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Expressions and correlation analysis of HIF-1α, survivin and VEGF in patients with hepatocarcinoma

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Abstract. – OBJECTIVE: To investigate the expressions of HIF-1α, surviving, and VEGF in patients with hepatocarcinoma as well as the correlation analysis among them.

PATIENTS AND METHODS: 65 patients, who were admitted to our hospital and diagnosed as hepatocarcinoma from January 2014 to October 2015, were selected as hepatocarcinoma group, while 50 healthy cases that do not have hepatocarcinoma were selected as normal control group. The expression levels of HIF-1α, surviving, and VEGF in hepatocarcinoma tissues of hepatocarcinoma group and normal liver tissues of control group were detected by immunohistochemical (SP) staining method; then, the correlation among them was explored. The expression levels of HIF-1α, surviving, and VEGF protein in hepatocarcinoma tissues and corresponding normal tissues were detected by Western blot.

RESULTS: The positive expression rate of HIF-1α, surviving, and VEGF in hepatocarcinoma tissues of hepatocarcinoma group was respectively 46.2%, 55.4%, and 61.5%, significantly higher than that in cancer adjacent normal liver tissues of control group which was 2%, 2%, and 2%, and the differences were statistically significant ($p<0.05$). The expressions of HIF-1α, surviving, and VEGF in hepatocarcinoma tissues of patients with hepatocarcinoma were correlated with clinical stage, tumor differentiation degree and extrahepatic metastasis ($p<0.05$), but were not related to gender and tumor size ($p>0.05$). By Spearman rank correlation analysis, it could be seen that HIF-1α expression was positively correlated with VEGF protein expression in hepatocarcinoma tissues ($r=0.683$, $p<0.05$). Survivin expression was positively correlated with VEGF protein expression ($r=0.717$, $p<0.05$). There was no significant correlation between HIF-1α expression and survivin expression ($p>0.05$). The relative quantitative value of HIF-1α, surviving, and VEGF in hepatocarcinoma tissues of hepatocarcinoma group was respectively 3.04±0.23, 2.26±0.31, and 2.57±0.36, significantly higher than that in cancer adjacent liver tissues of control group which was 1.07±0.17, 1.31±0.27, and 1.42±0.43, and the differences were statistically significant ($p<0.05$). From Western blot electrophoresis scanning, it could be seen that the expressions of HIF-1α, surviving, and VEGF in hepatocarcinoma tissues were higher than those in cancer adjacent normal liver tissues.

CONCLUSIONS: The expressions of HIF-1α, surviving, and VEGF played important roles in the occurrence, invasion, and metastasis of hepatocarcinoma. In hepatocarcinoma tissues, HIF-1α, and survivin protein expression was positively correlated with VEGF expression, but survivin protein was not related to HIF-1α expression, which indicated that HIF-1α and survivin may inhibit the apoptosis of hepatocarcinoma cells and promote tumor angiogenesis by up-regulating the expression of VEGF protein, thus accelerating the occurrence and development of hepatocarcinoma.

Key Words
HIF-1α, Survivin, VEGF, Hepatocarcinoma.

Introduction

The early stage of hepatocarcinoma may not be manifested by typical symptoms but may appear insidiously, so many definitely diagnosed patients are in the middle and late stage[1]. Previous studies[2,3] have indicated that, on one hand, VHL, p53, HFE, nm23-H1 and other multiple gene mutations can induce hepatocarcinoma; on the other hand, it is closely related to the occurrence, development, treatment, and prognosis of hepatocarcinoma. Therefore, from the perspective of

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molecular biology to actively explore the hepatocarcinoma-related gene is of great significance, which will be conducive to the early diagnosis and early treatment of hepatocarcinoma.

Vascular endothelial growth factor (VEGF) is a kind of proangiogenic factor, which can regulate the migration and proliferation of endothelial cells in the human blood vessels. Hypoxia-inducible factor (HIF-1α) can regulate the expression of multiple target genes in human body, such as the expression of vascular endothelial growth factor (VEGF)[4]. The survivin protein, which belongs to a kind of tumor inhibitor of apoptosis in recent years, is also actively involved in the formation of human blood vessels. At present, some studies[5-10] have reported the expressions of HIF-1α, surviving, and VEGF in bladder cancer, esophageal cancer, colonic adenocarcinoma, and other tumor tissues, but the research on their expressions and clinical significance in hepatocarcinoma is still few. This study aims to investigate the expressions of HIF-1α, surviving, and VEGF in hepatocarcinoma tissues and corresponding normal tissues, and to explore the mechanism of proliferation and apoptosis of hepatocarcinoma cells, in order to provide a new way for early diagnosis, treatment, and prognosis of hepatocarcinoma.

Patients and Methods

Patients

65 patients, who were admitted to our hospital and diagnosed as hepatocarcinoma from January 2014 to October 2015, were selected as hepatocarcinoma group. In the group, there were 38 cases of males and 27 cases of females. The age of patients ranged from 25 to 77 years old, with an average of (46.5±2.8). According to the clinicopathologic features, all patients were divided as follows. Clinical stage: 40 cases in Stage I+II, 25 cases in Stage III; Tumor cell differentiation: 42 cases of Grade I-II, 23 cases of Grade III-IV; Extrahepatic metastasis: 30 cases of metastasis, 35 cases of no metastasis; Tumor diameter: 27 cases of less than 5 cm, 38 cases of more than or equal to 5 cm. No intervention was performed before the operation, and the diagnosis was confirmed by pathological examination after the operation. Meanwhile, 50 healthy cases that do not have hepatocarcinoma were selected as normal control group. In the group, there were 32 cases of males and 18 cases of females. The age of research subjects ranged from 26 to 73 years old, with an average of (45.8±2.5). The study was approved by the Ethics Committee of the 4th Affiliated Hospital of Baotou Medical College. Signed written informed consents were obtained from all participants before the study.

Methods

The expression levels of HIF-1α, surviving, and VEGF in normal liver tissues and hepatocarcinoma tissues were detected by immunohistochemical (SP) staining method. The staining steps were operated in accordance with EnVision kit instructions of rabbit anti-human HIF-1α antibody, rabbit anti-human survivin antibody, and rabbit anti-human VEGF antibody, respectively (all purchased from Wanjiang Biotechnology Co., Ltd., Shanghai, China), and the staining results were observed.

The expression levels of HIF-1α, surviving, and VEGF protein in hepatocarcinoma tissues and corresponding normal tissues were detected by Western blot.

Evaluation Criterion

A) The combination of semi-quantitative scoring method and Berry grading method[11]: the positive expressions of HIF-1α, surviving, and VEGF were cytoplasmic staining, and according to the positive cell rate and staining intensity of positive cells, the scoring was performed, respectively. According to the degree of staining to determine the level of expression: staining was the same as negative control, 0 points; staining showed light yellow, 1 point; staining showed tan, 2 points; staining showed sepia, 3 points. According to the proportion of positive cells in the observed cells, the scoring was performed as follows: the number of positive cells ≤ 10%, 1 point; 11% < the number of positive cells < 50%, 2 points; 51% < the number of positive cells < 75%, 3 points; the number of positive cells > 75%, 4 points. The product of two item scores: 0-3 points, (-); 4-5 points, (+); 6-7 points, (++); 8 points or above, (+++). The final score less than or equal to 3 points was negative, and the score over than 3 points was positive.

B) Judgment method of Western blot result: thin layer chromatogram scanner was used to perform gray scale scanning for photographic film, and the gray value of each product was analyzed. Subsequently, each gray value was compared to the gray value of internal reference β-actin which regarded as an indicator to calculate the expression level of the samples.

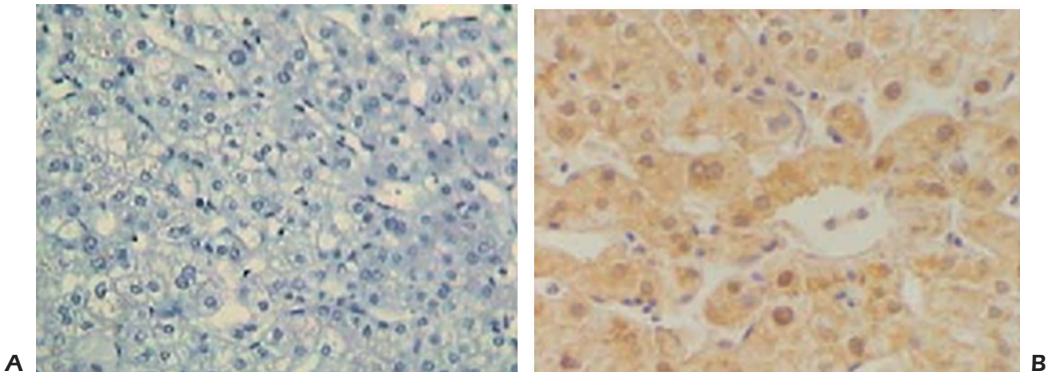

Figure 1. Expression of HIF-1α. **A**, Positive expression of HIF-1α in hepatocarcinoma tissues (400×). **B**, Negative expression of HIF-1α in hepatocarcinoma adjacent tissues (400×).

Statistical Analysis

SPSS20.0 (IBM, SPSS Statistics for Windows, Armonk, NY, USA) software was used for statistical analysis. The Chi-square test was used in the comparison of the positive rate among groups; the *t*-test was used in comparison among groups and Spearman rank correlation test was used for correlation analysis. α=0.05 was regarded as inspection level.

Results

Expressions of HIF-1α, Surviving, and VEGF in Hepatocarcinoma Tissues

The positive expression products of HIF-1α, surviving, and VEGF were brown yellow particles, mainly located in the cytoplasm (Figures 1-3). The positive expression rate of HIF-1α, surviving, and VEGF in hepatocarcinoma tissues of hepatocarcinoma group was respectively 46.2%, 55.4%, and 61.5%, significantly higher than that in cancer adjacent normal liver tissues of control group which was 2%, 2%, and 2%, and the differences were statistically significant ($p<0.05$) (Tables I-III).

Relationship Between HIF-1α, Survivin, VEGF, and Clinicopathologic Features of Patients with Hepatocarcinoma

The expressions of HIF-1α, surviving, and VEGF in hepatocarcinoma tissues of patients with hepatocarcinoma were correlated with clinical stage, tumor differentiation degree and extrahepatic metastasis ($p<0.05$), but were not related to gender and tumor size ($p>0.05$) (Table IV).

Correlation Analysis on the Expressions of HIF-1α, Surviving, and VEGF

By Spearman rank correlation analysis, it could be seen that HIF-1α expression was positively correlated with VEGF protein expression in hepatocarcinoma tissues (r=0.683, $p<0.05$) (Fig-

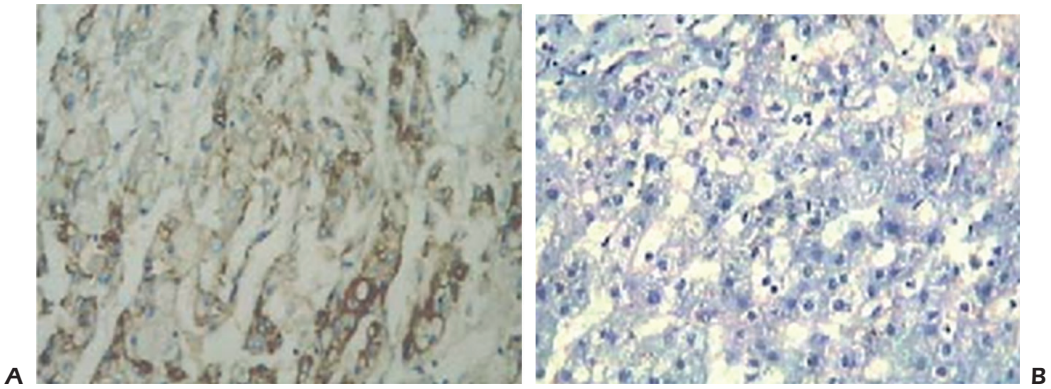

Figure 2. Expression of survivin. **A**, Positive expression of survivin in hepatocarcinoma tissues (400×). **B**, Negative expression of survivin in hepatocarcinoma adjacent tissues (400×).

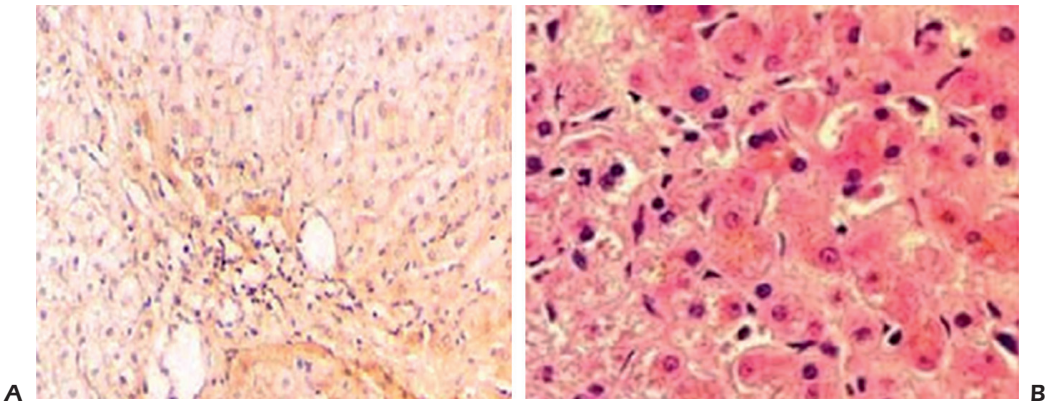

Figure 3. Expression of VEGF. **A**, Positive expression of VEGF in hepatocarcinoma tissues (400×). **B**, Negative expression of VEGF in hepatocarcinoma adjacent tissues (400×).

ure 4). Survivin expression was positively correlated with VEGF protein expression (r=0.717, $p<0.05$) (Figure 5). There was no significant correlation between HIF-1α expression and survivin expression ($p>0.05$).

Analysis of Western Blot Results

The relative quantitative value of HIF-1α, surviving, and VEGF in hepatocarcinoma tissues of hepatocarcinoma group was respectively 3.04±0.23, 2.26±0.31, and 2.57±0.36, significantly higher than that in cancer adjacent liver tissues of control group which was 1.07±0.17, 1.31±0.27, and 1.42±0.43, and the differences were statistically significant ($p<0.05$) (Table V).

From the part of Western blot electrophoresis scanning, it could be seen that the expressions of HIF-1α, surviving, and VEGF in hepatocarcinoma tissues were higher than those in cancer adjacent normal liver tissues (Figures 6-8).

Table I. Expressions of HIF-1α in the hepatocarcinoma tissues and cancer adjacent normal tissues (case).

Groups	No.	+	++	+++	Positive rate (%)	–
Hepatocarcinoma group	65	7	10	13	46.2	35
Control group	50	1	0	0	2	49
p						<0.05

Table II. Expressions of survivin in hepatocarcinoma tissues and cancer adjacent normal tissues (case).

Groups	No.	+	++	+++	Positive rate (%)	–
Hepatocarcinoma group	65	9	12	15	55.4	29
Control group	50	1	0	0	2	49
p						<0.05

Table III. Expressions of VEGF in hepatocarcinoma tissues and cancer adjacent normal tissues (case).

Groups	No.	+	++	+++	Positive rate (%)	–
Hepatocarcinoma group	65	11	12	17	61.5	25
Control group	50	1	0	0	2	49
p						<0.05

Table IV. Relationship between HIF-1α, survivin, VEGF and clinicopathologic features of patients with hepatocarcinoma.

Clinicopathologic features	No.	Positive cases of HIF-1α	*p*	Positive cases of survivin	*p*	Positive cases of VEGF	*p*
Gender							
Male	38	16	>0.05	18	>0.05	21	>0.05
Female	27	14		18		19	
Tumor size (cm)	27	15	>0.05	17		20	
< 5			>0.05		>0.05		
≥ 5	38	15		19		20	
Clinical stage							
I+II	40	11	<0.05	13	<0.05	15	<0.05
III	25	19		23		25	
Differentiation degree							
I-II	42	7	<0.05	13	<0.05	17	<0.05
III-IV	23	23		23		23	
Extrahepatic metastasis							
Yes	30	22	<0.05	29	<0.05	30	<0.05
No	35	8		7		10	

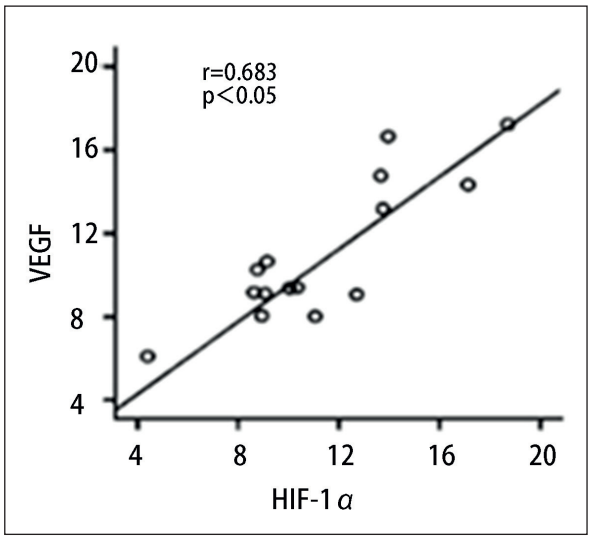

Figure 4. Correlation between HIF-1α expression and VEGF protein expression.

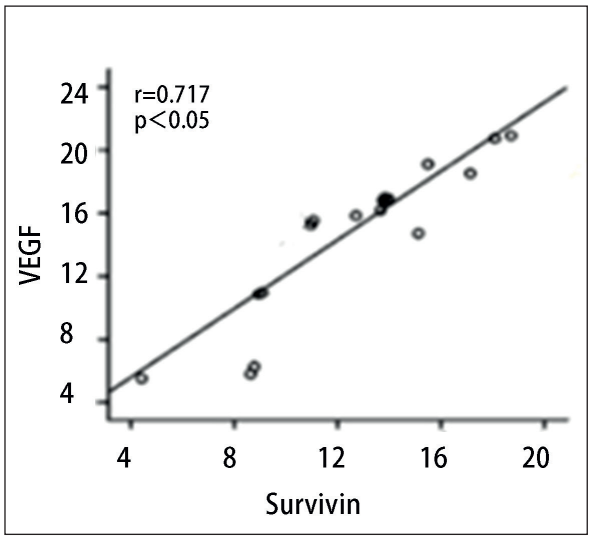

Figure 5. Correlation between survivin expression and VEGF protein expression.

Table V. The relative quantitative values of expressions of HIF-1α, survivin and VEGF protein in different liver tissues.

Groups	HIF-1α	Survivin	VEGF
Hepatocarcinoma group	3.04±0.23	2.26±0.31	2.57±0.36
Control group	1.07±0.17	1.31±0.27	1.42±0.43
p	<0.05	<0.05	<0.05

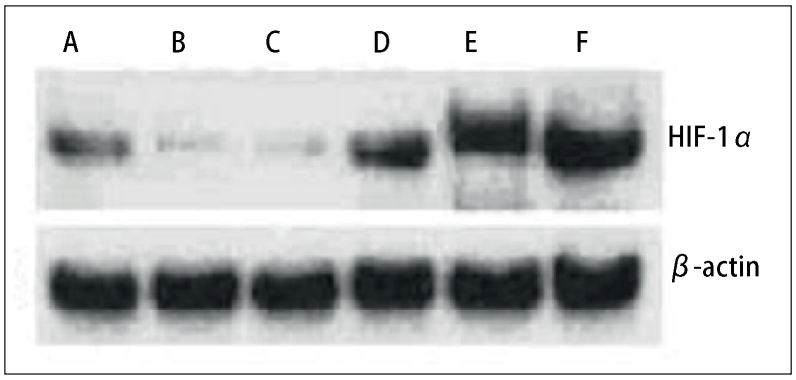

Figure 6. Electrophoresis scanning of HIF-1α. **A**, **B** and **C** were the hepatocarcinoma tissues; **D**, **E** and **F** were the cancer adjacent liver tissues.

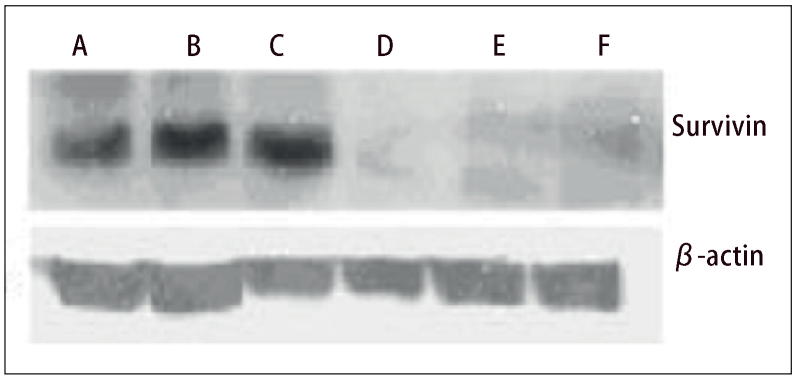

Figure 7. Electrophoresis scanning of surviving. **A**, **B** and **C** were the hepatocarcinoma tissues; **D**, **E** and **F** were the cancer adjacent liver tissues.

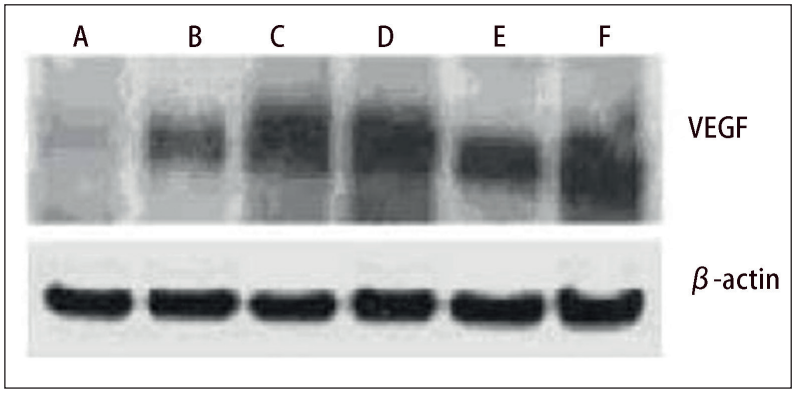

Figure 8. Electrophoresis scanning of VEGF. **A**, **B** and **C** were the cancer adjacent liver tissues; **D**, **E** and **F** were the hepatocarcinoma tissues.

Discussion

Some studies[12-14] demonstrated that HIF-1α was over-expressed in pancreatic cancer, esophageal cancer, non-small cell lung cancer, and other cancer tissues, and also found the expressions of HIF-1α in tumor necrosis tissues and tumor-infiltrating edge were significantly more, but no expression of HIF-1α was found in normal tissues. According to the detection of HIF-1α in cervical cancer tissues, some foreign scholars found that HIF-1α expression located in the cytoplasm or nucleus, and it was closely related to the up-regulation of VEGF and the increase of microvascular density (MVD)[15-18].

As one of the most important cell factors in the new vessels induced by cancer cells, VEGF plays a significant role in the occurrence, evolution, and metastasis of cancer cells. VEGF gene expression has been proved to be affected and regulated by various factors, such as cytokines, tumor suppressor gene product, ischemia, hypoxia, etc. (19-21). VEGF is one of the most important regulatory genes of HIF-1α. Survivin gene belongs to a new kind of apoptosis inhibitor gene, which can be expressed in a variety of human cancer tissues.

The expression levels of HIF-1α, surviving, and VEGF in hepatocarcinoma tissues and nor-

mal tissues were detected by immunohistochemical (SP) staining method. The results indicated that the positive expression rate of HIF-1α, surviving, and VEGF in hepatocarcinoma tissues of hepatocarcinoma group was respectively 46.2%, 55.4%, and 61.5%, significantly higher than that in cancer adjacent normal liver tissues of control group which was 2%, 2%, and 2%, and the differences were statistically significant ($p<0.05$). The expressions of HIF-1α, surviving, and VEGF in hepatocarcinoma tissues of patients with hepatocarcinoma were correlated with clinical stage, tumor differentiation degree and extrahepatic metastasis ($p<0.05$), but were not related to gender and tumor size ($p>0.05$). Meanwhile, from the detection result of Western blot, it could be seen that the expressions of HIF-1α, surviving, and VEGF in hepatocarcinoma tissues were higher than those in cancer adjacent normal liver tissues. The results suggested that HIF-1α, surviving, and VEGF protein have jointly participated in the occurrence and development of hepatocarcinoma.

A variety of tumor cells were able to secrete VEGF, and at the same time, the VEGF receptors on the endothelial cells of the tumor tissues also showed the characteristics of strong expression[22]. The combination of VEGF and receptors of vascular endothelial cells in cancer tissues can promote the proliferation of tumor vascular endothelial cells, stimulate the increase of vascular permeability, thus providing the material basis for the growth and infiltration, evolution and metastasis of cancer cells.

Excessive proliferation of cancer cells and the relative lack of blood vessel growth will induce local hypoxia in newborn tumor cells, while hypoxia will further stimulate the expression of VEGF and stimulate the further proliferation of blood vessels, which will create conditions for the further growth of cancer cells. Some studies[23] showed that the expression of VEGF was increased in the surrounding cells of the cancer tissue necrosis region, and the expression of VEGF increased with the increase of the severity of hypoxia in cancer cells. Previous investigations[24,25] have indicated that HIF-1α was an important link in the signal transduction pathway of regulating VEGF under hypoxic condition; HIF-1 could not only enhance the mRNA stability of VEGF, but also improve the transcriptional activity of VEGF. The up-regulation of VEGF expression was able to further stimulate the expression of survivin, so as to resist the apoptosis caused by hypoxia. In this study, by Spearman rank correlation analysis, it could be seen that HIF-1α expression was positively correlated with VEGF protein expression in hepatocarcinoma tissues ($r=0.683$, $p<0.05$). Survivin expression was positively correlated with VEGF protein expression ($r=0.717$, $p<0.05$). There was no significant correlation between HIF-1α expression and survivin expression ($p>0.05$). The results indicated that HIF-1α and survivin may inhibit the apoptosis of hepatocarcinoma cells and promote tumor angiogenesis by up-regulating the expression of VEGF protein, thus accelerating the occurrence and development of hepatocarcinoma.

Conclusions

We showed that the expressions of HIF-1α, surviving, and VEGF played important roles in the occurrence, invasion, and metastasis of hepatocarcinoma. In hepatocarcinoma tissues, HIF-1α, and survivin protein expression was positively correlated with VEGF expression, but survivin protein was not related to HIF-1α expression, which indicated that HIF-1α and survivin may inhibit the apoptosis of hepatocarcinoma cells and promote tumor angiogenesis by up-regulating the expression of VEGF protein, thus accelerating the occurrence and development of hepatocarcinoma.

Conflict of interest

The authors declare no Conflict of interest.

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