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Increased expression of insulin-like growth factor-1 receptor predicts poor prognosis in patients with hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is a lethal disease worldwide. In this study, we sought to explore the expression of insulin-like growth factor-1 receptor (IGF-1R) and its prognostic value in HCC.

The expressions of IGF-1R mRNA and protein were estimated using quantitative real-time polymerase chain reaction and immunohistochemistry assays, respectively. The association between IGF-1R expression and clinicopathologic characteristic of patients with HCC was analyzed through Chi-squared test. Kaplan–Meier analysis and multivariate Cox analysis were performed to analyze prognostic value of IGF-1R in HCC.

The IGF-1R was significantly upregulated in HCC tissues at both mRNA and protein levels compared with adjacent normal ones ($P < .01$). Its expression was associated with tumor node metastasis stage ($P = .037$) and lymph node metastasis ($P = .027$) of patients with HCC. Patients with HCC with high expression of IGF-1R had worse overall survival than those with low expression. IGF-1R might be a potential prognostic biomarker for HCC (hazard ratio [HR] = 1.912, 95% confidence interval [CI]: 1.023–3.572, $P = .042$).

The IGF-1R expression level is upregulated in HCC tissues and may act as a prognostic biomarker for the disease.

Abbreviations: AFP = alpha-fetoprotein, CI = confidence interval, DCP = Des-gamma-carboxy prothrombin, GBMs = glioblastomas, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, HR = hazard ratio, IGF-1 = insulin-like growth factor 1, IGF-1R = insulin-like growth factor-1 receptor, IGFs = insulin-like growth factors, qRT-PCR = quantitative real-time polymerase chain reaction, SD = standard deviation, TNM = tumor node metastasis.

Keywords: hepatocellular carcinoma, insulin-like growth factor-1 receptor, prognosis

1. Introduction

Hepatocellular carcinoma (HCC) is a lethal malignancy all over the world, especially in East Asia.[1,2] High incidence of HCC may be attributed to the infections of hepatitis B virus (HBV) and hepatitis C virus (HCV).[3,4] Radical resection is the 1st choice in treating HCC, but the 5-year overall survival is still very low due to postoperative recurrence and/or metastasis.[5,6] Despite recent improvements in radical surgery and other therapeutic techniques, overall prognosis of patients with HCC remains dismal. Therefore, it is vital to identify novel predictive biomarkers for HCC so as to improve the patients' prognosis.

Insulin-like growth factor-1 (IGF-1), an important growth stimulating factor, is synthesized and secreted by hepatocytes. The activity of IGF-1 is mainly mediated by insulin-like growth factor-1 receptor (IGF-1R). IGF-1R is a tyrosine kinase type receptor, composing of α and β subunits.[7] IGF-1R could activate PI3K/AKT and Ras/mitogen-activated protein kinase pathways, and play crucial roles in several tumor-related courses, such as tumor growth and metastasis, as well as drug resistance.[8] The knockdown of IGF-1R could suppress lung cancer progression in mouse model.[9] A meta-analysis constructed by Zhao et al reported that IGF-1R overexpression predicted poor clinical outcomes in patients with nonsmall cell lung cancer.[10] A study by Aleem et al concluded that the upregulation of IGF-1R might be an early event in hepatocarcinogensis.[11] The knockdown of IGF1R might inhibit HCC cell proliferation and migration.[12] Based on these findings, we hypothesized that the expression of IGF-1R may be associated with malignant behaviors of HCC cells and act as a potential biomarker for the patients' outcomes. However, few studies have investigated prognostic performance of IGF-1R in HCC, especially among Chinese Han population.

Therefore, in the present study, we investigated the expression of IGF-1R in HCC tissues and adjacent normal tissues, and analyzed its relationship with overall survival and clinical parameters of patients with HCC. Prognostic value of IGF-1R in HCC was also estimated.

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2. Materials and methods

2.1. Patients and specimens

A total of 136 pathologically diagnosed patients with HCC were recruited from Cangzhou Central Hospital. All fresh HCC tissues and adjacent noncancerous ones from surgery were put into liquid nitrogen immediately, and then stored at −80°C until RNA extraction. Clinicopathologic characteristics of patients with HCC were obtained from medical records and pathologic reports are listed in Table 1. A 5-years' follow-up was conducted.

This study was approved by the Medical Ethics Committee of Cangzhou Central Hospital. Written informed consent was signed by each patient prior to surgery.

2.2. RNA extraction and quantitative real-time polymerase chain reaction

Total RNA was isolated from both HCC and adjacent normal tissue specimens adopting TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Then total RNA was reversely transcribed into the 1st-strand of cDNA using the RevertAid First Strand cDNA synthesis kits (Fermentas) according to the manufacturer's protocol. Quantitative real-time polymerase chain reaction (qRT-PCR) reaction was performed using a SYBR Green Premix Ex Taq (Takara, Japan) on an ABI Prism 7900 instrument (Applied Biosystems, Foster City, CA). Relative expression of IGF-1R mRNA was calculated employing the comparative $2^{-\Delta\Delta Ct}$ (threshold) method. GADPH was used as internal control. Primer sequences were as follows: IGF-1R forward: 5′-GTACAACTACCGCTGCTGGA-3′, and reverse: 5′-TGGCAGCACTCATTGTTCTC-3′; GADPH: forward: 5′-TCTTCGCTTTGTCCTTTCGT-3′, and reverse: 5′-TGCTGTAGCCAAATTCGTTG-3′. All experiments were performed in triplicate.

2.3. Immunohistochemistry

The IGF-1R protein levels in collected 136 pairs of HCC tissues and adjacent normal tissues were estimated through immunohistochemistry (IHC) method, and experiments were performed following standard procedures. In brief, tissue specimens were cut into slices with a thickness of 4 μm. Then the tissue sections were deparaffinized, hydrated, and rehydrated. Next, antigen retrieval was performed. Later, the tissue section was incubated with primary anti-IGF-1R rabbit monoclonal antibody (G11; Ventana-Roche, Tuscon, AZ) at 4°C overnight. PBS buffer was used to wash the tissue sections 3 times, and then the tissues were incubated with secondary antibody at 37°C for 30 minutes. The results were analyzed using 3,3′-diaminobenzidine chromogen solution. Staining range was scored as follows: 0, <5%; 1, 5% to 25%; 2, 26% to 50%; 3, 51% to 75%; and 4, >75%. Staining intensity was scored as follows: 0, nonstaining; 1, light yellow; 2, brownish yellow; and 3, brown. Final scores were the sum of scores for staining range and staining intensity. A final score that was not >3 meant low expression, while a final score that was >3 signified high expression.

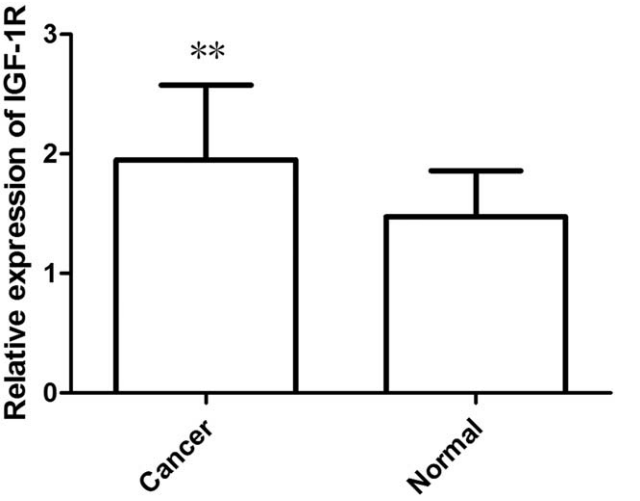

Figure 1. Insulin-like growth factor-1 receptor (IGF-1R) mRNA expressions in hepatocellular carcinoma (HCC) and normal tissues. Expression level of IGF-1R in HCC tissues was higher than that in adjacent normal tissues. $^{**}P < .01$.

2.4. Statistical analysis

All statistical analyses were performed using SPSS 21.0 software (SPSS Inc, Chicago, IL) and GraphPad Prism 5 (GraphPad Software, San Diego, CA). Data were from three independent experiments and expressed as mean ± standard deviation (SD). Difference between 2 groups was determined through Student *t* test. The associations between *IGF-1R* mRNA expression level and clinicopathologic factors were assessed using Chi-squared test. Kaplan–Meier method was applied to analyze overall survival of patients with HCC and the log-rank test was taken to assess differences in patients' overall survival. Multivariate Cox proportional hazards model was adopted to identify prognosis factors for HCC. Results were estimated using hazard ratio (HR) combined with 95% confidence interval (CI). *P*-value <.05 was considered to be statistically significant level.

3. Results

3.1. Upregulation of IGF-1R in HCC

The levels of IGF-1R mRNA in HCC tissues and adjacent normal ones were detected using qRT-PCR method. We found that the expression of IGF-1R mRNA was significantly higher in tumor tissues than in adjacent nontumor ones ($P < .01$, Fig. 1).

In addition, IGF-1R protein expression in collected tissue samples was also estimated using IHC method. Representative staining results for IGF-1R in HCC tissues and adjacent nontumor ones are displayed in Figure 2. Positive staining for

Table 1

The staining range and staining intensity of HCC tissues and adjacent normal tissues in immunohistochemistry assay.

	Staining range, %			Staining intensity (score)		
Tissue samples	Total	Low IGF-1R expression	High IGF-1R expression	Total	Low IGF-1R expression	High IGF-1R expression
HCC tissues	51.35 ± 22.95	50.26 ± 22.68	52.24 ± 23.29	2.11 ± 0.73	2.02 ± 0.65	2.19 ± 0.79
Adjacent tissues	22.82 ± 16.47*			1.22 ± 0.77*		

HCC = hepatocellular carcinoma, IGF-1R = insulin-like growth factor-1 receptor.
* $P < .05$ (HCC tissues vs adjacent tissues).

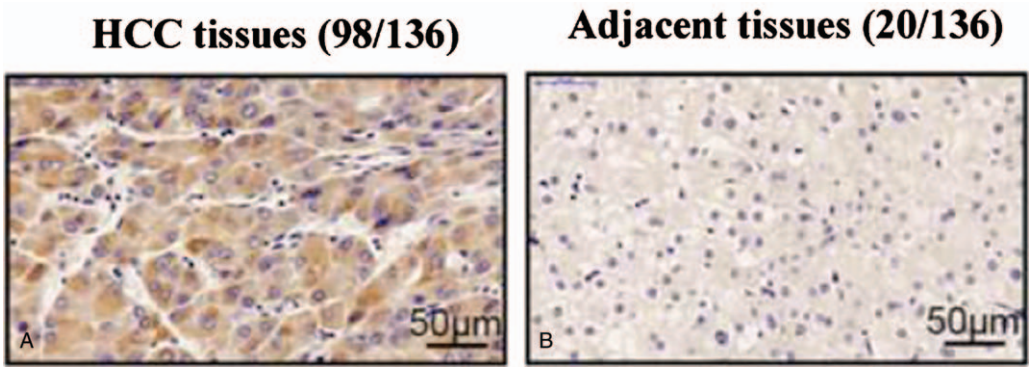

Figure 2. The expression of insulin-like growth factor-1 receptor (IGF-1R) protein in hepatocellular carcinoma (HCC) tissues (A) and adjacent noncancerous ones (B) was detected using the method of immunohistochemistry. IGF-1R showed positive staining results in HCC tissues, and negative results in nonmalignant controls.

IGF-1R protein was observed in 72.06% (98/136) of HCC tissues, while the positive rate was only 22.06% (20/136) in nonmalignant ones. IGF-1R protein levels were significantly higher in HCC tissues than in noncancerous samples ($P < .001$). The staining range and staining intensity in HCC tissues were respectively $51.35 \pm 22.95\%$ and 2.11 ± 0.73 scores, and $22.82 \pm 16.47\%$, 1.22 ± 0.77 scores in adjacent normal tissues. Both of staining range and staining intensity were significantly different between HCC tissues and adjacent normal tissues ($P < .05$, Table 1). Meanwhile, we also analyzed the staining range and staining intensity differences in HCC tissues with low- or high-IGF-1R expression, but the results showed there was no significant difference between low- and high- IGF-1R expression groups in staining range or staining intensity ($P > .05$).

3.2. Correlation between IGF-1R expression and clinicopathologic features in patients with HCC

The included patients were divided into 2 groups based on the mean expression level of IGF-1R mRNA in HCC tissue specimens. With a cutoff value of 1.95. That is, patients with HCC would be classified into high expression group (n = 75) when their IGF-1R levels were >1.95; otherwise, they would be into low expression group (n = 51). As shown in Table 2, the expression level of IGF-1R was significantly correlated with tumor node metastasis (TNM) stage ($P = .037$) and lymph node metastasis ($P = .027$) among patients with HCC, but not with age, gender, tumor size, or differentiation ($P > .05$ for all).

3.3. Correlation between IGF-1R expression and overall survival in patients with HCC

The influence of IGF-1R level on overall survival of patients with HCC was estimated using Kaplan–Meier analysis. Survival curve was plotted based on IGF-1R mRNA levels in HCC tissue samples. As shown in Figure 3, the patients with high IGF-1R expression showed poorer overall survival than those with low expression (Log-rank test: $P < .05$). We further evaluated the correlation for IGF-1R mRNA level with clinicopathologic parameters and patients' outcomes using multivariate Cox

Table 2

The relationship between IGF-1R expression and clinicopathologic features of patients with HCC.

Parameters	Cases (n = 136)	IGF-1R expression		χ^2	*P*
		Low	High		
Age, yr				0.684	.408
<50	66	32	34		
≥50	70	29	41		
Gender				0.874	.350
Male	72	35	37		
Female	64	26	38		
Tumor size, cm				0.107	.744
<5	69	30	39		
≥5	67	31	36		
Differentiation				2.909	.088
Moderate + well	76	39	37		
Poor	60	22	38		
TNM stage				4.355	.037
I–II	69	37	32		
III	67	24	43		
Lymph node metastasis				4.870	.027
Negative	66	36	30		
Positive	70	25	45		

HCC = hepatocellular carcinoma, IGF-1R = insulin-like growth factor-1 receptor, TNM = tumor node metastasis.

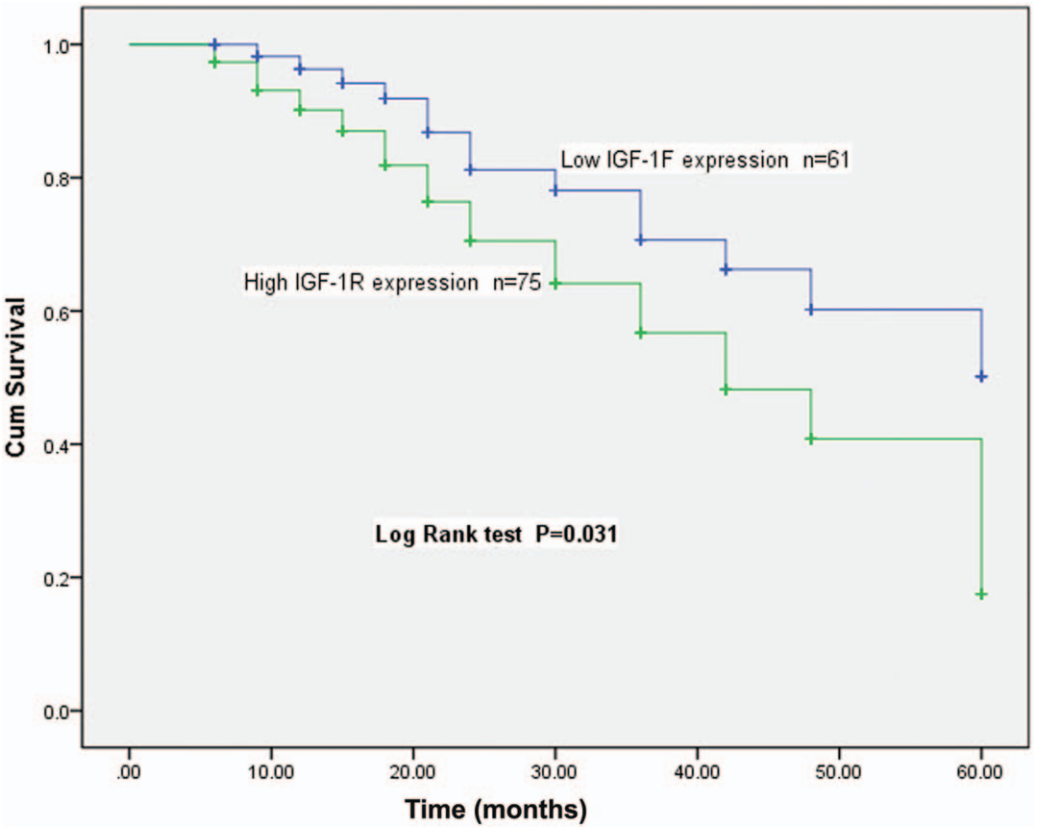

Figure 3. Kaplan–Meier analysis for overall survival of patients with hepatocellular carcinoma. Patients with high insulin-like growth factor-1 receptor (IGF-1R) expression had shorter overall survival than those with low expression (log rank test, $P < .05$).

regression analysis. The results suggested that the expression level of IGF-1R was significantly associated with the prognosis of patients with HCC (Table 3, HR = 1.912, 95% CI: 1.023–3.572, $P = .042$).

4. Discussion

The HCC is a malignant cancer characterized by rapid progression and poor prognosis. The infections of HBV and HCV are major reasons for HCC onset, with the former one as the leading cause in China.[13] Five-year survival rate of patients with HCC is reportedly <10%.[14] Biomarkers are beneficial to early diagnosis, prognosis evaluation, and treatment in cancers. So far, a variety of biomarkers have been proposed for HCC, such as alpha-fetoprotein (AFP), des-gamma-carboxy prothrombin, osteopontin, and GP73.[15] However, their clinical values are limited due to insufficient accuracy.[16] Therefore, it is important to find effective prognostic molecular markers so as to improve the outcome and treatment of HCC.

Table 3

Multivariate analysis adjusted for clinical variables for the prognostic value of IGF-1R in patients with HCC.

Variable	HR	95% CI	*P*
Low IGF-1R expression	–	–	–
High IGF-1R expression	1.912	1.023–3.572	.042

95% CI = 95% confidence interval, HCC = hepatocellular carcinoma, HR = hazard ratio, IGF-1R = insulin-like growth factor-1 receptor.

The IGF signaling pathway plays key roles in various physiologic and pathologic processes, and has been considered as a promising therapeutic target for diabetes and cancers.[17] IGF axis may promote tumor progression through directly activating PI3K/Akt signaling pathway or indirectly combining with other molecules involved in carcinogenesis.[18–20] IGF-1R, a receptor of IGF axis, is an attractive therapeutic target in antitumor treatment over the past 2 decades, and closely associated with tumor development and progression.[21,22] The upregulation of IGF-1R was observed in several cancers.[23–25] For instance, Maris et al found that both IGF-IR and IGF-IIR were overexpressed in glioblastomas (GBMs), and that IGF-1R could be an interesting target in GBM therapy.[24] Bieghs et al indicated that increased IGF-1R expression was correlated with poor prognosis in patients with multiple myeloma, which showed its potential in disease diagnosis and therapy.[25] Zhao et al found that the expression of IGF-1R might be correlated to the occurrence and development of breast cancer and might be able to predict treatment efficacy on the disease.[26] In the present study, we detected IGF-1R expression pattern in HCC tissues, as well as its association with clinical characteristics of the patients. IGF-1R was upregulated in HCC tissues compared to adjacent normal ones. Then we found that IGF-1R expression was significantly correlated with TNM stage and lymph node metastasis. The upregulation of IGF-1 predicted aggressive progression in HCC. Yue et al reported that the inhibition of IGF-1R could suppress HCC cell proliferation and promote apoptosis in vitro.[27] In addition, IGF-1R might contribute to HCC progression through enhancing the transcriptions of HCV.[28] IGF-1R might act as an oncogene in HCC.

Prognostic value of IGF-1R in HCC was also investigated in our study. Kaplan–Meier analysis showed that patients with HCC with high IGF-1R expression had poor overall survival. Multivariate analysis revealed that IGF-1R expression was an independent prognostic factor for HCC. Elevated expression of IGF-1R predicted unsatisfactory outcomes for patients with HCC. In addition to HCC, prognostic value of IGF-1R was also reported in other cancers, such as nonsmall cell lung cancer,[10] prostate cancer,[29] etc. The present study might be the 1st one to explore prognostic significance of IGF-1R in HCC.

Despite those encouraging results, several limitations in the present study should be stated. First, the sample size was relatively small that might reduce statistical power of our study. Second, the levels of IGF-1R exhibited minor statistically significant difference between HCC and noncancerous tissues, which might limit its clinical applications. Third, only activated IGF-1R protein played functional roles in tumorigenesis. The activity and function of the protein are dependent on its expression level and structure. In our study, we only demonstrated that mRNA and protein levels of IGF-1R were high in HCC tissues, but whether all IGF-1R protein was activated remained unclear. Further protein structure analyses should be performed to ascertain the activity of IGF-1R protein in HCC. In addition, cell experiments and animal models are required to state oncogenic mechanisms of IGF-1R in HCC.

In conclusion, IGF-1R expression is increased in HCC tissues, and influenced by some clinical factors. Taken together, IGF-1R may be a promising prognostic biomarker for HCC.

Author contributions

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Investigation: Tiejun Li.
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Project administration: Tiejun Li.
Resources: Tiejun Li.
Software: Tiejun Li.
Supervision: Tiejun Li.
Validation: Tiejun Li.
Visualization: Tiejun Li.
Writing – original draft: Bao Lei, Wei Chai, Ruhai Liu, Tiejun Li.
Writing – review & editing: Bao Lei, Wei Chai, Ruhai Liu, Tiejun Li.

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