KIAA1522 Promotes the Progression of Hepatocellular Carcinoma via the Activation of the Wnt/ β -Catenin Signaling Pathway

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Purpose: *KIAA1522* was previously identified to play a crucial role in cancer development and progression. However, its functions and underlying mechanisms in hepatocellular carcinoma (HCC) remain elusive.

Materials and Methods: To elucidate the role of *KIAA1522* in HCC, its expression was assessed using The Cancer Genome Atlas and GEPIA databases. Next, these results were validated by quantitative reverse transcription-polymerase chain reaction, Western blotting, and immunohistochemistry of HCC tissues and cell lines. Flow cytometry, CCK-8, EDU, colony formation, Transwell invasion, and wound healing assays were performed to explore the function of *KIAA1522* in HCC in vivo and in vitro. Finally, gene set enrichment analysis was used to identify the pathways involved.

Results: Our results demonstrated that *KIAA1522* was highly expressed in HCC tissues and cell lines. Furthermore, *KIAA1522* overexpression was associated with unfavorable clinicopathological characteristics. Survival analyses revealed that *KIAA1522* overexpression predicted lower recurrence-free and overall survival rates in patients with HCC. Functional studies suggested that *KIAA1522* facilitated HCC proliferation, migration, and invasion both in vitro and in vivo. Moreover, *KIAA1522* up-regulated the Wnt/ β -catenin signaling pathway, as confirmed by TOP-flash/FOP-flash luciferase reporter assays and Western blotting.

Conclusion: In conclusion, we highlighted the oncogenic role of *KIAA1522* in HCC and determined its potential as a therapeutic target for HCC.

Keywords: hepatocarcinogenesis, KIAA1522, prognosis, Wnt/ β -catenin signaling pathway

Introduction

Hepatocellular carcinoma (HCC) remains one of the deadliest diseases worldwide, especially in China.¹⁻³ Viral infections and liver fibrosis are the most common risk factors for HCC.⁴⁻⁶ Although surgical resection and liver transplantation have been developed to treat HCC, postoperative survival of HCC patients is still not ideal due to high recurrence and rate of metastasis of HCC.⁷⁻⁹ Therefore, there is an urgent need to understand the mechanisms underlying the initiation and development of HCC.

Several reports indicated that dysregulation of the uncharacterized protein *KIAA1522* was related to cancer development and progression.¹⁰ In breast cancer, *KIAA1522* was determined as a direct target of miR-125b-5p, and it promoted malignant progression of the cancer.¹¹ In esophageal cancer, *KIAA1522* overexpression contributed to the tumorigenicity and metastasis of the cancer by up-regulating *ERK* activity.¹² Moreover, *KIAA1522* could be used as an independent factor to predict

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poor survival and platinum-resistance in non-small cell lung cancer.¹³ In bronchial brushings of lung cancer patients, a six-protein panel, including *KIAA1522*, *KIAA0317*, *MCM6*, *MCM7*, *TP53*, and *Ki67*, was identified as a novel biomarker for early diagnosis of lung cancer.¹⁴ However, little is known about the expression patterns of *KIAA1522* in HCC, and hence, the potential mechanisms of its action must be investigated.

Here, we presented evidence that *KIAA1522* was overexpressed in HCC tissues and cell lines. Survival analyses revealed that *KIAA1522* overexpression predicted lower recurrence-free rates and poor overall survival in HCC patients. Furthermore, *KIAA1522* contributed to HCC progression both in vitro and in vivo by decreasing the proportion of cells undergoing G2/M phase arrest as well as cell apoptosis. Finally, gene set enrichment analysis (GSEA) revealed that *KIAA1522* up-regulated the Wnt/ β -catenin signaling pathway. In summary, this study revealed that *KIAA1522* could be a novel therapeutic target and prognostic biomarker for HCC.

Materials and Methods

Tissues and Cell Lines

Sixty paired tumor tissues were collected from HCC patients, who underwent surgical resection at the First People's Hospital of Lianyungang. Human hepatic cell line L02 was obtained from the American Type Culture Collection (Manassas, VA, USA). HepG2 and Huh7 cells were purchased from China Center for Type Culture Collection (Wuhan, China).

HCCLM3, SMMC7721, Focus, and MHCC97L were obtained from Key Laboratory on Living Donor liver transplantation, National Health and Family Planning Commission (Nanjing, China).

Lentiviral Transfection

Lentiviral vectors that encode *KIAA1522* (LV-*KIAA1522*), short hairpin RNAs (shRNAs) against *KIAA1522* (LV-sh*KIAA1522*), and corresponding empty vectors (LV-vector/LV-shNC) were purchased from GenePharma Biotech (Shanghai, China). Puromycin (5 μ g/mL, Sigma-Aldrich) was used to select the stable cell lines.

Cell-Counting Kit-8 (CCK-8) and Clonogenic Assays

CCK-8 cell proliferation/cytotoxicity assay kit (Dojindo, Kumamoto, Japan) was used to assess cell proliferation according to the manufacturer's protocol. Briefly, we

seeded stable cell lines into 96-well plates (500 cells/well) and added CCK-8 solution (10 μ L) to each well. Absorbance was measured at 450 nm after incubation for 2 h. For the clonogenic assay, we seeded stable cell lines into 6-well plates (500 cells/well). After 14 d of culture, proliferating colonies were identified using crystal violet dye solution (Beyotime, Guangzhou, China).

Apoptosis and Cell Cycle Assays

To identify apoptotic cells, stable cell lines were collected, and the cells were incubated with FITC-conjugated Annexin V (BD Pharmingen) and propidium iodide (PI) at 37°C in the dark for 15 min. This was followed by flow cytometry analysis. For the cell cycle assay, the cells were fixed with 75% ice-cold ethanol overnight, stained with PI staining solution (500 μ L), and incubated at 37°C in the dark for 15 min. The results were acquired using the FlowJo software (Tree Star Inc.).

Transwell Assays

Transwell plates (8- μ m pore size) were coated with or without Matrigel™ (BD Biosciences, San Jose, CA, USA) and used to observe cell invasion and migration, respectively. Serum-free medium (200 μ L) was added to the upper chamber, while medium supplemented with 10% fetal bovine serum (500 μ L) was added to the lower chamber. After incubation for 24 h, the cells were stained using crystal violet (Kaigen, Nanjing, China) for 15 min and visualized under a light microscope.

Wound Healing Assays

HCC cells were seeded into 6-well plates (5×10^5 cells/well). When these cells reached confluency, a uniform linear scratch was created using the tip of a sterile pipette (200 μ L) in the center of the well. A baseline was acquired at 0 h after rinsing the cells twice with phosphate-buffered saline (PBS). After 48 h, images of the same location were acquired.

Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from the HCC cells and tissues, and qRT-PCR was performed as previously described.¹⁵

Western Blotting

Extracted protein was used to perform Western blotting as previously described.¹⁵ The antibodies, which were diluted in primary antibody diluent, are listed below: *KIAA1522* (1/1000, ab122203; Abcam, Cambridge, UK), *Bcl-2* (1/1000,

ab196495; Abcam, Cambridge, UK), *Bax* (1/1000, ab53154; Abcam, Cambridge, UK), *CyclinD1* (1/1000, ab226977; Abcam, Cambridge, UK), *CD44* (1/1000, ab157107; Abcam, Cambridge, UK), *c-Met* (1/1000, ab74217; Abcam, Cambridge, UK), *cleaved caspase-3* (1/1000, 9661; Cell Signaling Technology, Beverly, MA, USA), *CDK2* (1/1000, 2546; Cell Signaling Technology, Beverly, MA, USA), *CDK4* (1/1000, 12790; Cell Signaling Technology, Beverly, MA, USA), *CDK6* (1/1000, 13331; Cell Signaling Technology, Beverly, MA, USA), *β-catenin* (1/1000, 8480; Cell Signaling Technology, Beverly, MA, USA), and *c-Jun* (1/1000, 9165; Cell Signaling Technology, Beverly, MA, USA). HRP-conjugated Affinipure goat anti-rabbit (1/5000, A0208; Beyotime, Shanghai, China), or anti-mouse IgG(H+L) (1/5000, A0216; Beyotime, Shanghai, China) was diluted in secondary antibody diluent and used to incubate the membranes.

Immunohistochemical (IHC) Staining

IHC staining was performed using the diaminobenzidine detection kit (Maixin-Bio, Fuzhou, China) and anti-*KIAA1522* antibodies, according to the manufacturer's protocol. The scoring system of IHC staining was published previously.¹⁶

Animal Experiments

For assessing subcutaneous tumor growth, 1×10^6 HCC cells were injected subcutaneously into female BALB/c nude mice (aged 4 weeks) ($n = 6$ per group). All mice were monitored once every 4 d and sacrificed after 24 d. For metastasis assays, 1×10^6 HCC cells that expressed luciferase were injected into the tail vein of nude mice ($n = 10$ per group). After 6 weeks, metastasis was observed using the IVIS 100 Imaging System, and the mice were sacrificed. The lungs of the mice were fixed in 4% paraformaldehyde and stained with hematoxylin and eosin (HE).

Statistical Analyses

Data were presented as mean $\pm$ standard error of the mean (SEM) and analyzed by Student's *t*-test or χ^2 -test. *P*-values < 0.05 were considered statistically significant.

Results

KIAA1522 Was Overexpressed in HCC, and It Correlated with Poor Prognosis

To obtain the expression pattern of *KIAA1522* in HCC, RNA sequencing data were downloaded from The Cancer Genome

Atlas (TCGA) database (371 HCC tissues and 50 non-tumor tissues). *KIAA1522* expression was up-regulated in HCC tissues compared to that in normal tissues (Figure 1A). Consistent with TCGA data, GEPIA data also revealed that *KIAA1522* expression was increased in HCC tissues (Figure 1B). Next, we evaluated *KIAA1522* expression in 60 paired HCC tissues and corresponding non-tumor tissues using qRT-PCR. We observed that *KIAA1522* mRNA levels were remarkably elevated in HCC tissues (Figure 1C). Western blotting and IHC staining verified that *KIAA1522* protein levels were up-regulated in six randomly selected paired HCC tissues (Figure 1D and E). Consistent with these results, *KIAA1522* mRNA and protein levels were elevated in seven human HCC cell lines, compared to that in normal human hepatic cell line L02 (Figure 1F and G). We also observed that *KIAA1522* expression levels correlated with larger tumor size, multiple tumors, microvascular invasion, and higher TNM and Edmonson stages (Table 1). Survival analysis, using the online bioinformatics tool GEPIA database and *KIAA1522* expression levels, revealed that patients with HCC and higher *KIAA1522* expression had lower recurrence-free rates and poor overall survival (Figure 1H and I). Collectively, these results indicated that *KIAA1522* was over-expressed in HCC in vivo and in vitro, and therefore, could be used as a novel prognostic biomarker for this disease.

KIAA1522 Contributed to the Malignant Process of HCC

HepG2 cells were chosen for experiments on *KIAA1522* down-regulation because this cell line, among the seven HCC cell lines tested, showed the highest expression levels of *KIAA1522*. According to the results of qRT-PCR and Western blotting, shRNA3 had the best knockdown efficiency, and hence, it was used in all subsequent experiments (Figure 2A and B). At the same time, the Hep3B cells, which showed low expression levels of *KIAA1522*, were transfected with LV-*KIAA1522* to up-regulate *KIAA1522* expression. *KIAA1522* overexpression was validated by qRT-PCR and Western blotting (Figure 2A and B). First, we analyzed the relationship between *KIAA1522* and the proliferation marker, proliferating cell nuclear antigen (PCNA), and observed that *KIAA1522* expression was positively associated with PCNA (Figure 2C). CCK-8 assays revealed that *KIAA1522* knock-down resulted in the inhibition of growth of HepG2 cells (Figure 2D). In line with this, the clonogenic survival of the HepG2 cells was significantly decreased when *KIAA1522* expression was knocked down (Figure 2F). EDU

Figure 1 KIAA1522 is overexpressed in HCC. (A) KIAA1522 expression in HCC and normal tissues is analyzed using TCGA data. (B) KIAA1522 expression in HCC and normal tissues is obtained from the GEPIA database. (C) qRT-PCR results of KIAA1522 expression in HCC and corresponding non-tumor tissues. (D) Western blotting results of KIAA1522 protein expression in six pairs of tumor tissues (T) and corresponding non-tumor tissues (N). (E) IHC staining of KIAA1522, derived from HCC and corresponding non-tumor tissues. (F) qRT-PCR results of KIAA1522 expression in L02 cells and seven HCC cell lines. (G) Expression level of KIAA1522 protein in seven HCC cell lines and L02 cells (Western blotting). (H and I) Kaplan-Meier analysis of the association of KIAA1522 with overall survival and recurrence-free survival rates in patients with HCC using the GEPIA database. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table I Correlations Between *KIAA1522* Expression and Clinical Characteristics in HCC Patients (n = 60)

Characteristics	Number	KIAA1522 Expression		P-value
		Low Group	High Group	
Age (years)				
<50	18	8	9	0.774
≥50	42	22	21	
Gender				
Female	25	10	15	0.190
Male	35	20	15	
Cirrhosis				
Present	49	27	22	0.095
Absent	11	3	8	
HBV infection				
Positive	48	22	26	0.197
Negative	12	8	4	
Tumor size (cm)				
<5	32	20	12	0.038*
≥5	28	10	18	
Microvascular invasion				
Presence	27	8	19	0.004**
Absence	33	22	11	
Tumor multiplicity				
Simple	38	25	13	0.001**
Multiple	22	5	17	
α-fetoprotein (ng/mL)				
≤20	27	15	12	0.436
>20	33	15	18	
TNM stage				
I	35	22	13	0.018*
II/III	25	8	17	
Edmonson stage				
I/II	45	26	19	0.037*
III/IV	15	4	11	

Note: Significant values are shown in bold; *P<0.05; **P<0.01.

proliferation assay, a more specific and sensitive assessment of proliferation, verified that *KIAA1522* knockdown resulted in the inhibition of cell proliferation (Figure 2G). To investigate whether *KIAA1522* knockdown affected HCC migration and invasion, wound healing and transwell assays were performed. The wound healing assay showed that *KIAA1522*

knockdown reduced the migratory ability of the HepG2 cells (Figure 2I). The transwell assays displayed that fewer HepG2 cells migrated across the membrane and invaded other tissues when *KIAA1522* expression was reduced (Figure 2K). To determine the function of *KIAA1522* further, the above assays were also performed using Hep3B cells with up-regulated *KIAA1522* expression. As expected, *KIAA1522* overexpression promoted cell proliferation (Figure 2E, F, and H). The wound healing assay showed that *KIAA1522* overexpression significantly increased cell migration (Figure 2J). Transwell assays further confirmed that *KIAA1522* overexpression augmented the ability of Hep3B cells to migrate and invade (Figure 2L).

KIAA1522 Overexpression Inhibited Apoptosis and Reduced the G0/G1 Cell Cycle Arrest in HCC Cells

Apoptosis rate and cell cycle alteration are two factors that may affect the progression and development of HCC.^{17,18} To detect the apoptosis rate and cell cycle distribution, flow cytometry experiments were performed. Our results showed that *KIAA1522* knockdown increased apoptosis of HepG2 cells, while *KIAA1522* overexpression decreased apoptosis of Hep3B cells (Figure 3A). We also found that the levels of apoptosis-related protein *Bax* and *cleaved caspase 3* were increased, while the that of anti-apoptotic protein *Bcl-2* was decreased in HepG2 cells with *KIAA1522* knockdown. Opposite results were observed in Hep3B cells with overexpressed *KIAA1522* (Figure 3B). Moreover, the percentage of HepG2 cells with *KIAA1522* knockdown in the G0/G1 phase was increased, while the percentage of cells in the S phase was decreased. The opposite was observed in Hep3B cells with overexpressed *KIAA1522*: a decreased percentage of cells in the G0/G1 phase and an increased percentage of cells in the S phase (Figure 3C). Consistently, the expression of checkpoint proteins in the G1/S phase (*CDK2*, *CDK4*, and *CDK6*) was significantly down-regulated when *KIAA1522* was silenced, while the opposite results were observed in Hep3B cells with overexpressed *KIAA1522* (Figure 3D).

KIAA1522 Contributed to HCC Growth and Metastasis in vivo

To evaluate the effect of *KIAA1522* on HCC growth in vivo, we used a subcutaneous xenograft mouse model

Figure 2 KIAA1522 promotes proliferation, migration, and invasion of HCC cells. (**A** and **B**) Knockdown and overexpression efficiencies of KIAA1522 are confirmed by qRT-PCR and Western blotting, respectively. (**C**) The association of KIAA1522 with PCNA is analyzed using the GEPIA database. (**D–H**) Proliferation of cell lines with knocked down or overexpressed KIAA1522 is assessed using CCK-8, colony formation, and EDU assays. (**I** and **J**) Migration of cell lines with knocked down or overexpressed KIAA1522 is detected using wound healing assays. (**K** and **L**) Migration and invasion of cell lines with knocked down or overexpressed KIAA1522 is determined using transwell assays. ** $P < 0.01$, *** $P < 0.001$.

Figure 3 KIAA1522 overexpression inhibits apoptosis and reduces the G0/G1 cell cycle arrest in HCC cells. (A) Flow cytometry analysis of apoptosis of cell lines with knocked down or overexpressed KIAA1522. (B) Apoptosis-related proteins are measured using Western blotting. (C) Flow cytometry analysis of the cell cycle of cell lines with knocked down or overexpressed KIAA1522. (D) The expression of checkpoint proteins in the G1/S phase is measured using Western blotting. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

of HCC. Tumors from mice injected with HepG2-sh3 cells grew slowly and had lower average tumor weight (Figure 4A, C, and E), while tumors from mice injected with Hep3B cells with overexpressed KIAA1522 showed increased tumor growth and weight (Figure 4B, D, and F). IHC staining experiments confirmed that KIAA1522 expression was decreased in the HepG2-sh3 group and increased in the Hep3B-KIAA1522 group, compared to the corresponding control groups (Figure 4G). Ki-67 and TUNEL staining demonstrated that HepG2-sh3 xenografts, compared to HepG2-shNC xenografts, showed lower proliferative activity and enhanced apoptosis. In contrast, overexpression of KIAA1522 increased proliferative

activity and decreased apoptosis activity in the Hep3B xenografts (Figure 4H and I).

To clarify the effects of KIAA1522 on HCC metastasis in vivo, the mice were intravenously injected with luciferase-expressing HCC cells. The mice injected with cells with KIAA1522 knockdown developed more lung metastases, whereas an inverse trend was noticed in the mice injected with overexpressed Hep3B-KIAA1522 cells (Figure 4J and K). These findings were further confirmed by HE staining of the lungs of the mice (Figure 4L). Taken together, our data suggested that KIAA1522 played a role in promoting the process of HCC growth and metastasis.

Figure 4 KIAA1522 contributes to HCC growth and metastasis in vivo. (**A** and **B**) Representative images of xenograft HCC tumors obtained from mice injected with HepG2-sh3 and -shNC or Hep3B-KIAA1522 and -vector. (**C** and **D**) Tumor growth curves of the experiments shown in (**A** and **B**). (**E** and **F**) Weight of the xenograft HCC tumors. (**G–I**) Representative images of the xenograft HCC tumors stained with anti-KIAA1522, anti-Ki-67, and anti-TUNEL antibodies. (**J**) Representative bioluminescence imaging of the tail vein injection mouse model. (**K**) The number of mice with pulmonary metastases is shown in the table. (**L**) Representative images of lung metastatic nodules that are stained with HE in the mouse model. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 5 KIAA1522 activates the Wnt/β-catenin signaling pathway. **(A)** GSEA is performed using TCGA data to analyze the signaling pathways that are affected by KIAA1522. **(B)** TOP-flash/FOP-flash luciferase activity was measured in HepG2 and Hep3B cells. **(C)** The expression levels of several key proteins involved in the Wnt/β-catenin signaling pathway are detected using Western blotting. **P < 0.01, ***P < 0.001.

KIAA1522 Stimulated the Wnt/β-Catenin Signaling Pathway

To identify the potential signaling pathways affected by KIAA1522, we performed GSEA by analyzing the data obtained from TCGA. We noticed that the activity of the Wnt/β-catenin signaling pathway was positively correlated

with KIAA1522 expression levels in 371 HCC tissues (Figure 5A). To verify these GSEA results, TOP-flash/FOP-flash luciferase assay was performed using HCC cells. We found that KIAA1522 knockdown decreased the TOP/FOP transcriptional activity, whereas KIAA1522 overexpression enhanced relative luciferase activity, suggesting that

KIAA1522 was implicated in the positive regulation of the Wnt/ β -catenin signaling pathway (Figure 5B). We also detected several key proteins involved in the Wnt/ β -catenin signaling pathway, and they included β -catenin, cyclin D1, CD44, TCF-1, *c-Met*, and *c-Jun*. We found that these genes were down-regulated in the cells with knocked down *KIAA1522* expression, while they were up-regulated in the cells with *KIAA1522* overexpression (Figure 5C). Our results suggested that *KIAA1522* positively regulated the Wnt/ β -catenin signaling pathway.

Discussion

Tumorigenesis and metastasis of HCC is a complicated and multi-faceted process, and is affected by a variety of factors, including epigenetic and genetic factors.^{19,20} The *KIAA1522* gene was first discovered by sequencing.²¹ To date, very few studies have reported its function, especially in HCC. As a result, the molecular mechanisms of how *KIAA1522* influences HCC progression and development remain unknown. In this study, we evaluated *KIAA1522* expression in HCC using TCGA and GEPIA databases and subsequently validated its overexpression in HCC tissues and cell lines. Clinical data showed that *KIAA1522* expression was closely correlated to larger tumor size ($p = 0.038$), multiple tumors ($p = 0.001$), microvascular invasion ($p = 0.004$), and higher TNM ($p = 0.018$) and Edmonson ($p = 0.037$) stages. Survival analysis revealed that HCC patients with higher *KIAA1522* expression had lower recurrence-free rates (hazard ratio = 1.5; $p = 0.01$) and poor overall survival (hazard ratio = 1.6; $p = 0.012$). Considering that *KIAA1522* is associated with unfavorable clinicopathological features, we evaluated the effect of *KIAA1522* on the malignant process of HCC both in vitro and in vivo. Functional experiments demonstrated that *KIAA1522* contributed to proliferation, migration, invasion, and metastasis of HCC cells. Apoptosis and the cell cycle are two significant factors that regulate the progression and development of HCC. Our data revealed that overexpression of *KIAA1522* inhibited the G0/G1 arrest and apoptosis, suggesting that *KIAA1522* could affect the malignant biological behavior of HCC cells by modulating apoptosis and the cell cycle.

It was reported that *KIAA1522* acted as an oncogene in esophageal cancer by strengthening the ERK activity.¹² To determine the downstream signaling pathways affected by *KIAA1522* in HCC, we performed GSEA and observed that the Wnt/ β -catenin signaling pathway was strongly associated with higher *KIAA1522* expression levels. The Wnt/ β -catenin signaling pathway is highly conserved, and its dysregulation

is associated with various pathologies.^{22–25} Dysregulation of the Wnt/ β -catenin signaling pathway has been widely accepted to influence the malignant process by affecting cell proliferation, cell apoptosis, the cell cycle, metastasis, stemness, drug resistance, and metabolic reprogramming.^{26–30} Wnt ligands bind to Frizzled receptor complexes and activate Frizzled, stabilizing the cytoplasmic β -catenin protein by inhibiting the protein destruction complex (*APC*, *axin*, *GSK3 β* , and *CK1*). Down-regulation of *APC* contributes to the nuclear accumulation of β -catenin, which subsequently interacts with a *LEF/TCF* transcription factor to activate the transcription of target genes, including apoptosis- and cell cycle-related proteins.³¹ In this study, we observed that *KIAA1522* could stimulate the Wnt/ β -catenin signaling pathway to promote HCC progression. Nonetheless, the precise signaling pathways (direct or indirect) that *KIAA1522* could regulate remain unknown and must be delineated in further studies.

Conclusion

Here, we presented evidence that *KIAA1522* was overexpressed in HCC tissues and cell lines, and this overexpression was closely related to adverse clinicopathological characteristics and lower recurrence-free and overall survival rates in patients with HCC. Functional studies confirmed the oncogenic role of *KIAA1522* in HCC. We also identified the *KIAA1522*/Wnt- β -catenin signaling pathway axis as a critical mediator in the malignant biological behavior of HCC. Future studies will focus on the *KIAA1522*/Wnt- β -catenin signaling pathway axis to develop a possible novel therapeutic strategy to treat HCC.

Ethics Approval and Consent to Participate

The present study was approved by the Ethics Committee of The First People's Hospital of Lianyungang, affiliated with the Kangda College of Nanjing Medical University. All experiments were performed according to the National Institutes of Health guide for the care and use of laboratory animals. Patients or their relatives were informed of the proper usage of the human samples, and they gave written consent before sample acquisition.

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Author Contributions

All authors contributed to data analysis and drafting or revision of the article, gave final approval for the version to be published, and agreed to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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