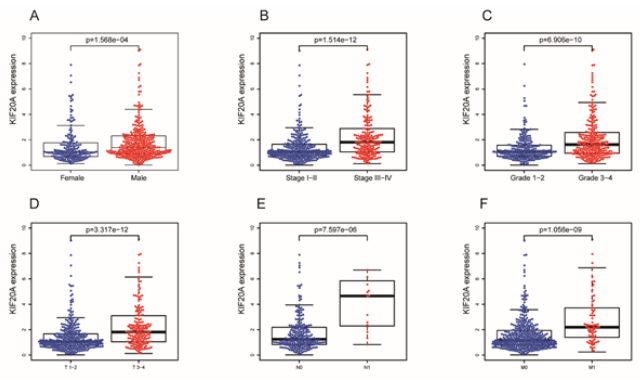

Fig. 1. KIF20A expression is associated with clinicopathological features. KIF20A mRNA expression level is higher in male vs. female (A), in advanced clinical stage patients vs. low clinical stage patients (B), in patients with higher histological grade vs. lower histological grade, and in lower TNM classification vs. higher TNM classification (D–E).

Table 2. Logistic regression analysis of KIF20A expression and clinical features.

Clinical feature	Number	Odds ratio in KIF20A expression	P
Sex (male vs. female)	530	2.024 (1.409–2.920)	0.001
Histological grade			
Grade 2 vs. 1	241	1.469 (0.475–5.489)	0.527
Grade 3 vs. 1	220	3.159 (1.020–11.818)	0.059
Grade 4 vs. 1	89	8.529 (2.518–34.394)	0.001
Clinical stage			
Stage II vs. I	322	1.053 (0.579–1.887)	0.861
Stage III vs. I	388	3.011 (1.938–4.725)	0.001
Stage IV vs. I	347	5.560 (3.198–10.054)	0.001
T classification			
T2 vs. T1	340	1.201 (0.698–2.051)	0.504
T3 vs. T1	450	3.735 (2.512–5.612)	0.001
T4 vs. T1	282	16.569 (3.106–306.314)	0.008
N classification (N1 vs. N0)	255	17.009 (3.366–310.019)	0.006
M classification (M1 vs. M0)	498	4.074 (2.371–7.320)	0.001

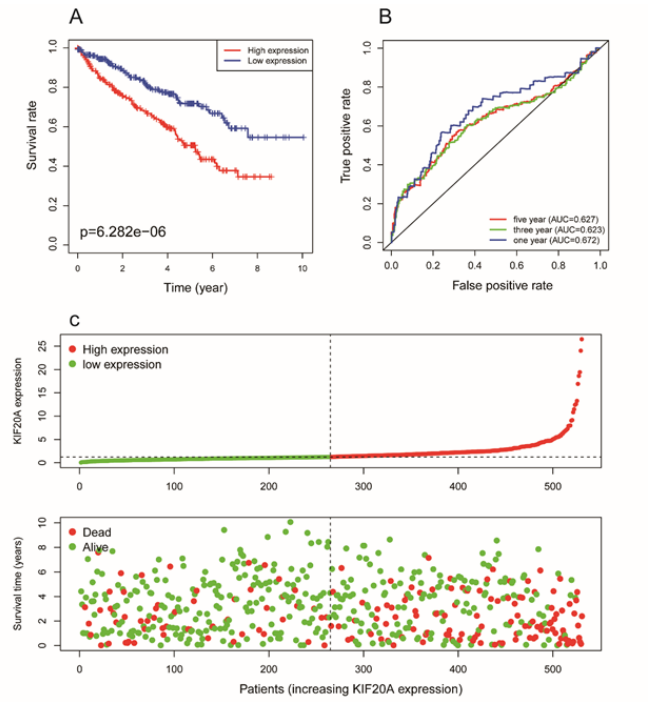

Fig. 2. Survival analysis of ccRCC patients relative to KIF20A expression. (A) The patient group with low KIF20A expression shows better overall survival than patient group with high KIF20A expression by the Kaplan-Meier curve. (B) ROC curve analysis of KIF20A expression for the prediction of one-year, three-year, and five-year survival. (C) Distribution of KIF20A expression and survival status of the 530 ccRCC patients in the TCGA database. Abbreviations: ccRCC, clear cell renal cell carcinoma; ROC, receiver operating characteristic.

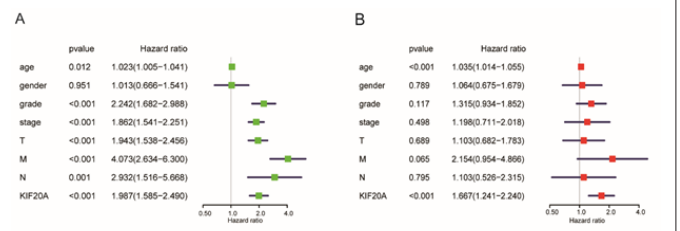

Fig. 3. Univariate and multivariate Cox regression analysis of KIF20A and clinical variables with survival status show that age, histological grade, clinical stage, TNM classification, and KIF20A expression are associated with poor OS in univariate analysis (A), while age and KIF20A expression are independently associated with unfavorable OS in multivariate analysis (B). Abbreviations: OS, overall survival; TNM, Tumor Node Metastasis.

expression and survival status of the 530 patients with ccRCC.

In univariate cox analysis (Figure 3A), high KIF20A expression (HR = 1.987, P<0.001), age (HR = 1.023; P = 0.012), histological grade (HR = 2.242; P <0.001), clinical stage (HR = 1.862; P <0.001), T classification (HR = 1.943; P<0.001), N classification (HR = 2.932; P = 0.001), and M classification (HR = 4.073; P<0.001) were related with poor OS. By multivariate Cox analysis, age (HR = 1.035; P<0.001) and KIF20A high expression (HR = 1.667; P <0.001) were related to dissatisfactory OS (Figure 3B). With the data shown above, it can be concluded that KIF20A is an independent predictor for poor prognosis of ccRCC.

3.2. PPI network construction, GO enrichment, and KEGG pathway analysis

To investigate the potential KIF20A-related signaling pathways in ccRCC, we identified and constructed a PPI network of KIF20A and 20 functional partners of KIF20A using the STRING database (Figure 4A). To confirm the effect and signaling pathways associated with KIF20A and its interaction partners, we performed GO and KEGG analysis using the cluster Profiler-R package. We found the biological processes (BP) of these genes were mainly chromosome segregation, nuclear division, and mitotic nuclear division (Figure 4B). The genes were highly enriched in cellular components (CC) related to the chromosome, centromeric region, and condensed chromosome. Their molecular functions (MF) were associated with microtubule and tubulin binding, which are consistent with the functions of KIF20A as a microtubule-dependent molecular motor. Moreover, KEGG pathway analysis showed that these genes were significantly involved in various tumor-related pathways, such as regulation of the cell cycle and p53 and FoxO signal transduction (Figure 4C).

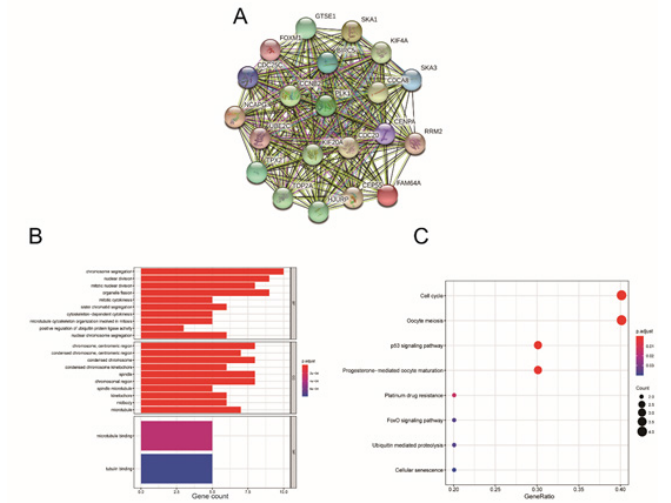

Fig. 4. PPI network construction and functional enrichment analyses. (A) The PPI network of KIF20A and 20 functional partners of KIF20A identified using the STRING database are constructed. (B) GO enrichment analysis of KIF20A and 20 co-regulated genes in the BP, CC, and MF categories. (C) KEGG pathway enrichment analysis of the 21 genes. Abbreviations: GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein-protein interaction; STRING, Retrieval of Interacting Genes Search Tool.

3.3. Co-expression analysis of KIF20A

Using cBioPortal and GEPIA database, co-expression analysis was performed to identify the most functionally related partners of KIF20A in ccRCC and TPX2 showed the strongest positive association with KIF20A in ccRCC (Figure 5A–C). Further analysis showed that TPX2 was also highly expressed in ccRCC relative to the normal renal samples. Increased TPX2 expression was significantly associated with clinically advanced stage and low survival of ccRCC (Figure 6A–D).

3.4. Identification of KIF20A and TPX2-related signaling pathways

For identifying the potential mechanisms of KIF20A and TPX2 that influence the survival of ccRCC patients, we performed GSEA to find the gene sets enriched in the KIF20A and TPX2 high-expression groups. As shown in Figures 7 & 8 and Table 3, genes in both the KIF20A and TPX2 high-expression groups were enriched in the cell cycle, DNA duplication, p53 signaling pathway, and homologous recombination. Cytokine-cytokine receptor interaction and base excision repair genes were enriched in the KIF20A high-expression group. In contrast, genes associated with natural killer cell-mediated cytotoxicity and systemic lupus erythematosus were enriched in the TPX2 high-expression group. The above gene sets are generally associated with the initiation and progression of tumors.

3.5. Association between KIF20A and TPX2 expression levels and immune permeation

Using the TIMER database, the association was analyzed between KIF20A and TPX2 expression with the purity of tumor and tumor-infiltrating immune cells (including $CD4^+$ T cells, B cells, $CD8^+$ T cells, dendritic cells, neutrophils, and macrophages) in the tumor microenvironment of ccRCC. Based on our results, both KIF20A expression and TPX2 expression were negatively correlated with ccRCC tumor purity. Upregulation of KIF20A and

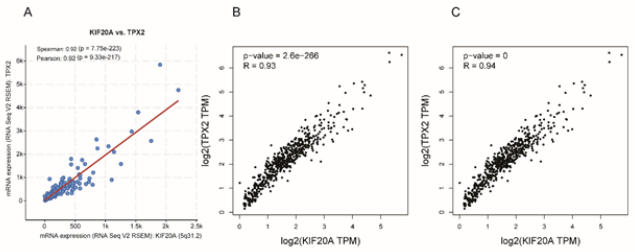

Fig. 5. Co-expression analysis of KIF20A and TPX2. Abbreviation: TPX2, targeting protein for Xenopus kinesin-like protein 2.

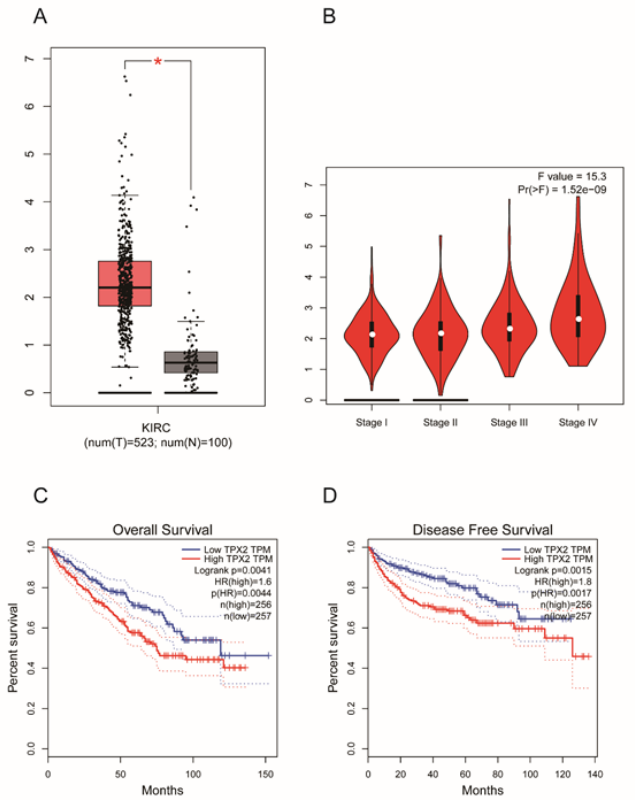

Fig. 6. Expression of TPX2 and its relationship with clinical features and survival status. (A) TPX2 mRNA level in ccRCC samples is significantly higher than in normal samples. *P < 0.05. (B) Relationship between TPX2 expression and clinical stage of ccRCC. OS (C) and disease-free survival (D) Kaplan-Meier curves for low and high TPX2 expression groups in ccRCC from the GEPIA database. Abbreviations: ccRCC, clear cell renal cell carcinoma; OS, overall survival; TPX2, targeting protein for Xenopus kinesin-like protein 2.

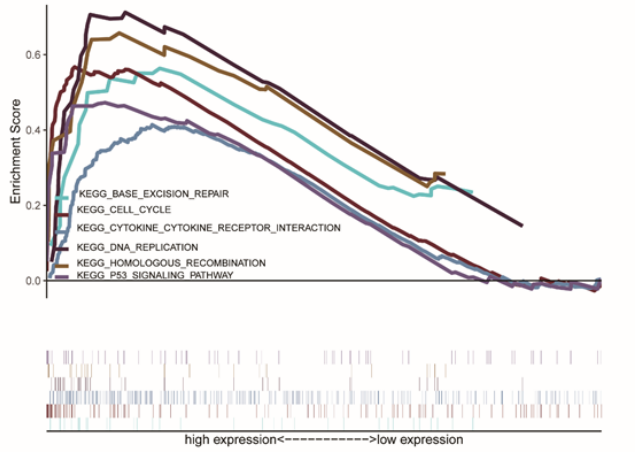

Fig. 7. GSEA of KIF20A high-expression group. Abbreviation: GSEA, gene set enrichment analysis.

Table 3. Gene set enrichment analysis of KIF20A high-expression group and TPX2 high-expression group.

Gene set name	NES	FDR q	NOM P
KIF20A high-expression group			
KEGG_CELL_CYCLE	1.976	0.004	0.111
KEGG_DNA_REPLICATION	1.909	0.004	0.126
KEGG_HOMOLOGOUS_RECOMBINATION	1.852	0.008	0.146
KEGG_P53_SIGNALING_PATHWAY	1.759	0.010	0.194
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	1.706	0.014	0.209
KEGG_BASE_EXCISION_REPAIR	1.712	0.025	0.234
TPX2 high-expression group			
KEGG_CELL_CYCLE	2.126	0.000	0.026
KEGG_DNA_REPLICATION	2.075	0.022	0.000
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	1.994	0.047	0.006
KEGG_HOMOLOGOUS_RECOMBINATION	1.977	0.043	0.002
KEGG_P53_SIGNALING_PATHWAY	1.955	0.042	0.002
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	1.939	0.043	0.008

Abbreviations: FDR, false discovery rate; NES, normalized enrichment score; NOM, nominal.

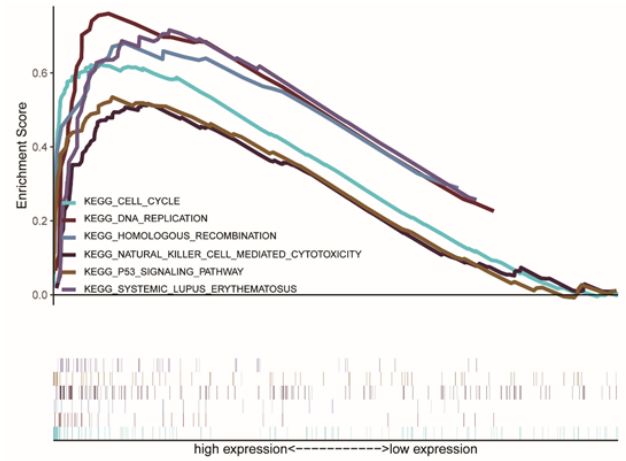

Fig. 8. GSEA of TPX2 high-expression group. Abbreviation: GSEA, gene set enrichment analysis.

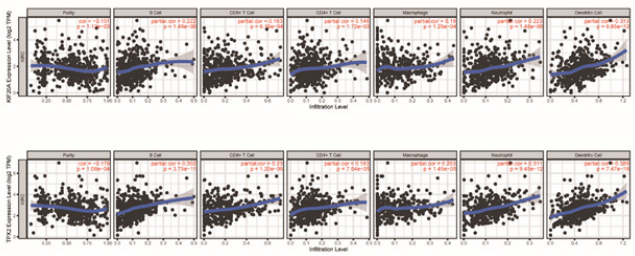

Fig. 8. Immune infiltration analysis of KIF20A and TPX2 in ccRCC. Abbreviations: ccRCC, clear cell renal cell carcinoma; TPX2, targeting protein for Xenopus kinesin-like protein 2.

TPX2 expression were markedly associated with infiltration of B cells (r = 0.222, P <0.001 and r = 0.302, P <0.001, respectively), $CD4^+$ T cells (r = 0.146, P = 0.002 and r = 0.183, P <0.001, respectively), $CD8^+$ T cells (r = 0.163, P <0.001 and r = 0.230, P <0.001, respectively), macrophages (r = 0.180, P <0.001 and r = 0.203, P <0.001, respectively), neutrophils (r = 0.223, P <0.001 and r = 0.311, P<0.001, respectively), and dendritic cells (r = 0.313, P = 0.885 and r = 0.389, P<0.001, respectively) (Figure 9). These data suggest that KIF20A and TPX2 have specific roles in regulating immune infiltration in ccRCC.

4. Discussion

ccRCC is a malignancy that often occurs in the urinary system. It is generally poorly responsive to chemotherapeutics and targeted therapies have been explored as non-surgical treatments but with limited efficacy [41]. Many biomarkers for ccRCC have been discovered, including von Hippel-Lindau tumor suppressor (VHL), vascular endothelial growth factor (VEGF), polybromo 1 (PBRM1), and BRCA1 related protein 1 (BAP1) [42]. Current opinion believes that cancerization is not the consequence of the deregulation of a few oncogenes or tumor suppressors. On the contrary, a network of various complex mechanisms is involved [43]. Thus, it is imperative to seek new diagnostic and therapeutic molecular biomarkers for ccRCC that can enhance our understanding of its pathogenesis and provide personalized treatment.

The proper regulation of mitosis and cytoplasmic division are critical for cell survival. KIF20A plays an important role in the mitotic process and has been reported to serve as an oncogene in various types of human cancers [16-18,44]. Currently, the specific ccRCC molecular mechanism and its relationship with clinical characteristics remain undefined. The rapid technological progress of next-generation sequencing has greatly advanced the study of clinical symptoms and pathogenesis in various cancers. However, more research has focused attention on identifying differential expression of genes and performing the cross-impact analysis of genes, which was investigated in this study. Using the expression profiles from multiple databases, we confirmed that KIF20A expression was significantly elevated in various cancer types, including kidney cancer and ccRCC cancer tissues, compared with paracancerous normal tissues. Of note, overexpression of KIF20A indicated poor clinical outcomes and prognosis of ccRCC patients.

Furthermore, we found that the expression of KIF20A and TPX2 were strongly positively correlated in ccRCC. TPX2 has a higher expression signal in ccRCC cells than in healthy kidney cells, and enhanced TPX2 expression was related to advanced cancer stage and shorter overall and disease-free survival. Both TPX2 and KIF20A are

intimately involved in microtubule-related cellular functions, which may explain why the regulation of these two proteins is highly correlated. Clinical relationship analysis revealed strong relationships between KIF20A and TPX2 expression to the TNM stage and other clinicopathological features. Not only that, the patients with high KIF20A and TPX2 expression appeared to have a poor prognosis in comparison with the low-expressing groups, indicating that KIF20A and TPX2 may have a prognostic value.

To further understand the potential action mechanism of KIF20A in ccRCC, a PPI network was constructed to screen closely related genes to KIF20A. GO and KEGG pathway enrichment analyses were then performed to define the biological aspects associated with KIF20A and correlated genes. In addition, GSEA revealed various cancer-related gene networks, including cell cycle, DNA replication, p53 signaling, cytokine-cytokine receptor interaction, base excising and repairing, and natural killer cell-mediated cytotoxicity. All of these gene networks have been reported to engage in the development and progression of malignancy in humans. For example, altered cell cycle regulation leads to deregulated proliferation of cells and promotes tumorigenesis [45]. Accurate DNA replication is essential for cell proliferation and genome stability. Any hindrance to DNA replication leads to replication stress, which if not relieved timely may result in gene mutations and chromosomal rearrangements found frequently in human cancers [46,47]. Homologous recombination is the dominant function for fixing DNA duplex structure breaks in mammalian cells. Genetic and epigenetic inactivation of homologous recombination components is common in sporadic cancers [48,49]. As the most well-known cancer-related pathway, p53 signaling is also correlative in the initiation and progression of ccRCC [50]. Cytokine-cytokine receptor interaction has been demonstrated to play a vital role in the autocatalysis and transport of inflammatory mediators and the progression and metastasis of tumor cells [51]. Base excision repair is an essential pathway to maintain the stability of the genome by removing endogenously damaged DNA bases produced daily. Therefore, changes to this pathway have the potential to promote tumorigenesis [52]. Natural killer cells play an important role in human cancer immune surveillance. Patients with Natural Killer cell defects have accelerated tumor progression or increased cancer incidence, unveiling an essential function of NK cell-mediated cytotoxicity in tumor immunity [53,54]. Kinesins function as microtubule motors in the cell cycle, and they have been found to function in DNA synthesis and homologous recombination [55,56]. The p53 signaling pathway plays a central role in mitotic regulation [57]. Recent findings suggest that microtubules are a potential player in the innate and adaptive immune system [58], and NK cell-mediated cytotoxicity is closely associated with microtubules and kinesins [59-61]. These data verify the reliability of our analysis results that these pathways are enriched by KIF20A and its related genes. The enriched pathways in KIF20A and TPX2 high-expression groups were not precisely overlapping, indicating that the MF of KIF20A and TPX2 are not redundant and they may promote ccRCC progression through regulating different signaling pathways.

It has been shown that high levels of lymphocyte infiltration are negatively correlated with the prognosis of ccRCC [62]. For the first time, we used the TIMER database to evaluate the expression of KIF20A and TPX2 in association with immune infiltration in ccRCC. Our data revealed that KIF20A and TPX2 expression were highly correlated with the infiltration of immune cells in the ccRCC tumor microenvironment, including B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils, and dendritic cells, leading to a general increase in immune infiltration. Based on what is known in the tumor microenvironment in immunology, these results suggest that high KIF20A expression is an unfavorable prognostic factor for patients with ccRCC. They also suggest that KIF20A may serve as a functional reference for ccRCC patients in immunotherapy.

The results from our study will enhance our understanding of the influence of KIF20A in ccRCC. However, there are some limitations in this study. First, our analytical results are mainly based on data from the TCGA database, which has not been verified in clinical samples. Second, the detailed biological mechanism of KIF20A in ccRCC could not be demonstrably illustrated by the TCGA database. Thus, subsequent *in vivo* and *in vitro* studies are required to explicitly investigate the underlying mechanism by which KIF20A affects renal cancer tenor. Third, the translatability of ccRCC data in the public database is still insufficient. When we used the clinicopathological parameters, some potential errors and biases likely existed during the analysis. Finally, the relationship between KIF20A and immune infiltration needed to be further validated in cellular and molecular experimental research.

In summary, KIF20A is upregulated in ccRCC cells and is a candidate for a prognostic marker. With its effects on immune cell infiltration into the tumor, KIF20A may serve as a new ccRCC target for immunotherapy.

Conflict of interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

No human or animals were used in the present research.

Informed Consent

The authors declare not to use any patients in this research.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request

Authors' contributions

Cankun Xie and Wingkeung Yiu: Conceptualization, methodology, writing original draft preparation. Cankun Xie, Wingkeung Yiu and Yijiang Mo: Investigation, software, statistical analysis. Yijiang Mo: Reviewing and editing, funding acquisition, supervision. All authors read and approved the final manuscript.

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