Correlation of serum ASPH and 5′-NT contents with angiogenesis and cancer cell proliferation in patients with liver cancer

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ABSTRACT

Objective: To proliferation in patients with liver cancer. Methods: Patients with primary liver cancer who underwent surgical resection in Yulin Third Hospital between December 2013 and December 2016 were selected as the liver cancer group of the research, and healthy volunteers who received physical examination in Yulin Third Hospital over the same period were selected as the control group of the research. Serum was collected from both groups to test the contents of ASPH and 5′-NT as well as liver cancer cell proliferation molecules; liver cancer lesions and para-carcinoma lesions were collected from liver cancer group to detect the contents of angiogenesis molecules and cancer cell proliferation molecules. Results: Serum ASPH and 5′-NT contents in liver cancer group were significantly higher than those in control group; VEGF, VEGFR1, VEGFR2, HGF, EGFR, Bcl-2, Bcl-x and Bcl-w contents in liver cancer lesions were significantly higher than those in para-carcinoma lesions and positively correlated with serum ASPH and 5′-NT contents in patients with liver cancer while Bax and Bak contents in liver cancer lesions were significantly lower than those in para-carcinoma lesions and negatively correlated with serum ASPH and 5′-NT contents in patients with liver cancer; serum AFP, GP73, GPC3 and DKK1 contents in liver cancer group were significantly higher than those in control group and positively correlated with serum ASPH and 5′-NT contents in patients with liver cancer. Conclusion: Serum ASPH and 5′-NT contents increase significantly in patients with liver cancer and can accurately evaluate angiogenesis and cancer cell proliferation.

1. Introduction

Liver cancer is a common malignant tumor in the digestive system, which is with insidious onset and atypical early clinical symptoms, and has mostly progressed to middle-advanced stage at the time of diagnosis. As advanced liver cancer is with extremely high malignant degree and strong proliferation and invasion, the prognosis of patients is generally poor. Current clinical experience holds that early diagnosing liver cancer and taking aggressive treatment is an effective method for improving the prognosis of liver cancer, but the common serum markers AFP, GP73 and so on of liver cancer are poor in sensitivity and specificity, and difficult to early detect hepatocellular carcinoma. Aspartate-β-hydroxylase (ASPH) is a kind of -ketoglutaric acid-dependent deoxygenase that regulates the cell growth and angiogenesis within the tumor lesions[1]; 5′-nucleotidase (5′-NT) is a highly specific phosphatase in liver cells, and it is closely associated with the changes of mitochondria function and the proliferation of cells[2]. In the following study, the correlation of serum ASPH and 5′-NT contents with angiogenesis and cancer cell proliferation in patients with liver cancer was analyzed in order to determine the diagnostic value of serum ASPH and 5′-NT detection for liver cancer.

2. Case information, research materials and research methods

2.1 Case data time

Patients with primary liver cancer who underwent surgical resection in Yulin Third Hospital between December 2013 and December 2016 were selected as the liver cancer group of the research, all patients were diagnosed with primary liver cancer...
by ultrasound examination, imaging examination and pathological examination, in conformity with the indications of surgical resection, and not treated with radiotherapy and chemotherapy as well targeted drug therapy before, there were a total of 35 cases, 22 cases were male and 13 cases were female, and they were 45-65 years old. Healthy volunteers who received physical examination in Yulin Third Hospital over the same period were selected as the control group of the research, all healthy volunteers were confirmed to be healthy and without previous history of hepatitis, fatty liver or alcoholic liver, there were a total of 55 cases, 33 cases were male and 22 cases were female, and they were 42-62 years old. There was no significant difference in general data between the two groups ($P>0.05$).

2.2 Research materials

Protein lysis buffer was bought in Beyotime Company in Shanghai, BCA protein quantitative kit and enzyme-linked immunosorbent assay kit were purchased from Shanghai Westang Biological Company, desk centrifuge was purchased in Thermo Company, and multifunctional microplate reader was purchased in Bio-tek Company.

2.3 Clinical research methods

2.3.1 Clinical sample collecting and preserving methods

3-5 mL cubital venous blood was collected from liver cancer group before surgery, 3-5 mL cubital venous blood was collected from control group of volunteers in physical examination, and the peripheral blood was let stand at room temperature for 30 min, and then centrifuged at 3 000 r/min for 20 min to separate upper serum and store it in the -80℃ refrigerator. After surgical resection, right amount of liver cancer lesions and para-carcinoma lesions were collected from liver cancer group, frozen with liquid nitrogen for 20-30 min and then stored in -80℃ refrigerator after the tissue nature was confirmed by pathological examination.

2.3.2 Detection of molecule contents in serum samples and lesion samples

Serum specimens were taken out of the -80℃ fridge and thawed at 4℃, and then enzyme-linked immunosorbent assay kit was used to determine ASPH, 5'-NT, AFP, GP73, GPC3 and DKK1 contents. Lesions specimens were taken out of the -80℃ fridge, added in protein lysis buffer and homogenized, BCA kit was used to determine the total protein content, enzyme-linked immunosorbent assay kit was used to determine Bcl-2, Bcl-x, Bcl-w, Bax and Bak contents and calculate Bcl-2, Bcl-x, Bcl-w, Bax and Bak contents per mg total protein samples, and the VEGF, VEGFR1, VEGFR2, HGF and EGFR contents in placenta homogenate samples were determined by Xi’an KingMed Diagnostics Company.

2.4 Statistical methods

SPSS 19.0 software was used to input and analyze data, measurement data analysis between two groups were by t test and $P<0.05$ indicated statistical significance in differences.

3. Results

3.1 Serum ASPH and 5'-NT contents

Serum ASPH contents in liver cancer group and control group were (68.23±9.13) pg/mL and (25.58±3.52) pg/mL respectively, and 5'-NT contents were (125.53±17.39) U/L and (10.38±1.85) U/L respectively. After t test, serum ASPH and 5'-NT contents in liver cancer group were significantly higher than those in control group. Differences in serum ASPH and 5'-NT contents were statistically significant between liver cancer group and control group ($P<0.05$).

3.2 Angiogenesis molecule contents in liver cancer lesions

Analysis of angiogenesis molecules VEGF (ng/mg), VEGFR1 (pg/mg), VEGFR2 (pg/mg), HGF (ng/mg) and EGFR (pg/mg) contents in liver cancer lesions and para-carcinoma lesions was as follows: VEGF, VEGFR1, VEGFR2, HGF and EGFR contents in liver cancer lesions were significantly higher than those in para-carcinoma lesions. Differences were statistically significant in VEGF, VEGFR1, VEGFR2, HGF and EGFR contents in liver cancer lesions and para-carcinoma lesions ($P<0.05$). Pearson correlation analysis showed that serum ASPH and 5'-NT contents in patients with liver cancer were positively correlated with VEGF, VEGFR1, VEGFR2, HGF and EGFR contents in liver cancer lesions.

3.3 Cancer cell proliferation molecule contents in serum and liver cancer lesions

Analysis of serum cancer cell proliferation molecules AFP, GP73, GPC3 and DKK1 contents between liver cancer group and control group was as follows: serum AFP, GP73, GPC3 and DKK1 contents in liver cancer group were significantly higher than those in control group, and differences in serum AFP, GP73, GPC3 and DKK1 contents were statistically significant between liver cancer group and control group ($P<0.05$). Pearson correlation analysis showed that serum ASPH and 5'-NT contents in patients with liver cancer were positively correlated with serum AFP, GP73, GPC3 and DKK1 contents.

Table 1.

Comparison of angiogenesis molecule contents in liver cancer lesions and para-carcinoma lesions.

<table>
<thead>
<tr>
<th>Lesion origin</th>
<th>n</th>
<th>VEGF</th>
<th>VEGFR1</th>
<th>VEGFR2</th>
<th>HGF</th>
<th>EGFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver cancer lesion</td>
<td>35</td>
<td>9.38±1.15</td>
<td>142.32±7.93</td>
<td>258.79±33.41</td>
<td>5.23±0.77</td>
<td>93.53±11.39</td>
</tr>
<tr>
<td>Para-carcinoma lesion</td>
<td>35</td>
<td>3.48±0.52</td>
<td>56.48±7.88</td>
<td>115.29±15.28</td>
<td>2.04±0.36</td>
<td>38.69±5.48</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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</table>
that the serum levels of ASPH and 5'-NT were significantly higher than those in control group. This indicates healthy volunteers were first analyzed in the study, and the results contents of these two molecules in the liver cancer patients and ASPH and 5'-NT are related to the occurrence and development of liver cancer. Further analysis of the correlation of serum ASPH and 5'-NT contents in patients with liver cancer with angiogenesis in liver cancer lesions showed that serum ASPH and 5'-NT contents were positively correlated with VEGF, VEGFR1, VEGFR2, HGF and EGFR contents in liver cancer lesions. This indicates that the increase of serum ASPH and 5'-NT contents in patients with liver cancer is closely related to the angiogenesis in the liver cancer lesions. The proliferation of cancer cells is a key biological behavior causing the growth of the tumor lesions, and in the process of liver cancer cell proliferation, a variety of molecules are abnormally synthesized and secreted into the blood circulation. AFP is the most common serum molecule to reflect the proliferation of liver cancer cells; GP73 is a transmembrane protein located in the cis-face of the golgi membrane, which is transferred out of the cells during the proliferation of liver cancer cells and secreted into the extracellular matrix[10]; GPC3 is a kind of glycoprotein outside the cell membrane, it is connected to the cell membrane through the carbon-terminal glycosylphosphatidylinositol, and it will become soluble form and be secreted into the blood circulation under the action of the new markers of liver cancer discovered in recent years. ASPH is a catalytic enzyme highly expressed in a variety of malignant tumor cells, and liver cells can express 5'-HT in large quantities during the canceration process; at the same time, the cancer cells proliferate, block the liver and cause intrahepatic cholestasis, causing hematopoietic cell damage and increasing 5'-HT secretion into the blood circulation[5]. In order to define the ASPH and 5'-NT value for the diagnosis of liver cancer, serum contents of these two molecules in the liver cancer patients and healthy volunteers were first analyzed in the study, and the results showed that serum ASPH and 5'-NT contents in liver cancer group were significantly higher than those in control group. This indicates that the serum levels of ASPH and 5'-NT increase significantly in patients with hepatocellular carcinoma, and ASPH and 5'-NT can be used in the diagnosis of liver cancer.

Angiogenesis is an important malignant biological behavior in the process of liver cancer progress, and the increased number of new blood vessels can provide nutrients for the proliferation and invasion of cancer cells. VEGF and HGF are the important cytokines mediating the angiogenesis process[6]. VEGF is the most specific cytokine that regulate angiogenesis, which can be combined with receptors VEGFR1 and VEGFR2 to promote endothelial cell growth and vascular structure formation[7,8]. HGF participates in the regulation of both liver cell growth and angiogenesis, the regulating effect on angiogenesis is realized through the combination with Kringle-1 domain EGFR, and it can not only recruit endothelial cells to participate in the formation of new blood vessels, but can also promote the production of VEGF. In the study, analysis of the expression of these new molecules in liver cancer lesions showed that VEGF, VEGFR1, VEGFR2, HGF and EGFR contents in liver cancer lesions were significantly higher than those in para-carcinoma lesions. This shows that the angiogenesis mediated by VEGF and HGF is closely related to the occurrence and development of liver cancer. Further analysis of the correlation of serum ASPH and 5'-NT contents in patients with liver cancer with angiogenesis in liver cancer lesions showed that serum ASPH and 5'-NT contents were positively correlated with VEGF, VEGFR1, VEGFR2, HGF and EGFR contents in liver cancer lesions. This indicates that the increase of serum ASPH and 5'-NT contents in patients with liver cancer is closely related to the angiogenesis in the liver cancer lesions.

4. Discussion

The early diagnosis of liver cancer and the accurate assessment of the disease can provide the basis for the formulation of therapy, and is also an effective way to improve the prognosis. The detection of serum tumor markers is a convenient complementary test for early screening of malignant tumors, and ASPH and 5'-HT are the new markers of liver cancer discovered in recent years. ASPH is a catalytic enzyme highly expressed in a variety of malignant tumor cells, it is mainly responsible for protein modification after translation, and it can cause the hydroxylation of aspartyl residues of epidermal growth factor-like domain in protein structure, and thus promote cell growth and invasion[3,4]. 5'-NT is a catalytic enzyme expressed in the liver cells, and liver cells can express 5'-HT in large quantities during the canceration process; at the same time, the cancer cells proliferate, block the liver and cause intrahepatic cholestasis, causing hematopoietic cell damage and increasing 5'-HT secretion into the blood circulation[5]. In order to define the ASPH and 5'-NT value for the diagnosis of liver cancer, serum contents of these two molecules in the liver cancer patients and healthy volunteers were first analyzed in the study, and the results showed that serum ASPH and 5'-NT contents in liver cancer group were significantly higher than those in control group. This indicates that the serum levels of ASPH and 5'-NT increase significantly in patients with hepatocellular carcinoma, and ASPH and 5'-NT can be used in the diagnosis of liver cancer.
of proteases in malignant tumor cells[11]; DKK1 is an important secretory protein in cells that can regulate the proliferation of the cells by Wnt/β-catenin pathway[12]. In the study, analysis of the contents of these cancer cell proliferation molecules in liver cancer lesions showed that serum AFP, GP73, GPC3 and DKK1 contents in liver cancer group were significantly higher than those in control group. Further analysis of the correlation of serum ASPH and 5'-NT contents in patients with liver cancer with these cancer cell proliferation molecule contents showed that serum ASPH and 5'-NT contents were positively correlated with serum AFP, GP73, GPC3 and DKK1 contents. This indicates that the rise of serum ASPH and 5'-NT contents in patients with liver cancer is closely related to the proliferation of cells in liver cancer.

The cell proliferation in liver cancer lesions can not only cause the changes in serum levels of a variety of molecules, but will also cause the changes in the expression of a variety of proliferation and apoptosis molecules in lesions. Mitochondrial pathway apoptosis is an important regulatory pathway for apoptosis, and Bcl-2 family molecules on the mitochondrial membrane are involved in the regulation of mitochondrial pathway apoptosis. The pro-apoptotic molecules Bax and Bak in Bcl-2 family can form oligomers and provide channel for the cytochrome C from the mitochondria to the cytoplasm[13]; the cytochrome C in the cytoplasm induces apoptosis through the cascade activation response of the caspase family. Anti-apoptotic molecules Bcl-2, Bcl-x and Bcl-w in Bcl-2 family can inhibit Bax and Bak from forming oligomers, thus reduce the release of cytochrome C and antagonize apoptosis[14,15]. In the study, analysis of the expression of these cell proliferation molecules in hepatocellular carcinoma lesions showed that Bcl-2, Bcl-x and Bcl-w contents in liver cancer lesions were significantly higher than those in para-carcinoma lesions while Bax and Bak contents were significantly lower than those in para-carcinoma lesions. This indicates that the inhibition of mitochondrial apoptosis is closely related to the occurrence and development of liver cancer. Further analysis of the correlation of serum ASPH and 5'-NT contents in patients with liver cancer with mitochondrial apoptosis molecules in liver cancer lesions showed that serum ASPH and 5'-NT contents were positively correlated with Bcl-2, Bcl-x and Bcl-w contents in liver cancer lesions, and negatively correlated with Bax and Bak contents in liver cancer lesions. This suggests that the rise of serum ASPH and 5'-NT contents in patients with liver cancer is closely related to the inhibition of mitochondrial apoptosis in the liver cancer lesions.

To sum up, it is believed that the serum ASPH and 5'-NT contents increase significantly in patients with liver cancer; the abnormally elevated serum ASPH and 5'-NT contents are closely related to the angiogenesis activity and cell proliferation activity in liver cancer lesions.

References