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Study on the Expression, Prognosis, and Clinical Features of HOXA6 in Liver Cancer

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Abstract: *Objective:* To study and analyze the expression level of homeobox A6 (HOXA6) in patients with liver cancer and its correlation with prognosis. *Methods:* From January 2020 to July 2021, 43 patients with liver cancer who underwent surgery without prior radiotherapy or chemotherapy were selected. Liver cancer tissues and adjacent tissues were collected, and the expression levels of HOXA6 mRNA and protein were measured using RT-qPCR and Western blot. The expression level of HOXA6 in tumor tissues (without radiotherapy and chemotherapy) was compared with that in adjacent tissues. Additionally, the prognosis and clinical characteristics of patients with low HOXA6 expression were compared to those with high HOXA6 expression, and the factors influencing high HOXA6 expression in liver cancer patients were analyzed. *Results:* HOXA6 mRNA and protein expression levels in tumor tissues were significantly higher than in adjacent tissues ($P < 0.05$). There was no significant difference in the 1-year and 2-year survival rates between the high HOXA6 expression group and the low HOXA6 expression group ($P > 0.05$). However, the 3-year survival rate was lower in the high HOXA6 expression group compared to the low HOXA6 expression group ($P < 0.05$). There were no statistically significant differences between the two groups in terms of gender, age, tumor diameter, alpha-fetoprotein levels, or hepatitis B virus DNA levels ($P > 0.05$). However, significant differences were found in the number of tumor lesions, degree of differentiation, and the proportion of tumor metastasis between the two groups ($P < 0.05$). Multivariate logistic regression analysis revealed that the number of tumor lesions, degree of differentiation, and tumor metastasis were influencing factors for high HOXA6 expression in liver cancer patients ($P < 0.05$). *Conclusion:* HOXA6 expression levels are abnormally elevated in liver cancer patients, and higher HOXA6 expression is associated with a worse 3-year survival rate. The factors influencing HOXA6 expression include the number of tumor lesions, degree of differentiation, and tumor metastasis.

Keywords: Liver cancer; HOXA6; Expression level; Clinical features; Influencing factors

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1. Introduction

Hepatocellular carcinoma (HCC) is a malignant tumor that occurs in hepatocytes or intrahepatic bile duct epithelial cells. It arises from multiple factors, including hepatitis virus, alcohol, high-fat diet, cirrhosis, and environmental influences. In the early stages, clinical symptoms are not specific, while in the middle and late stages, symptoms such as wasting, fatigue, poor appetite, abdominal distension, and hepatic pain become

more prominent, leading to a low survival rate among patients. Early diagnosis is crucial in enabling timely interventions. Analyzing the gene expression profiles of cancer cells plays a significant role in cancer diagnosis and treatment.

HOX genes are essential regulators in vertebrate embryonic development and organ formation, with a role in regulating transcription [1,2]. Homeobox A6 (*HOXA6*), an important member of the HOX family, not only encodes and regulates gene expression but also governs transcription factors involved in cell differentiation. It plays a key role in cell differentiation, proliferation, and invasion [3]. Studies have shown that HOXA6 is implicated in glioma and other cancer types, with overexpression observed in tumor tissues of cancer patients, which can promote tumor cell proliferation [4]. However, the expression of HOXA6 in liver cancer and its impact on prognosis remains unclear. Identifying tumor markers related to liver cancer development and prognosis is crucial for understanding its pathogenesis, guiding treatment plans, and evaluating patient outcomes.

In this study, 43 liver cancer patients were selected, and the diagnosis and treatment were conducted at our hospital between January 2020 and July 2021. RT-qPCR and Western blot were employed to detect the expression levels of HOXA6 mRNA and protein, aiming to analyze the expression of HOXA6 in liver cancer and its correlation with patient prognosis.

2. Materials and methods

2.1. General information

A total of 43 patients with liver cancer were selected for the study. The male-to-female ratio was 24:19, with a mean age of 48.36 ± 3.26 years. The study protocol was approved by the Medical Ethics Committee.

Inclusion criteria: (1) Liver cancer was pathologically confirmed, meeting the diagnostic criteria outlined in the Code for Diagnosis and Treatment of Primary Liver Cancer (2019 Edition) [5]; (2) Patients had surgical indications and voluntarily underwent hepatocellular carcinoma resection; (3) Patients were fully informed about the study protocol; (4) Patients cooperated with all required examinations.

Exclusion criteria: (1) Patients who received immunotherapy, radiotherapy, or chemotherapy before surgery; (2) Patients with other malignant wasting diseases; (3) Patients with incomplete clinical or follow-up data; (4) Patients with inflammation in adjacent tissues; (5) Patients with uncontrolled infectious lesions.

2.2. Methods

2.2.1. Immunohistochemistry of paraffin-embedded tissue

Liver cancer tissues and adjacent normal tissues were collected, fixed in formalin, and embedded in paraffin. After sectioning, the samples were dewaxed, and hydrated, and antigen retrieval was performed using sodium citrate buffer (boiling for 15 minutes). After cooling to room temperature, the samples were incubated with 3% hydrogen peroxide for 15 minutes to remove endogenous peroxidase, followed by sealing with goat serum for 40 minutes. The primary antibody (1:150) was incubated overnight at 4°C and then rewarmed. The secondary antibody was incubated at 37°C for 1 hour. Following color development using DAB, the tissues were restained with hematoxylin, dehydrated, and sealed. Scoring of the staining depth was independently conducted by two pathologists. A score of 2–3 indicated high HOXA6 expression, while a score of 0–1 indicated low HOXA6 expression.

2.2.2. RT-qPCR method

Total RNA was extracted from liver cancer tissues and adjacent tissues using the Trizol RNA extraction

kit. cDNA was synthesized using a reverse transcription kit. After reverse transcription into cDNA, qRT-PCR was performed. HOXA6 primers: upstream 5'-TacACGCGCTACCagAC-3', downstream 5'-GCGTGGAATTGATGAGCTTGTTT-3'. The reaction system was as follows: upstream and downstream primers (0.5 μL each), 10× amplification buffer and dNTP (2 μL total), 0.2 μg template DNA, Taq DNA polymerase (0.2 μL), Mg^{2+} (1.5 μL, 1.5 mmol/L), and 20 μL double-distilled water. Reaction conditions: 94°C for 10 minutes, followed by 30 cycles of 94°C for 15 seconds, and annealing/extension at 60°C for 32 seconds. The CT values for each sample were calculated, and the expression level of HOXA6 mRNA was determined using the $2^{-\Delta\Delta CT}$ method.

2.2.3 Western blot

Total protein was extracted from liver cancer and adjacent tissues using the RIPA method, and protein concentration was determined using the BCA method. A 20 μg protein sample was subjected to SDS-PAGE, transferred to a PVDF membrane, and blocked overnight with 50g/L skim milk powder at 4°C. The primary antibody was incubated overnight at 4°C. After incubation with the secondary antibody at room temperature for 2 hours, strip scanning was performed following development with an ECL luminescence kit. ImageJ software was used for analysis.

2.3. Observation indicators

(1) The prognosis of patients with low HOXA6 expression was compared with that of patients with high HOXA6 expression. The 1-year, 2-year, and 3-year survival rates were compared between the two groups.

(2) Clinical characteristics, including gender, age, number of tumor lesions, tumor diameter, degree of differentiation, tumor metastasis, and the expression levels of alpha-fetoprotein (AFP) and hepatitis B virus (HBV DNA), were compared between the low and high HOXA6 expression groups.

(3) Factors influencing high HOXA6 expression in liver cancer patients were analyzed.

2.4. Statistical analysis

SPSS 25.0 software was used for data analysis. The results were expressed as [*n* (%)] and mean ± standard deviation (SD). Factors influencing high HOXA6 expression in liver cancer patients were analyzed using χ^2 test data and multivariate logistic regression. A *P*-value < 0.05 indicated statistical significance.

3. Results

3.1. HOXA6 expression levels in tumor tissues versus adjacent tissues

Compared to adjacent non-cancerous tissues, both HOXA6 mRNA and protein expression levels were significantly higher in tumor tissues of liver cancer patients, with statistical significance ($P < 0.05$). See **Table 1**.

Table 1. Comparison of HOXA6 expression levels between tumor tissues and adjacent tissues (mean ± SD)

Groups	Number of cases	HOXA6 mRNA	HOXA6 protein
Adjacent tissues	43	157.36 ± 20.13	4.25 ± 1.03
Tumor tissues	43	183.19 ± 21.04	6.37 ± 0.98
t		5.817	9.778
P		0.000	0.000

3.2. Prognosis comparison between low and high HOXA6 expression groups

No significant differences were observed in 1-year and 2-year survival rates between the low and high HOXA6 expression groups ($P > 0.05$). However, the 3-year survival rate was significantly lower in the high HOXA6 expression group compared to the low expression group ($P < 0.05$). See **Table 2**.

Table 2. Prognosis comparison between low and high HOXA6 expression groups [*n* (%)]

Groups	Number of cases	1-year survival rate	2-year survival rate
Low HOXA6 expression group	20	18 (90.00)	15 (75.00)
High HOXA6 expression group	23	21 (91.30)	13 (56.52)
χ^2		0.022	1.608
P		0.883	0.205

3.3. Comparison of clinical features between low and high HOXA6 expression groups

There were no statistically significant differences in gender, age, tumor diameter, AFP, or HBV DNA levels between the high and low HOXA6 expression groups ($P > 0.05$). However, statistically significant differences were found in the number of tumor lesions, degree of differentiation, and tumor metastasis between the two groups ($P < 0.05$). See **Table 3**.

Table 3. Comparison of clinical features between low and high HOXA6 expression groups [*n* (%)]

Outcome measures		HOXA6 high expression group ($n = 23$)	HOXA6 low expression group ($n = 20$)	χ^2	*w*
Gender	Male	13 (56.52)	11 (55.00)	0.010	0.920
	Female	10 (43.48)	9 (45.00)		
Age (years)	< 60	14 (60.87)	10 (50.00)	0.513	0.474
	≥ 60	9 (39.13)	10 (50.00)		
Number of tumor lesions	Single	14 (60.87)	5 (25.00)	5.581	0.018
	Multiple	9 (39.13)	15 (75.00)		
Tumor diameter (cm)	≤ 5	9 (39.13)	8 (40.00)	0.003	0.954
	> 5	14 (60.87)	12 (60.00)		
Degree of differentiation	Low differentiation	5 (21.74)	14 (70.00)	10.103	0.001
	Medium-high differentiation	18 (78.26)	6 (30.00)		
Tumor lesion metastasis	Yes	6 (26.09)	12 (60.00)	5.055	0.025
	No	17 (73.91)	8 (40.00)		
AFP (ng/mL)	≤ 400	10 (43.48)	11 (55.00)	0.568	0.451
	> 400	13 (56.52)	9 (45.00)		
HBV DNA (IU/mL)	≤ 400	6 (26.09)	9 (45.00)	01.685	0.194
	> 1000	17 (73.91)	11 (55.00)		

3.4. Factors influencing high HOXA6 expression in liver cancer patients

Using variables that showed statistical significance in univariate analysis, a multivariate logistic regression was performed. The results indicated that the number of tumor lesions, degree of differentiation, and tumor metastasis were factors influencing the high expression of HOXA6 in liver cancer patients ($P < 0.05$). See **Table 4**.

Table 4. Influencing factors of HOXA6 expression in liver cancer patients

Influencing factors	β	SE	Wald	*P*	OR	95% CI
Number of tumor lesions	0.711	0.322	4.887	0.027	2.037	1.084–3.828
Degree of differentiation	0.631	0.315	4.004	0.045	1.879	1.013–3.485
Tumor focus metastasis	1.046	0.510	4.211	0.040	2.845	1.048–7.723

4. Discussion

Hepatocellular carcinoma is among the most prevalent malignant tumors worldwide, with hepatocellular carcinoma being the most common form of primary liver cancer. Its clinical course is highly variable, and its pathological process mirrors embryonic mechanisms. The *HOX* gene plays a crucial role in early liver development, and mutations in *HOX* genes may significantly impact the progression of liver cancer [6]. The *HOX* gene family is largely characterized by gene clusters, with the *HOXA* gene cluster located on chromosome 7. This cluster contains six long-chain non-coding RNAs and 11 protein-coding genes, which are essential in the development of vertebrate organs, limbs, axons, and the central nervous system. Abnormal gene regulation may play a pivotal role in the onset of malignant tumors [7,8]. The *HOX* gene can act as either an oncogene or a tumor suppressor, and its expression level is prone to alterations in cancers such as colon cancer, lung cancer, brain tumors, breast cancer, leukemia, and others [9,10].

HOXA6 encodes transcription factors and plays a regulatory role in cell differentiation and gene expression. However, its role varies across different cancers [11]. Research has shown that *HOXA6* is hypermethylated in certain malignant tumors, and its expression is downregulated in breast cancer tissues [12,13]. In this study, HOXA6 mRNA and protein expression levels in liver cancer tumor tissues were significantly higher than in adjacent non-cancerous tissues ($P < 0.05$), suggesting that HOXA6 plays an important role in the onset and progression of liver cancer. Patients with high HOXA6 expression levels had a lower 3-year survival rate compared to those with low HOXA6 expression levels ($P < 0.05$). Detecting HOXA6 expression levels could provide valuable insights for clinical evaluation of patient prognosis and survival prediction.

The HOX family plays an important role in tumor regulation. Studies have demonstrated that HOXA6 is highly expressed in various malignant tumors, correlating with cell invasion, proliferation, and chemoresistance [11]. For instance, HOXA6 has been shown to promote the proliferation, invasion, and metastasis of colorectal cancer cells [3,14]. In this study, multivariate logistic regression analysis indicated that the number of tumor lesions, the degree of differentiation, and tumor metastasis were the key factors influencing the high expression of HOXA6 in liver cancer patients ($P < 0.05$). Clinically, HOXA6 expression in liver cancer patients can be assessed by observing the number of tumor lesions, the degree of tumor differentiation, and the presence of metastasis [15].

In conclusion, HOXA6 expression is abnormally elevated in liver cancer patients and is correlated with the 3-year survival rate. Factors influencing HOXA6 expression include the number of tumor lesions, differentiation degree, and metastasis. Detecting HOXA6 can help elucidate the mechanisms of tumor development and progression. Additionally, it can aid in assessing disease severity and guide clinical diagnosis of liver cancer. Therefore, clinical detection of HOXA6 expression should be enhanced, and patient prognosis should be evaluated based on HOXA6 expression levels to adjust treatment strategies accordingly.

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Disclosure statement

The author declares no conflict of interest.

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