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Correlation of serum GP73, SOD and GPC3 contents with cell proliferation and angiogenesis in liver cancer lesion

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ABSTRACT

Objective: To study the correlation of serum GP73, SOD and GPC3 contents with cell proliferation and angiogenesis in liver cancer lesion. **Methods:** Patients who were diagnosed with primary liver cancer in Jianghan Oilfield General Hospital between June 2014 and February 2017 were selected as liver cancer group, and healthy subjects who received physical examination in Jianghan Oilfield General Hospital during the same period were selected as control group. Serum was collected from two groups of subjects to determine the contents of GP73, SOD and GPC3; liver cancer lesion and adjacent lesion were collected from liver cancer group to determine the expression of cell proliferation molecules and angiogenesis molecules. **Results:** Serum GP73 and GPC3 levels of liver cancer group were obviously higher than those of control group while SOD content was obviously lower than that of control group; DNMT3B, STC2, SIRT6, LETM1, EphB4, SULT2B1, HIF-1 , VEGF, Ang-2, HGF and TGF- β 1 protein expression levels in liver cancer lesion of liver cancer group were significantly higher than those in adjacent lesion; DNMT3B, STC2, SIRT6, LETM1, EphB4, SULT2B1, HIF-1 , VEGF, Ang-2, HGF and TGF- β 1 protein expression levels in liver cancer lesion of liver cancer group were positively correlated with serum GP73 and GPC3 levels, and negatively correlated with serum SOD level. **Conclusion:** The changes of GP73, SOD and GPC3 levels in the serum of patients with liver cancer are closely related to the cell proliferation and angiogenesis in liver cancer lesion.

1. Introduction

Primary liver cancer is one of the most common malignant tumors of digestive system in China. It is characterized by high degree of malignancy, rapid progression of disease, high recurrence rate and high metastasis rate, and the overall prognosis is poor. Alpha fetoprotein (AFP) is the most common indicator for clinical liver cancer screening at present, but the AFP generation in the body is affected by many factors, and the sensitivity and specificity of AFP alone are not ideal for hepatocellular carcinoma diagnosis[1,2]. Golgi protein 73 (GP73), superoxide dismutase (SOD) and glypican 3 (GPC3) are the liver cancer markers developed in recent years, and their combination can enhance the sensitivity and specificity of liver cancer diagnosis[3−5]. However, it is not clear about the correlation of liver cancer markers GP73, SOD and GPC3 with the malignant degree and malignant biological behavior of liver cancer. The malignant proliferation of cancer cells and the increase of new blood vessels are the important malignant biological behaviors in the development of liver cancer. In the following study, in order to clarify the assessment value of above three liver cancer markers for the malignant biological behavior of liver cancer, we specifically analyzed the correlation of serum GP73, SOD and GPC3 contents with cell proliferation and angiogenesis in liver cancer lesion of patients with liver cancer.

2. Research subjects and methods

2.1 General information of research subjects

Patients who were diagnosed with primary liver cancer in Jianghan Oilfield General Hospital between June 2014 and February 2017 were selected as liver cancer group, all patients were diagnosed by

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liver biopsy and complied with the indications for surgical resection, and a total of 62 cases were enrolled and included 39 male cases and 23 female cases that were 41-65 years old; healthy subjects who received physical examination in Jianghan Oilfield General Hospital during the same period were selected as control group, they were healthy and without history of hepatitis, and a total of 55 cases were enrolled and included 32 male cases and 23 female cases that were 38-62 years old. There was no significant difference in general data between the two groups (P>0.05).

2.2 Research methods

2.2.1 Serum sample collection and index collection

3 mL of peripheral blood was collected from liver cancer group before operation, 3 mL of peripheral blood was collected from control group during physical examination, the blood was centrifuged to get serum, and the kits were used for enzyme-linked immunosorbent assay experiment to determine serum GP73, SOD and GPC3 contents.

2.2.2 Liver cancer lesion and adjacent lesion collection and index detection

Right amount of liver cancer lesion and adjacent lesion were collected after surgical resection, RIPA lysate was used to separate the protein in tissue, and the kits were used for enzyme-linked immunosorbent assay experiment to determine the SULT2B1, HIF-1α, VEGF, Ang-2, HGF and TGF-β1 protein expression in tissue protein specimens.

2.3 Statistical methods

SPSS 22.0 software was used for the t test of the differences between two groups and the Pearson test of the correlation between two measurement data, and P<0.05 indicated that the differences in test results were statistically significant.

3. Results

3.1 Serum GP73, SOD and GPC3 contents in patients with liver cancer

Analysis of serum GP73 (ng/mL), SOD (U/mL) and GPC3 (ng/mL) contents between two groups of subjects was as follows: serum GP73 and GPC3 levels of liver cancer group were obviously higher than those of control group while SOD content was obviously lower than that of control group. Differences in serum GP73, SOD and GPC3 contents were statistically significant between liver cancer group and control group (P<0.05).

3.2 Cell proliferation molecule expression in liver cancer lesions

Analysis of cell proliferation molecules DNMT3B (ng/mL), STC2 (pg/mL), SIRT6 (ng/mL), LETM1 (ng/mL) and EphB4 (pg/mL) expression in liver cancer lesion and adjacent lesion was as follows: DNMT3B, STC2, SIRT6, LETM1 and EphB4 protein expression levels in liver cancer lesion were significantly higher than those in adjacent lesion. Pearson test showed that serum GP73 and GPC3 contents in patients with liver cancer were positively correlated with DNMT3B, STC2, SIRT6, LETM1 and EphB4 protein expression levels in liver cancer lesion, and serum SOD level was negatively correlated with DNMT3B, STC2, SIRT6, LETM1 and EphB4 protein expression levels in liver cancer lesion.

3.3 Angiogenesis molecule expression in liver cancer lesion

Analysis of angiogenesis molecules SULT2B1, HIF-1α, VEGF, Ang-2, HGF and TGF-β1 expression in liver cancer lesion and adjacent lesion was as follows: SULT2B1, HIF-1α, VEGF, Ang-2, HGF and TGF-β1 protein expression levels in liver cancer lesion were significantly higher than those in adjacent lesion. Pearson test showed that serum GP73 and GPC3 contents in patients with liver cancer were positively correlated with SULT2B1, HIF-1α,

Table 1.

Serum GP73, SOD and GPC3 contents in two groups of subjects.

Groups	n	GP73	SOD	GPC3
Liver cancer group	62	251.24±33.93	55.92±8.24	0.89±0.11
Control group	55	62.96±9.51	203.51±33.92	0.27±0.05
t		36.958	29.191	26.482
P		<0.05	<0.05	<0.05

Table 2.

Cell proliferation molecule expression in liver cancer lesion and adjacent lesion.

Tissue origin	n	DNMT3B	STC2	SIRT6	LETM1	EphB4
Liver cancer lesion	62	1.95±0.26	193.66±23.62	3.28±0.55	1.15±0.18	252.93±33.68
Adjacent lesion	62	0.89±0.12	103.25±13.58	1.54±0.24	0.58±0.09	103.52±15.86
t		11.290	9.109	10.298	9.875	14.582
P		<0.05	<0.05	<0.05	<0.05	<0.05

Table 3.

Angiogenesis molecule expression in liver cancer lesion and adjacent lesion.

Tissue origin	*n*	SULT2B1	HIF-1	VEGF	Ang-2	HGF	TGF-β1
Liver cancer lesion	62	1.99±0.25	1.52±0.19	3.28±0.62	231.25±33.95	304.61±42.94	2.26±0.35
Adjacent lesion	62	0.72±0.09	0.80±0.11	1.42±0.18	109.36±14.72	137.58±18.82	1.03±0.18
t		14.576	8.018	13.217	12.598	14.108	12.331
P		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

VEGF, Ang-2, HGF and TGF-β1 protein expression levels in liver cancer lesion, and serum SOD level was negatively correlated with SULT2B1, HIF-1α, VEGF, Ang-2, HGF and TGF-β1 protein expression levels in liver cancer lesion.

4. Discussion

Liver cancer is the malignant tumor of digestive system with high malignant degree and poor prognosis, targeted therapy, surgical resection and other means have been developing in recent years, but the metastasis rate and mortality rate of patients with liver cancer are still high, and the overall outcome is not optimistic. Early screening and diagnosis of disease is an effective means to improve the prognosis of liver cancer patients. Serum tumor marker detection plays an important role in early screening of tumor. GP73, SOD and GPC3 are newly developed liver cancer markers in recent years, and their combination with traditional liver cancer marker AFP can improve the diagnostic sensitivity and specificity of disease. GP73 is a kind of transmembrane protein located in Golgi body, which can maintain the integrity of the Golgi body, and be secreted out of cells during the malignant proliferation of cells[6]; SOD is an intracellular antioxidant enzyme involved in oxygen radical scavenging, and there is generally excessive generation of oxygen free radicals in the malignant tumor cells, which can on the one hand, cause cellular damage, and on the other hand, consume SOD and reduce its content[7]; GPC3 is a heparan sulfate proteoglycan, which can be combined with multiple heparin-binding growth factors and promote the growth of cells[8]. The analysis of the changes in serum levels of above three tumor markers in patients with liver cancer showed that serum GP73 and GPC3 contents of liver cancer group were significantly higher than those of control group whereas SOD content was significantly lower than that of control group. This means that the contents of tumor markers GP73, SOD and GPC3 change dramatically in the occurrence and development of liver cancer, and serum GP73, SOD and GPC3 contents can provide the basis for the diagnosis of liver cancer and the evaluation of its illness. At present, it is still unclear about the evaluation value of GP73, SOD and GPC3 contents for malignant biological behavior in the development of hepatocellular carcinoma. Malignant proliferation is one of the important biological behaviors of hepatocellular carcinoma cells, and the abnormal expression of multiple cell proliferation molecules is closely related to it. DNMT3B is a catalytic enzyme that catalyzes the de novo methylation of genes, which can make tumor suppressor genes methylated and inactivated so as to promote cell proliferation[9]; STC2 is a kind of secreted glycoprotein that can enhance cell proliferation by PKB/ERK pathway[10]; SIRT6 is a Sirtuin family member located in the nucleus, which participates in the development of cell cycle and promotes cell growth[11,12]; LETM1 is a kind of mitochondrial membrane-binding protein that can enhance the function of PI3K/AKT pathway and promote cell proliferation[13,14]; EphB4 is a Ephrin family member that participates in the activation of the downstream Rac1 signaling pathway, and it has tyrosine kinase activity and can promote cell growth[15]. Analysis of the change in the expression of above cell proliferation molecules in liver cancer lesion in the study showed that DNMT3B, STC2, SIRT6, LETM1 and EphB4 protein expression levels in liver cancer lesion were significantly higher than those in adjacent lesion. This indicates that the high expression of the pro-proliferation molecules is closely related to the occurrence of liver cancer. Further analysis of the correlation of serum GP73, SOD and GPC3 contents with cell proliferation in patients with liver cancer showed that DNMT3B, STC2, SIRT6, LETM1 and EphB4 protein expression levels in liver cancer lesion were positively correlated with serum GP73 and GPC3 levels, and negatively correlated with serum SOD level. This means that the change of serum GP73, SOD and GPC3 contents in patients with liver cancer is closely related to the change of cell proliferation molecule expression, and serum GP73, SOD and GPC3 contents can reflect liver cancer cell proliferation activity.

The liver tissue receives two sets of blood supply from the portal vein and hepatic artery, and it has extremely abundant blood perfusion. In the growth of liver cancer, many kinds of molecules that are synthesized and secreted by cancer cells can further promote the angiogenesis and increase the blood supply in the lesion. SULT2B1 is a kind of metabolic enzyme that catalyzes the sulfation of oxysteroids, which can increase the expression of hypoxia-related regulating factor HIF-1 in malignant tumor lesions, and then up-regulate the expression of pro-angiogenesis molecule VEGF and increase the number of new blood vessels through the roles of HIF-1α[16,17]. Ang-2 is the Ang family member involved in the regulation of angiogenesis, which can make endothelial cells proliferate and form new vascular structure in the presence of VEGF[18]. Both HGF and TGF-β1 are the cytokines with wide pro-proliferation effect, the former activates downstream c-Met genes to participate in the process of angiogenesis, and the latter activates downstream Smad family molecules to participate in the process of angiogenesis[19,20]. Analysis of the changes in the expression of the above angiogenesis

molecules in liver cancer lesion showed that SULT2B1, HIF-1α, VEGF, Ang-2, HGF and TGF-β1 protein expression levels in liver cancer lesion were significantly higher than those in adjacent lesion. This indicates that the high expression of angiogenesis molecules is closely related to the occurrence of liver cancer. Further analysis of the correlation of serum GP73, SOD and GPC3 contents with angiogenesis in patients with liver cancer showed that SULT2B1, HIF-1α, VEGF, Ang-2, HGF and TGF-β1 protein expression levels in liver cancer lesion were positively correlated with serum GP73 and GPC3 levels, and negatively correlated with serum SOD level. This means that the change of serum GP73, SOD and GPC3 contents in patients with liver cancer is closely related to the change of angiogenesis molecule expression, and serum GP73, SOD and GPC3 contents can reflect the angiogenesis activity in liver cancer lesions.

To sum up, it can be concluded that serum GP73 and GPC3 levels significantly increase whereas SOD level significantly decreases in patients with liver cancer; the change of GP73, SOD and GPC3 contents is closely related to the cell proliferation and angiogenesis in liver cancer lesions, and serum GP73, SOD and GPC3 contents can reflect the activity of cell proliferation and angiogenesis in liver cancer lesions.

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