

Co-expression of *KIAA1199* and hypoxia-inducible factor 1 α is a biomarker for an unfavorable prognosis in hepatocellular carcinoma

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Abstract

Advanced studies demonstrated that hypoxic stress induced *KIAA1199* expression leading to enhanced cell migration. *KIAA1199* is a protein related with cancer metastasis. Hypoxia inducible factor 1 α (HIF-1 α) is a transcriptional factor that maintains oxygen homeostasis. Both *KIAA1199* and *HIF-1 α* were upregulated in many human cancers. In the present study, co-expression of *KIAA1199* and *HIF-1 α* was evaluated for the clinicopathological characteristics and survival in hepatocellular carcinoma (HCC). Clinical-pathological information and follow-up data were collected from 152 HCC patients. *KIAA1199* and *HIF-1 α* expression were scored based on the percentage and intensity of immunohistochemical staining in pathological slide. Correlations between clinical features and the expression of *KIAA1199* and *HIF-1 α* were evaluated by Chi-square test, Kaplan-Meier curves and multivariate Cox regression analysis. The frequency of *KIAA1199* high expression was higher in HCC than adjacent tissue. *KIAA1199(H)/HIF-1 α (H)* tumors were more frequently of TNM ($P=.011$), tumor size ($P=.021$), vascular invasion ($P=.002$) and HBV ($P=.001$). In survival analysis, *KIAA1199(H)/HIF-1 α (H)* patients had the worst prognosis. Using the combination of the two parameters increased the prognostic value ($P<.01$ vs $P=.03$). *KIAA1199* in combination with *HIF-1 α* expression tends to indicate a more accurate prognosis.

Abbreviations: HCC = hepatocellular carcinoma, HIF-1 α = hypoxia inducible factor 1 α .

Keywords: hepatocellular carcinoma, *HIF-1 α* , *KIAA1199*, prognosis

1. Introduction

KIAA1199 was first described as an inner-ear-specific gene. *KIAA1199* mutations are associated with nonsyndromic hearing loss.^[1] *KIAA1199*, also defined as cell migration inducing protein (CEMIP), plays a role in the development and maintenance of

cancer metastasis. Yoshida *et al.* discovered that *KIAA1199* mediated depolymerization of hyaluronic acid via the cell membrane-associated clathrin-coated pit endocytic pathway, which would endow the tumor with an aggressive phenotype.^[2,3]

As we know, hypoxia is one of the most important factors that related to the tumor microenvironment.^[4] Because of aberrant vascularization and a poor blood supply, it is common that most solid tumors indeed have subjected to hypoxic stress permanently or transiently. The hypoxic response is mainly ascribed to hypoxia-inducible factors (HIFs).^[5] Oxygen sensitive HIFs family was constituted by O₂-labile alpha subunit (HIF1 α , -2 α and -3 α) and a stable beta subunit (HIF1 β , also known as ARNT).^[6] Of interest, it has been reported that hypoxia promotes *KIAA1199* expression in invasive cancer cells.^[7] HIF-2 α binds directly to the *KIAA1199* promoter region resulting in increased *KIAA1199* expression.^[7] While HIF-2 α displays a more tissue specific expression profile, HIF-1 α is expressed ubiquitously under hypoxic activation.^[8] The target genes of HIF-1 α encode proteins that play important roles in multiple aspects of tumorigenesis.^[9,10] Genome-wide chromatin immunoprecipitation reported that many loci bound both isoforms with similar affinity.^[11] HIF-1 α and -2 α are closely related and share a consensus hypoxia responsive elements.^[12]

Recently, emerging researches have demonstrated that *KIAA1199* overexpression was associated with tumor progression and poor prognosis in numerous cancers, such as colorectal,^[13,14] gastric,^[15] breast,^[16] pancreatic,^[17] cervical^[18] and liver,^[19] cancers. It also has reported that *KIAA1199* knockdown inhibited the growth and metastasis of HCC.^[20] *KIAA1199* plays an indispensable role in maintaining sorafenib-resistant HCC cell metastasis.^[21] Although numbers of studies have revealed that increased *HIF-1 α* level in the primary tumor was associated with increased mortality in most human cancers, the interaction of *KIAA1199* and *HIF-1 α* , which affects tumor

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growth and the clinical outcome of HCC patients, is still obscure to some extent.^[22] The aim of our study was to investigate the effects of *KIAA1199* and *HIF-1 α* expression on the prognosis in HCC patients.

2. Patients and methods

2.1. Study populations

This study included 152 unselected Chinese HCC patients, all of whom underwent surgery at the Affiliated Hospital of Nantong University from 2004 to 2010. No patient treated preoperatively with radiotherapy, chemotherapy, or immunotherapy. Clinical-pathological variables and follow-up data were: gender, age at diagnosis, grade, vessel invasion, TNM, hepatitis B virus (HBV) infection, tumor size, AFP value and cirrhosis. Included in this study were 117 males and 35 females. The average of age was 53.18 years. The detail Characteristics were present in Table 1. The study was approved by the Ethics Committee of the Human Research Ethics Committee of the Affiliated Hospital of Nantong University (2017-K036). Written informed consent was obtained from the patients for publication of this study.

2.2. *KIAA1199* and *HIF-1 α* staining

Tissue samples were collected from HCC patients who visited Affiliated Hospital of Nantong University from 2004 to 2010. A thorough histologic examination was made by tissue microarray slides from patients. Both *KIAA1199* and *HIF-1 α* staining were performed by Tissue Microarray System (Quick-Ray, UT06, UNITMA, Korea). Core tissue biopsies (2 mm in diameter) were taken from individual paraffin-embedded sections and arranged in the new recipient paraffin blocks. Immunohistochemistry (IHC) analysis was performed as previously described.^[23] The slides were incubated with the primary antibodies against *KIAA1199* (Cat No: 21129-1-AP, ProteinTech Group, Chicago, IL, USA) or *HIF-1 α* (Cat No: ab1, Abcam, Cambridge, MA, USA) at 4°C overnight. The images were acquired and analyzed by the Vectra 3 System (PerkinElmer, USA). Two variables were estimated: intensity (0 to 3 as negative, weak, moderate or strong) and percentage (0% to 100%). The final staining score of each tissue sample was generated by multiplying the intensity and percentage scores. The scores ranged from 0 (no staining) to 300 (100% of cells with strong staining intensity).

The cutoff points for *KIAA1199* and *HIF-1 α* expression were set by X-tile software (<http://medicine.yale.edu/lab/rimm/research/software.aspx>; Rimm lab at Yale University). The degree of *KIAA1199* and *HIF-1 α* expression were quantified using a two-level grading system defined as follows: score less than or equal to cutoff point (score = 80) as low (L), otherwise defined as high (H).

2.3. 2.3 Cell culture and hypoxia treatment

HepG2 cells were maintained in 1640 medium (HyClone, UT, USA) containing 10% fetal bovine serum (HyClone, UT, USA) and were cultured at 37°C in an incubator with 5% CO₂. Cells were subjected to hypoxic treatment of 1% O₂ – 5% CO₂ in hypoxia chamber (Invivo₂ 400, Ruskinn Technologies, Leeds, UK) or cultured under normoxic conditions of 21% O₂ to 5% CO₂.

Table 1

Characteristics of the populations studied.

Characteristic	Detail
N	152
Age (mean)	53.18 ± 9.94 years (range 31–79 years)*
Sex	117 male, 35 female
Follow-up (mean)	44.81 ± 30.37 months*

* $\bar{x} \pm SD$; range in parentheses.

2.4. 2.4 Western blot

Cells or tissues were lysed with the cell lysis buffer (Beyotime, Shanghai, China). Whole cell or tissue extracts were resolved by 10% SDS-PAGE and electrophoretically transferred to polyvinylidene difluoride membranes (Roche Diagnostics, Mannheim, Germany). The membranes were blocked, and then incubated with anti-*KIAA1199* (Cat No: 21129-1-AP, ProteinTech Group, Chicago, IL), anti- β -actin (Cat No: ab8226, Abcam, Cambridge, MA) or anti-*HIF-1 α* antibodies (Cat No: ab1, Abcam, Cambridge, MA) at 4°C overnight, followed by an incubation with the appropriate horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch, PA). The chemiluminescence reaction was performed using ECL reagent (Thermo Scientific, IL). The specificity of *KIAA1199* and *HIF1 α* antibodies by RNA interference were confirmed by RNA interference (Supplementary Fig. 1, <http://links.lww.com/MD/F257>).

2.5. Statistical analyses

The Chi-square and Fisher exact test were used to assess correlations between clinicopathologic features and expression of *KIAA1199* and *HIF-1 α* . Survival time was calculated from date of diagnosis to date of death/censoring. Overall survival curves were constructed according to Kaplan–Meier method. And the log-rank test was applied to examine the survival difference. The univariate and multivariate survival analysis were performed with Cox regression. All *P* values reported are from two-sided tests and the threshold for significance was set at .05.

3. Results

3.1. *KIAA1199* expression in HCC and adjacent normal tissue

First, we examined the *KIAA1199* protein expression in 48 pairs of HCC and adjacent non-cancerous tissues. The *KIAA1199* expression levels were significantly higher in tumor tissues than in non-tumor tissues (Fig. 1A and B). We further evaluated the *KIAA1199* expression in HCC and non-tumor tissues by IHC analysis. Representative images of *KIAA1199* staining are shown in Figure 1C. Positive *KIAA1199* staining was predominantly localized to the cellular membrane and cytoplasm. The expression level of *KIAA1199* protein in adjacent tissue was clearly lower than that of cancerous tissues.

3.2. Association between the expression of *KIAA1199*/*HIF-1 α* and clinicopathological parameters in HCC

To determine the expression of *KIAA1199* and *HIF-1 α* in response to hypoxia, HepG2 cells were exposed to hypoxia (1% oxygen) for periods from 1 to 12 hours. As shown in Figure 2,

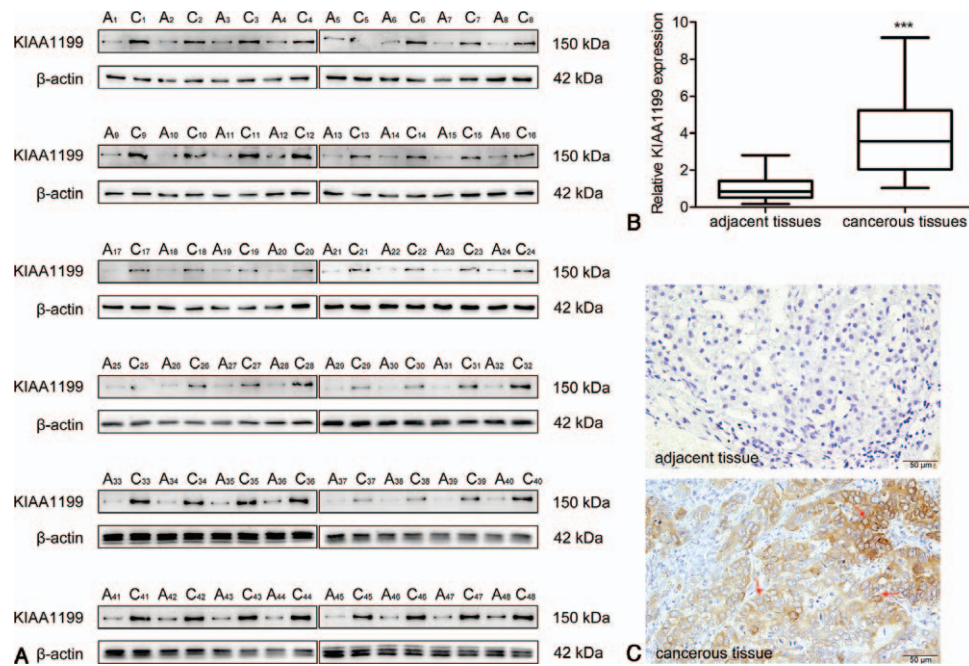


Figure 1. The expression of *KIAA1199* in HCC and adjacent tissues. The *KIAA1199* expression in individual 48 HCC patients were analyzed by Western blot (A) and quantified (B) using β -actin as control. (C) Immunohistochemistry for *KIAA1199* expression in adjacent and cancerous tissue (bar = 50 μ m). The *KIAA1199* positive expression were indicated by red arrows. A: adjacent tissue; C: cancerous tissue; *** $P < .001$ compared with the control.

hypoxia upregulated both the *KIAA1199* and *HIF-1 α* in a time-dependent manner. Then we analyze the relationship with the clinicopathologic features and *KIAA1199* expression (Table 2). A total of 103 (67.76%) patients showed high expression of

KIAA1199, while 49 (32.24%) showed low or no expression of *KIAA1199*. High *KIAA1199* expression was significantly associated with vascular invasion ($P = .024$), TNM ($P = .034$), HBV ($P = .001$), tumor size ($P = .034$) and cirrhosis ($P = .021$). By

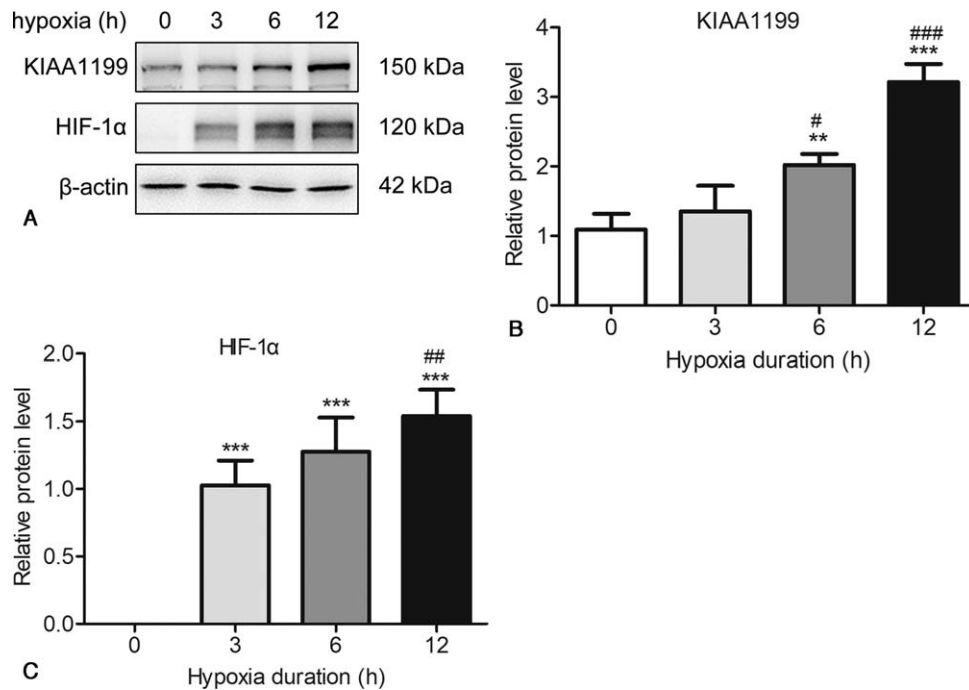


Figure 2. The expression of *KIAA1199* and *HIF-1 α* in respond to hypoxia. HepG2 cells were exposed to hypoxia for 0, 3, 6, 9 and 12 h. The expressions of *KIAA1199* and *HIF-1 α* were measured by Western blotting (A) and quantified (B, C) using β -actin as control. The data are presented as the means \pm SD, $n = 3$. ** $P < .01$ & *** $P < .001$ compared to 0 hour.

Table 2**KIAA1199 expression and clinical variables in hepatocellular carcinoma.**

Total	KIAA1199		P
	Low 49 (32.24%)	High 103 (67.76%)	
Gender			.91
female	11 (31.43%)	24 (68.57%)	
male	38 (32.48%)	79 (67.52%)	
Age			.40
≤ 50	16 (28.07%)	41 (71.93%)	
> 50	33 (34.74%)	62 (65.26%)	
Grade			.49
well	4 (23.53%)	13 (76.47%)	
moderate	33 (31.43%)	72 (68.57%)	
poor	12 (40.00%)	18 (60.00%)	
Vascular invasion			.02*
no	45 (59.21%)	45 (59.21%)	
yes	58 (76.32%)	58 (76.32%)	
TNM			.03*
I	28 (41.18%)	40 (58.82%)	
II & III	21 (25.00%)	63 (75.00%)	
HBV			.001***
no	15 (62.50%)	9 (37.50%)	
yes	34 (26.56%)	94 (73.44%)	
Tumor size			.03*
≤ 5 cm	29 (40.85%)	42 (59.15%)	
> 5 cm	20 (24.69%)	61 (75.31%)	
AFP			.48
≤400 μg/L	28 (30.11%)	65 (69.89%)	
>400 μg/L	21 (25.59%)	38 (64.41%)	
Cirrhosis			.02*
no	22 (44.90%)	27 (55.10%)	
yes	27 (26.21%)	76 (73.79%)	

* $P < .05$.*** $P < .01$.

contrast, no correlation ($P > .05$) was observed between KIAA1199 expression and other clinical parameters, such as sex, age at diagnosis, histopathology grading and AFP value.

It has been reported that *HIF-1α* expression strongly was associated with clinical features in HCC patients.^[24,25] Thus, we also detected the correlation of KIAA1199 and *HIF-1α* expression. We found that there is a strong correlation of KIAA1199 expression with levels of *HIF-1α* ($P = .0005$) (Table 3).

Furthermore, we analyze the combination expression of KIAA1199 and *HIF-1α* in HCC (Table 4). A total of 74 (74/152, 48.68%) patients showed high expression of both KIAA1199 and *HIF-1α*, while 28 (28/152, 18.42%) showed low expression of both KIAA1199 and *HIF-1α*. KIAA1199(H)/*HIF-1α*(H) expression was associated with vascular invasion ($P = .002$), TNM ($P = .011$), HBV ($P = .001$), tumor size ($P = .021$) and cirrhosis ($P = .032$).

3.3. Survival analysis according to KIAA1199/HIF-1α expression for HCC patients

Finally, we carried out survival analyses in these HCC patients. Patients in the high KIAA1199 expression group had worse overall survival than the low expression group (Fig. 3A). The 5-year overall survival rate for patients with high expression of KIAA1199 and for patients with low expression of KIAA1199

Table 3**The correlation of KIAA1199 and HIF-1α expression in hepatocellular carcinoma.**

HIF-1α		KIAA1199		P
		Low (n)	High (n)	
HIF-1α	Low (n)	28	29	.000***
	High (n)	21	74	

*** $P < .001$.

was 42.22% and 68.57% ($P = 0.028$), respectively. While, there was no difference in 1-year, 3-year and 5-year overall survival rate (Table 5). As shown in Figure 3B, we found that patients with high expression levels for both KIAA1199 and *HIF-1α* had the worst overall survival rate. The 3- and 5-year overall survival rate of KIAA1199(H)/*HIF-1α*(H) patients are much worse than that of KIAA1199(L)/*HIF-1α*(L) group ($P = .041$ and $.004$, respectively) (Table 6). Thus, using the combination with the two parameters increased the prognostic value, compared with KIAA1199 alone.

Next, a multivariate Cox regression analysis was performed to search for an independent prognostic factor (Table 7). After adjustment for clinical variables, cirrhosis ($HR_{adj} = 2.26$; 95% CI = 1.11 – 4.62) and *HIF-1α* ($HR_{adj} = 2.61$; 95% CI = 1.29 – 5.28) were all found to be independent prognostic factors.

4. Discussion

HCC is the most frequent primary liver cancer and represents a major medical problem.^[26,27] Only surgery provides a suitable therapeutic method for HCC to prolong life span or provide better prognosis in the last few years. However, it is difficult to deal with the cases that at terminal stage of HCC. Therefore, the identification of biological markers would clearly be of great benefit. Our research revealed that increased expression of KIAA1199/*HIF-1α* was significantly correlated with vascular invasion, tumor TNM stage, HBV infection, tumor size and cirrhosis. KIAA1199/*HIF-1α* high staining was associated with poor prognosis in HCC patients. The disparity in the prognosis between KIAA1199(H)/*HIF-1α*(H) and KIAA1199(L)/*HIF-1α*(L) patient was much more apparent than the disparity observed when evaluating KIAA1199 alone.

KIAA1199 has been reported to not be expressed only in tumor tissues^[1]. While, tumor hypoxia also represents a remarkably exploitable target for cancer therapy, because hypoxia encountered in solid tumors are not observed elsewhere in healthy tissues.^[28,29] Therefore, it is possible to develop reagents inhibiting KIAA1199 expression by a hypoxia-triggered liposome platform to avoid side effects.

Some research reported that KIAA1199 could promote cancer cell migration and invasion through different signaling pathways.^[18,30,31] In contrast, Tiwari *et al.* argued that KIAA1199 overexpression reduced SW480 cell invasive ability and cell proliferation, but did not alter migratory ability.^[32] Similarly, *HIF-1α* is overexpressed in many human cancers, such as brain (oligodendroglioma), breast, cervix, oropharynx, ovary and uterus (endometrial) cancer. Whereas it was reported that *HIF-1α* overexpression patients had increased survival time in oropharyngeal, head and neck, non-small-cell lung and ovarian cancer. This discrepancy of KIAA1199 and *HIF-1α* overexpression could be due to cancer type, experimental models, cell lines, and ectopic/endogenous expression, and the presence or absence of genetic alterations. Furthermore, the effects of gain or

Table 4
KIAA1199/HIF-1 α expression and clinical variables in hepatocellular carcinoma.

	KIAA1199(L)/HIF-1 α (L) n (%)	KIAA1199(L)/HIF-1 α (H) n (%)	KIAA1199(H)/HIF-1 α (L) n (%)	KIAA1199(H)/HIF-1 α (H) n (%)	P
Total	28 (18.42)	21 (13.82)	29 (19.08)	74 (48.68)	
Gender					.16
female	7 (20.00)	4 (11.43)	11 (31.43)	13 (37.14)	
male	21 (17.95)	17 (14.53)	18 (15.38)	61 (52.14)	
Age					.03
≤ 50	9 (15.79)	7 (12.28)	9 (15.79)	32 (56.14)	
> 50	19 (20.00)	14 (14.74)	20 (21.05)	42 (44.21)	
Grade					.02
well & moderate	20 (16.39)	17 (13.93)	22 (18.03)	63 (51.64)	
poor	8 (26.67)	4 (13.33)	7 (23.33)	11 (36.67)	
Vascular invasion					.002**
no	20 (26.32)	11 (14.47)	19 (25.00)	26 (34.21)	
yes	8 (10.53)	10 (13.16)	10 (13.16)	48 (63.16)	
TNM					.011*
I	17 (25.00)	11 (16.18)	17 (25.00)	23 (33.82)	
II & III	11 (13.10)	10 (11.90)	12 (14.29)	51 (60.71)	
HBV					.001**
no	10 (41.67)	5 (20.83)	5 (20.83)	4 (16.67)	
yes	18 (14.06)	16 (12.50)	24 (18.75)	70 (54.69)	
Tumor size					.02*
≤ 5 cm	17 (23.94)	12 (16.90)	17 (23.94)	25 (35.21)	
> 5 cm	11 (13.58)	9 (11.11)	12 (14.81)	49 (60.49)	
AFP					.58
≤ 400 μ g/L	16 (17.20)	12 (12.90)	21 (22.58)	44 (47.31)	
> 400 μ g/L	12 (20.34)	9 (15.25)	8 (13.56)	30 (50.85)	
Cirrhosis					.03*
no	15 (30.61)	7 (14.29)	10 (20.41)	17 (34.69)	
yes	13 (12.62)	14 (13.59)	19 (18.45)	57 (55.34)	

H = high, L = low.
* $P < .05$.
** $P < .01$.

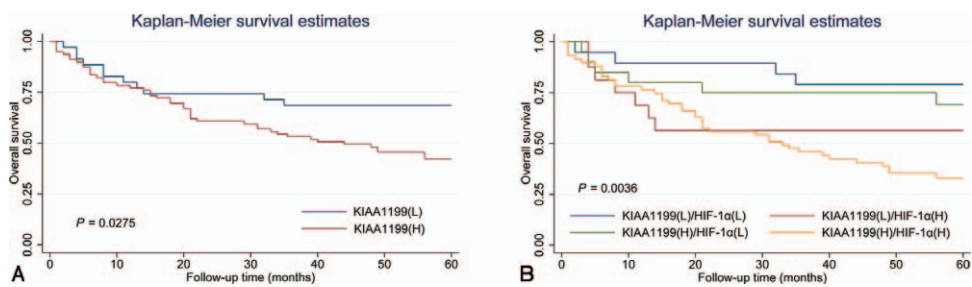


Figure 3. Survival curves for HCC patients, in relation to KIAA1199 and HIF-1 α expressions. Overall survival of HCC patient calculated by the Kaplan-Meier method. Overall survival in dependence on KIAA1199 (A) and in KIAA1199/HIF-1 α (B). L: low; H: high.

Table 5
Relationship between KIAA1199 expression and survival rate in hepatocellular carcinoma.

Survival measurement	KIAA1199 density [†]		P
	Low	High	
1-year overall survival (%)	80.00 \pm 6.67	77.22 \pm 4.72	.70
3-year overall survival (%)	68.57 \pm 7.85	53.16 \pm 5.61	.16
5-year overall survival (%)	68.57 \pm 7.85	42.22 \pm 5.66	.03*

[†] $\bar{x} \pm SE$.
* $P < 0.05$.

Table 6
Relationship between KIAA1199/HIF-1 α expression and survival rate in hepatocellular carcinoma.

Survival measurement	KIAA1199/HIF-1 α density [†]		P
	KIAA1199(L)/HIF-1 α (L)	KIAA1199(H)/HIF-1 α (H)	
1-year overall survival (%)	89.47 \pm 7.04	76.27 \pm 5.54	.53
3-year overall survival (%)	78.95 \pm 9.35	45.76 \pm 6.49	.04*
5-year overall survival (%)	78.95 \pm 9.35	33.01 \pm 6.26	.004**

[†] $\bar{x} \pm SE$.
* $P < .05$.
** $P < .01$.

Table 7
Cox regression analysis of prognostic factors for 5-year survival in hepatocellular carcinoma.

	Univariate analysis			Multivariate analysis		
	P	HR	95% CI	P	HR	95% CI
Gender	.33	0.75	0.42–1.34			
male vs female						
Age	.44	1.24	0.72–2.14			
< 55 vs ≥ 55						
Grade	.48	0.84	0.52–1.37			
well & moderate vs poor						
Vessel invasion	.61	1.15	0.68–1.95			
no vs yes						
TNM	.74	1.10	0.64–1.87	.33	1.20	0.83–1.72
I vs II & III						
HBV	.56	1.35	0.49–3.74			
no vs yes						
Tumor size	.051	1.72	1.00–2.98			
≤ 5 cm vs > 5 cm						
AFP	.51	1.19	0.71–2.02			
≤ 400 μg/L vs > 400 μg/L						
Cirrhosis	.02*	2.42	1.18–4.94	.03*	2.26	1.11–4.62
no vs yes						
HIF-1α	.001**	3.09	1.55–6.13	.007**	2.61	1.29–5.28
low vs high						
KIAA1199	.03*	2.06	1.06–3.99	.17	1.60	0.81–3.14
low vs high						

*P < .05.

**P < .01.

loss of protein function can vary according to the stage of cancer progression. Although biopsy immunohistochemistry is a common approach to analyze the association between altered expression of proteins and clinical features, it does not reveal whether the protein carries any mutations or has been post-translationally modified, which could affect its function. Combination with the two or more parameters would significantly improve the prognostic value and diagnostic accuracy.

Several factors could limit the outcomes of this study. Firstly, the sample sizes were small. In such cases, large sample sizes will be necessary for reliable interpretation. Secondly, this study doesn't contain the variables like race.

5. Conclusion

Here, we concluded that *KIAA1199* expression correlation with clinical variables in HCC, and in combination with *HIF-1α* expression tends to indicate a more accurate prognosis.

Author contributions

Conceptualization: Dan Wang, Hui Zhao.

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Formal analysis: Dan Wang, Shu Lu.

Funding acquisition: Hui Zhao, Dan Wang.

Investigation: Dan Wang, Shu Lu.

Methodology: Xiaojing Zhang, Linlin Huang.

Project administration: Dan Wang, Shu Lu, Xiaojing Zhang.

Resources: Hui Zhao.

Software: Dan Wang.

Supervision: Hui Zhao.

Validation: Dan Wang, Shu Lu, Xiaojing Zhang, Linlin Huang, Hui Zhao.

Writing – original draft: Dan Wang.

Writing – review & editing: Hui Zhao.

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