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Relationship of ultrasonic shear wave velocity with oncogene and tumor suppressor gene expression in primary liver cancer lesions as well as angiogenesis factor contents

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ABSTRACT

Objective: To discuss the relationship of ultrasonic shear wave velocity (SWV) with oncogene and tumor suppressor gene expression in primary liver cancer lesions as well as angiogenesis factor contents. **Methods:** 100 patients with primary liver cancer who underwent surgical treatment in our hospital between March 2014 and September 2016 were collected as observation group, and 50 healthy subjects who received physical examination in our hospital during the same period were collected as normal control group. The ultrasonic SWV levels of two groups of subjects were measured before the operation, and the observation groups were further divided into high SWV group and low SWV group, 50 cases in each group. Intraoperative tumor tissue samples were kept and fluorescence quantitative PCR was used to determine the mRNA expression of oncogenes and tumor suppressor genes. Enzyme-linked immunosorbent assay was used to determine serum contents of angiogenesis factors in observation group before operation. **Results:** Hepatic ultrasonic SWV level in observation group was significantly higher than that in normal control group; proto-oncogene CK, Ki67, Gly-3, Survivin and Pokemon mRNA expression in tumor tissue of high SWV group were higher than those of low SWV group while tumor suppressor genes Tg737, p16, p27, PTEN and runx3 mRNA expression were lower than those of low SWV group; serum angiogenesis factors VEGF, MMP-9 and IGF-1R contents were higher than those in low SWV group. **Conclusion:** The hepatic ultrasonic SWV level increases in patients with primary liver cancer, and the SWV level is directly correlated with oncogene and tumor suppressor gene expression as well as angiogenesis factor contents.

1. Introduction

The primary liver cancer is the malignant tumor disease with higher incidence in our country, and early diagnosis and determining malignant degree can lay the ground for later reasonable treatment scheme selection[1,2]. Elasticity imaging techniques can detect the the hardness information in region of interest and reflect the pathological changes of tissue, so many scholars recommend early elasticity imaging examination for patients with suspected liver lesions. Shear wave velocity (SWV) is an important part of virtual touch tissue quantification (VTQ), it is the reliable index to quantitatively reflect the tissue hardness, the tissue hardness increases as SWV levels rise, and it has been successfully applied in the examination of the superficial small organs and deeper abdominal organs[3,4]. At present, there is not much research about the SWV application value for primary liver cancer, liver SWV levels in patients with primary liver cancer and normal people were detected in the study, and the inner link between SWV levels and primary liver cancer illness was further explored in order to lay the foundation for the long-term diagnosis of same diseases and judgment of illness, now reported as follows.

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2. Information and methods

2.1 Case information

100 patients with primary liver cancer who underwent surgical treatment in our hospital between March 2014 and September 2016 were collected as observation group, and 50 healthy subjects who received physical examination in our hospital during the same period were collected as normal control group. Observation group included 51 male cases and 49 female cases that were 47-79 years old; normal control group included 27 male cases and 23 female cases that were 45-76 years old. The gender and age distribution of the two groups were not statistically different (P>0.05), and the hospital ethics committee approved the study.

2.2 Ultrasonic SWV detection

The two groups of subjects received hepatic VTQ examination, the frequency of the probe was set to 4.5MHz, and the patients took the left lateral position. The tumor lesions and area of interest were determined, VTQ system was started, and SWV level was calculated.

2.3 Oncogene and tumor suppressor gene expression

Tumor tissue samples were collected from patients with primary liver cancer during operation, Trizol reagent (Shanghai Genmed Genetic Pharmaceutical Technology Co., Ltd., the article number GMS12279) was used to split cells, chloroform (Shanghai Hengfei Biotechnology Co., Ltd., the article number 0757) was added, and the mixture was centrifuged at 4 ℃ and high speed to get upper clear water phase. Same volume of isopropanol (Wuhan Yuancheng Science and Technology Development Co., Ltd., the article number 5484) was added to mix and precipitate the total RNA in it, and 75% ethanol (Beijing Genia Biotechnology Co., Ltd., the article number E1214) was used to clean the precipitation and air dry it at room temperature. Reverse transcription kit (Beijing Biodragon Immunotechnologies Co., Ltd., the article number BDIT0035) instructions were followed to synthesize sample cDNA, and the fluorescence quantitative PCR kit (Thermo Fisher Company, article number MJA-981) instructions were followed for the mRNA amplification of oncogenes CK, Ki67, Gly-3, Survivin and Pokemon as well as tumor suppressor genes Tg737, p16, p27, PTEN and runx3. The corresponding PCR amplification curves were obtained, and the mRNA expression of above target genes were calculated.

2.4 Serum angiogenesis factors

2.0 mL fasting cubital venous blood was extracted from patients with primary liver cancer, anti-coagulated with heparin sodium (Chengdu Hepatunn Pharmaceutical Co., Ltd., approved by H51021210), let stand at room temperature for stratification and centrifuged at 2 500 r/min for 10-15 min to take the upper serum. Enzyme-linked immunosorbent assay method was used to detect the levels of the angiogenesis factors, including vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9) and insulin-like growth factor 1 receptor (IGF-1R).

2.5 Statistical processing

Data in the study were recorded and analyzed by specially-assigned person, and statistical software was SPSS 20.0. SWV levels, proto-oncogene and tumor suppressor gene mRNA expression, serum angiogenesis factor contents and other measurement data were in terms of mean ± standard deviation, and comparison between groups was by t test. Statistics P<0.05 was the standard of statistical significance in differences.

3. Results

3.1 SWV levels

Comparison of hepatic ultrasonic SWV levels between two groups of research subjects was as follows: the ultrasonic SWV level in observation group was (2.83±0.35) m/s, and the ultrasonic SWV level in normal control group was (1.39±0.18) m/s. Hepatic ultrasonic SWV level in observation group was significantly higher than that in normal control group, and differences were statistically significant (P<0.05). The median of hepatic ultrasonic SWV level in observation group was 2.76 and used as boundary to divide the patients with primary liver cancer into high SWV group and low SWV group, 50 cases in each group.

3.2 Proto-oncogene mRNA expression

Comparison of proto-oncogene CK, Ki67, Gly-3, Survivin and Pokemon mRNA expression in tumor tissue of primary liver cancer patients with different hepatic ultrasonic SWV levels was as follows: proto-oncogene CK, Ki67, Gly-3, Survivin and Pokemon mRNA

Table 1.
Comparison of proto-oncogene mRNA expression in primary liver cancer tissue.

Groups	n	CK	Ki67	Gly-3	Survivin	Pokemon
Low SWV group	50	93.27±10.82	102.48±15.29	97.65±11.47	101.38±13.75	99.72±10.69
High SWV group	50	163.28±20.15	148.23±18.55	139.75±15.47	172.48±18.66	141.56±17.29
T		13.284	12.948	15.472	18.938	15.486
P		<0.05	<0.05	<0.05	<0.05	<0.05

Table 2.
Comparison of tumor suppressor gene mRNA expression in primary liver cancer tissue.

Groups	*n*	Tg737	p16	p27	PTEN	runx3
Low SWV group	50	99.17±10.54	102.37±14.28	96.83±11.54	98.47±10.23	95.24±11.63
High SWV group	50	67.21±7.95	73.55±8.47	60.17±7.43	52.85±6.73	70.92±8.61
T		11.215	13.284	12.673	11.293	9.872
P		<0.05	<0.05	<0.05	<0.05	<0.05

Table 3.
Comparison of serum angiogenesis factor contents in patients with primary liver cancer.

Groups	*n*	VEGF	MMP-9	IGF-1R
Low SWV group	50	30.48±4.51	3.42±0.46	2.17±0.34
High SWV group	50	45.76±6.98	5.88±0.72	3.84±0.45
T		13.287	9.387	6.483
P		<0.05	<0.05	<0.05

expression in tumor tissue of high SWV group were significantly higher than those of low SWV group, and differences in CK, Ki67, Gly-3, Survivin and Pokemon mRNA expression in tumor tissue were statistically significant between primary liver cancer patients with different hepatic ultrasonic SWV levels (P<0.05), shown in Table 1.

3.3 Tumor suppressor gene mRNA expression

Comparison of tumor suppressor genes Tg737, p16, p27, PTEN and runx3 mRNA expression in tumor tissue of primary liver cancer patients with different hepatic ultrasonic SWV levels was as follows: tumor suppressor genes Tg737, p16, p27, PTEN and runx3 mRNA expression in tumor tissue of high SWV group were significantly lower than those of low SWV group, and differences in Tg737, p16, p27, PTEN and runx3 mRNA expression in tumor tissue were statistically significant between primary liver cancer patients with different hepatic ultrasonic SWV levels (P<0.05), shown in Table 2.

3.4 Serum angiogenesis factors

Comparison of preoperative serum angiogenesis factors VEGF (ng/L), MMP-9 (ng/L) and IGF-1R (ng/mL) contents between primary liver cancer patients with different hepatic ultrasonic SWV levels was as follows: preoperative serum VEGF, MMP-9 and IGF-1R contents in high SWV group were significantly higher than those in low SWV group, and differences in serum VEGF, MMP-9 and IGF-1R contents were statistically significant between primary liver cancer patients with different hepatic ultrasonic SWV levels (P<0.05), shown in Table 3.

4. Discussion

VTQ is a simple method to noninvasively assess the tissue texture, which transmits acoustic pulse wave by ultrasonic probe to cause small deformation in the target tissue, produces the shear wave spreading transversely during this period, uses the probe to accept the signal, and finally calculates the SWV[5,6]. SWV is the indicator most closely related to tissue hardness, and the higher the SWV, the harder the tissue. Both fibrosis and massive angiogenesis in local tumor tissue of patients with primary liver cancer can cause the tissue elasticity change, and the change was captured by Doppler ultrasound and transferred into the change of SWV levels, which can reflect the liver disease accurately in real time[7,8]. In the study, hepatic SWV levels were first compared between patients with primary liver cancer and normal people, and it was found that compared with normal control group, observation group of patients were with higher hepatic SWV level, confirming that the SWV level abnormally increases in patients with primary liver cancer. In order to further clarify the inner link between SWV level and the degree of the hepatic lesions, the median SWV level in observation group was used in the study to divided them into high SWV group and low SWV group, the changes in tumor tissue-related gene expression and serum angiogenesis indexes under different levels of SWV were analyzed.

Oncogene is the core factor causing all kinds of malignant tumors, its expression in the body is in the relative inhibitory state under physiological conditions, and the abnormal increase of its expression can induce malignant transformation of cells. CK is the main skeleton protein in the keratinocyte and plays an important role in maintaining the integrity and continuity of epithelium; Ki67 is a cell proliferation nuclear antigen, and increase in its expression can promote tumor cell proliferation; Gly-3 plays a negative role in apoptosis induction, it can promote tumor cell proliferation, and it has been confirmed in many studies that the high expression of above three kinds of proto-oncogenes in liver cancer tissue are the important causes of primary liver cancer[9,10]. Survivin is the most powerful anti-apoptotic protein at present, it is widely expressed in various tumor tissues, and study has shown that the survival time is shorter in patients with high Survivin expression[11]. Pokemon is a newly discovered proto-oncogene in recent years, which is a transcription suppressor and can induce rapid cell proliferation at a low rate of differentiation[12]. In the study, above proto-oncogene mRNA expression in tumor tissue were compared between primary liver cancer patients with different levels of SWV, and it was found that compared with low SWV group, high SWV group were with higher mRNA expression of CK, Ki67, Gly-3, Survivin and Pokemon. This indicates that the the ultrasonic hepatic SWV level in patients with primary liver cancer is positively correlated with the expression of proto-oncogenes.

Proto-oncogene/tumor suppressor gene expression imbalance is involved in the whole occurrence and development process of malignant tumor, in this paper, it has been made clear that there is abnormally high expression of proto-oncogenes in primary liver cancer tissue, and the tumor suppressor gene expression changes were further studied in the study. Tg737, p16, p27, PTEN and runx3 are the commonly studied clinical tumor suppressor genes at present, and both cell experiments and animal experiments show that increasing the tumor-suppressor gene expression can effectively reduce the proliferation/invasion vitality of tumor cells, and inhibit tumor progression[13,14]. In the study, the tumor-suppressor gene mRNA expression in tumor tissue were compared between primary liver cancer patients with different levels of SWV, and it was found that compared with low SWV group, high SWV group were with lower mRNA expression of Tg737, p16, p27, PTEN and runx3 in tumor tissue. This indicates that the the ultrasonic hepatic SWV level is negatively correlated with the expression of tumor suppressor genes.

Tumor angiogenesis is the basis for tumor cells to absorb oxygen and nutrients, so there is the high expression of a large amount of pro-angiogenesis factors in patients, and detecting their contents can also indirectly reflect the malignant degree of tumor. VEGF is the most powerful factor known to promote angiogenesis at present, and its high expression has been found in almost all malignant tumor tissues[15]. MMP-9 is a member of the matrix metalprotease family that combines extracellular matrix decomposition and angiogenesis promotion, and it is massively expressed under the induction of VEGF[16]. IGF-1R is the receptor for IGF-1, and the combination of the two can increase the VEGF expression and promote tumor angiogenesis[17,18]. In the study, preoperative serum levels of angiogenesis factors were compared between primary liver cancer patients with different SWV levels, and it was found that compared with low SWV group, high SWV group were with higher preoperative serum VEGF, MMP-9 and IGF-1R contents, showing that ultrasound liver SWV level in patients with primary liver cancer is positively correlated the contents of angiogenesis factors.

Thus, hepatic ultrasonic SWV level in patients with primary liver cancer is higher than that in normal people, its level is directly related to the oncogene and tumor suppressor gene expression in tumor tissue as well as angiogenesis factor contents in serum, and it can be used as the reliable means for early diagnosis of advanced primary liver cancer, judgment of curative effect and so on.

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