The correlation of the quantitative ultrasonography parameters with the liver cancer cell proliferation activity and intralesional angiogenesis

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ARTICLE INFO

Article history:
Received 14 Jul 2017
Received in revised form 17 Jul 2017
Accepted 24 Jul 2017
Available online 28 Jul 2017

Keywords:
Liver cancer
Ultrasonography
Proliferation
Apoptosis
Angiogenesis

ABSTRACT

Objective: To study the correlation of the quantitative ultrasonography parameters with liver cancer cell proliferation activity and intralesional angiogenesis. Methods: A total of 46 patients with liver cancer who received surgical resection in Hancheng People’s Hospital between June 2014 and March 2017 as well as 28 patients who received partial hepatectomy for abdominal trauma during the same period were selected, the patients with liver cancer received ultrasonography, and the quantitative parameters were calculated; the liver cancer tissue and adjacent tissue of patients with liver cancer as well as the normal liver tissue of patients with abdominal trauma were collected to determine the expression of proliferation genes, tumor suppressor genes and angiogenesis genes. Results: The IMAX of liver cancer tissue was significantly higher than that of adjacent tissue while the TTP, WT and mTT were significantly shorter than those of adjacent tissue; RNF180, Merlin, PICK1 and PTEN protein expression in liver cancer tissue and adjacent tissue were significantly lower than those in normal liver tissue, negatively correlated with the IMAX of liver cancer tissue, and positively correlated with the TTP, WT and mTT of liver cancer tissue; EphB4, SALL4, Cripto-1, VEGF, PDGF, EGFR and Ang-2 protein expression in liver cancer tissue and adjacent tissue were significantly higher than those in normal liver tissue, positively correlated with the IMAX of liver cancer tissue, and negatively correlated with the TTP, WT and mTT of liver cancer tissue. Conclusion: The changes in the quantitative parameters of liver cancer ultrasonography are valuable for evaluating the cell proliferation and angiogenesis.

1. Introduction

Primary liver cancer is a common gastrointestinal malignancy in China with high malignant degree, low surgical resection rate and poor prognosis. The occurrence and development of liver cancer is a complicated process involving many link and many genes, and the activation of proto-oncogenes, the inactivation of tumor suppressor genes and the increase in the number of new blood vessels are closely related to the occurrence of liver cancer[1,2]. The blood supply of liver cancer tissue is abundant, and the significantly increased new blood vessels in local lesions can provide the necessary nutrients for the proliferation and invasion of cancer cells. In clinical practice, accurately assessing the blood supply of liver cancer tissue can provide a basis for the diagnosis and treatment of the disease. Ultrasonography is an ultrasound method developed in recent years, which uses the contrast agent to quantitatively evaluate the blood supply of local tissues[3,4]. In the following studies, we analyzed the correlation of the quantitative ultrasonography parameters of liver cancer lesions with cancer cell proliferation activity and intralesional angiogenesis.

2. Research subjects and research methods

2.1 General information of research subjects

A total of 46 patients with liver cancer who received surgical resection in Hancheng People’s Hospital between June 2014 and
March 2017 were selected, and all patients were diagnosed with primary liver cancer by postoperative pathology examination, and included 29 men and 17 women that were 41-64 years old. 28 patients who received partial hepatectomy for abdominal trauma in Hancheng People’s Hospital during the same period were selected, and they were without history of hepatitis, liver cirrhosis, fatty liver, liver cancer and so on, and included 19 men and 9 women that were 35-62 years old. There was no significant difference in the general data between patients with liver cancer and abdominal trauma ($P<0.05$).

### 2.2 Ultrasonography

HI VISION Ascendus diasonograph was used for inspection, the convex array probe was selected, the frequency was set to 1-5 MHz, conventional two-dimensional ultrasound scanning was performed to confirm the location of lesions, then contrast agent Sonovue1.5 mL was quickly injected via the cubital vein, and after that, diasonograph was used to record 120 seconds of lesion imaging process. After the dynamic images were input, the automatic image analysis function was started to generate time - intensity curve, and the peak intensity IMAX, time to peak TTP, washout time WT and mean transit time mTT were calculated.

### 2.3 Gene expression detection

Liver cancer tissues and adjacent tissues surgically removed from patients with liver cancer were collected, normal liver tissues surgically removed from patients with abdominal trauma were collected, the tissues were washed with saline, then added in RIPA lysis buffer and fully grinded, grinding fluid was obtained and centrifuged at 4 °C and 12 000 r/min for 20 min to separate supernatant, and the enzyme-linked immunosorbent assay kit was used to determine RNF180, Merlin, PICK1, PTEN, EphB4, SALL4, Cripto-1, VEGF, PDGF, EGFR and Ang-2 levels.

### 2.4 Statistical methods

SPSS 19.0 software was used to input and analyze data, the comparison of ultrasonography parameters and gene expression data among three groups was by variance analysis and $P<0.05$ indicated statistical significance in differences in the analysis results.

### 3. Results

#### 3.1 Ultrasonography parameters of liver cancer tissue and adjacent tissue

Analysis of ultrasonography parameters IMAX, TTP, WT and mTT of liver cancer tissue and adjacent tissue in patients with liver cancer was as follows: the IMAX of liver cancer tissue was significantly higher than that of adjacent tissue while the TTP, WT and mTT were significantly shorter than those of adjacent tissue. Differences were statistically significant in IMAX, TTP, WT and mTT levels of liver cancer tissue and adjacent tissue in patients with liver cancer ($P<0.05$).

##### Table 1.

Differences in ultrasonography parameters of different tissues.

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>n</th>
<th>IMAX</th>
<th>TTP (ng/mL)</th>
<th>WT (pg/mL)</th>
<th>mTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver cancer tissue</td>
<td>46</td>
<td>142.31±16.64</td>
<td>12.03±1.58</td>
<td>20.35±3.32</td>
<td>79.35±10.25</td>
</tr>
<tr>
<td>Adjacent tissue</td>
<td>46</td>
<td>81.25±10.55</td>
<td>16.57±2.04</td>
<td>27.61±3.95</td>
<td>95.61±11.28</td>
</tr>
<tr>
<td>$T$</td>
<td>8.968</td>
<td>7.182</td>
<td>7.968</td>
<td>6.698</td>
<td></td>
</tr>
<tr>
<td>$P$</td>
<td>$&lt;0.05$</td>
<td>$&lt;0.05$</td>
<td>$&lt;0.05$</td>
<td>$&lt;0.05$</td>
<td></td>
</tr>
</tbody>
</table>

#### 3.2 Tumor suppressor gene expression in liver cancer tissue, adjacent tissue and normal liver tissue

Analysis of tumor suppressor genes RNF180 (ng/mL), Merlin (ng/mL), PICK1 (pg/mL) and PTEN (pg/mL) expression in liver cancer tissue, adjacent tissue and normal liver tissue was as follows: RNF180, Merlin, PICK1 and PTEN protein expression in liver cancer tissue and adjacent tissue were significantly lower than those in normal liver tissue, and RNF180, Merlin, PICK1 and PTEN protein expression in liver cancer tissue were significantly lower than those in adjacent tissue. Differences were statistically significant in pair-wise comparison of RNF180, Merlin, PICK1 and PTEN protein expression in liver cancer tissue, adjacent tissue and normal liver tissue ($P<0.05$). Pearson correlation analysis showed that the IMAX of liver cancer tissue was negatively correlated with RNF180, Merlin, PICK1 and PTEN protein expression in liver cancer tissue and adjacent tissue, and the TTP, WT and mTT of liver cancer tissue were positively correlated with RNF180, Merlin, PICK1 and PTEN protein expression in liver cancer tissue.

##### Table 2.

Differences in tumor suppressor gene expression in different tissues.

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>n</th>
<th>RNF180</th>
<th>Merlin</th>
<th>PICK1</th>
<th>PTEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver cancer tissue</td>
<td>46</td>
<td>96.62±10.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.52±9.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.28±0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.75±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adjacent tissue</td>
<td>46</td>
<td>144.26±17.85&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>103.48±13.25&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.74±0.24&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.27±0.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal liver</td>
<td>28</td>
<td>285.51±34.52</td>
<td>193.57±23.67</td>
<td>3.27±0.41</td>
<td>2.32±0.34</td>
</tr>
</tbody>
</table>

<sup>a</sup>: compared with normal liver tissue, $P<0.05$; <sup>b</sup>: compared with adjacent tissue, $P<0.05$.

#### 3.3 Proliferation gene expression in liver cancer tissue, adjacent tissue and normal liver tissue

Analysis of proliferation genes EphB4, SALL4 and Cripto-1 expression in liver cancer tissue, adjacent tissue and normal liver tissue was as follows: EphB4, SALL4 and Cripto-1 protein expression in liver cancer tissue and adjacent tissue were significantly higher than those in normal liver tissue, and EphB4,
SALL4 and Cripto-1 protein expression in liver cancer tissue were significantly higher than those in adjacent tissue. Differences were statistically significant in pair-wise comparison of EphB4, SALL4 and Cripto-1 protein expression in liver cancer tissue, adjacent tissue and normal liver tissue (P<0.05). Pearson correlation analysis showed that the IMAX of liver cancer tissue was positively correlated with EphB4, SALL4 and Cripto-1 protein expression in liver cancer tissue, and the TTP, WT and mTT of liver cancer tissue were negatively correlated with EphB4, SALL4 and Cripto-1 protein expression in liver cancer tissue.

### 3.4 Angiogenesis gene expression in liver cancer tissue, adjacent tissue and normal liver tissue

Analysis of angiogenesis genes VEGF, PDGF, EGFR and Ang-2 expression in liver cancer tissue, adjacent tissue and normal liver tissue was as follows: VEGF, PDGF, EGFR and Ang-2 protein expression in liver cancer tissue and adjacent tissue were significantly higher than those in normal liver tissue, and VEGF, PDGF, EGFR and Ang-2 protein expression in liver cancer tissue were significantly higher than those in adjacent tissue. Differences were statistically significant in pair-wise comparison of VEGF, PDGF, EGFR and Ang-2 protein expression in liver cancer tissue, adjacent tissue and normal liver tissue (P<0.05). Pearson correlation analysis showed that the IMAX of liver cancer tissue was positively correlated with VEGF, PDGF, EGFR and Ang-2 protein expression in liver cancer tissue, and the TTP, WT and mTT of liver cancer tissue were negatively correlated with VEGF, PDGF, EGFR and Ang-2 protein expression in liver cancer tissue.

### 4. Discussion

The abundant blood supply and the strong angiogenesis process in hepatocellular carcinoma are clear, and a large number of new blood vessels can provide the nutrients for the growth of tumor cells. The liver cancer is with high malignant degree and poor prognosis, and the recurrence rate and mortality rate are both high after treatment. In the clinical practice, the accurate assessment of the blood supply of liver cancer lesions can provide a basis for the judgment of disease severity and also provide a reference for the selection of treatment options. Ultrasoundography is the ultrasound method developed in recent years, and the adopted ultrasound contrast agent is a kind of micron-grade pure blood vessel contrast agent that can well reflect the blood flow in local tissue, and then quantitative parameters were determined to evaluate the characteristics of the blood flow[5,6]. In the study, the analysis of the differences in quantitative ultrasoundography parameters of liver cancer tissues and adjacent tissues showed that the IMAX of liver cancer tissue was significantly higher than that of adjacent tissue while the TTP, WT and mTT were significantly shorter than those of adjacent tissue. This indicates that the overall blood supply of liver cancer tissue is more abundant than that of adjacent tissue, and the blood supply shows the pattern of fast in and fast out, flows in quickly, reaches the peak rapidly and flows out rapidly.

The abundant blood supply in liver cancer is conducive to the proliferation of cancer cells, and the deletion of tumor suppressor genes is considered to be an important link in the occurrence of liver cancer and the infinite proliferation of cancer cells. RNF180 encodes a kind of protein with ubiquitin ligase activity, which can regulate the ubiquitination of protein substrate and cause apoptosis[7]; Merlin encodes the product that can inhibit the growth of cells by molecules such as KSR1 and FAK[8]; PICK1 is a molecule in the cells with protein kinase function, which can inhibit the expression of CyclinD1, c-myc and so on through the PDZ domain so as to hinder the process of cell cycle; PTEN has dual phosphatase activity, which can lead to PIP3 dephosphorylation, affect the activation of downstream pro-proliferation pathway PI3K/Akt and inhibit cell proliferation[9,10]. In the study, the analysis of the expression of these tumor suppressor genes in liver cancer tissues showed that RNF180, Merlin, PICK1 and PTEN protein expression in liver cancer tissue were significantly lower than those in adjacent tissue and normal liver tissue. This indicates that the expression deletion of multiple tumor suppressor genes is closely related to the occurrence of liver cancer. Further analysis of the correlation between blood supply of liver cancer lesions and tumor suppressor gene expression showed that the IMAX of liver cancer tissue was negatively correlated with RNF180, Merlin, PICK1 and PTEN protein expression in liver cancer tissue, and the TTP, WT and mTT were positively correlated with RNF180, Merlin, PICK1 and PTEN protein expression in liver cancer tissue. This indicates that the increased blood supply in liver cancer tissue can decrease the expression of tumor suppressor genes.
Liver cancer cell proliferation is influenced by both tumor suppressor gene expression deletion and proto-oncogene expression increase, and multiple pro-proliferation genes in cells are considered to be the proto-oncogenes that play an important part in the development of liver cancer. EphB4 is a type of tyrosine kinase in Ephrin family, which can not only act on the Rac1/Akt pathway and promote cell proliferation, but can also act on the epithelial-mesenchymal transition and promote the invasive growth of cells[11]; SALL4 is a class of zinc finger protein transcription factor that can activate the expression of stem cell genes such as SOX2, Nanog and Oct3 in the cells, and then maintain the characteristics of infinite proliferation and self-renewal of cells[12,13]; Cripto-1 is the membrane protein expressed on the surface of the cell membrane, which can mediate downstream signaling pathway transduction and increase the expression of MMP2 and MMP9 so as to promote the degradation of extracellular matrix and the invasive growth of cells[14]. In the study, analysis of the proliferation gene expression in liver cancer tissue showed that EphB4, SALL4 and Cripto-1 protein expression in liver cancer tissue were significantly higher than those in adjacent tissue and normal liver tissue. This indicates that the increased expression of multiple genes is closely related to the occurrence of liver cancer. Further analysis of the correlation between the blood supply of liver cancer lesions and the proliferation gene expression showed that the IMAX of liver cancer tissue was positively correlated with VEGF, PDGF, EGFR and Ang-2 protein expression in liver cancer tissue, and the TTP, WT and mTT were negatively correlated with VEGF, PDGF, EGFR and Ang-2 protein expression in liver cancer tissue. This indicates that the increase in blood supply of hepatocellular carcinoma is closely related to the increase in angiogenesis gene expression, and the increase in blood supply can reduce the expression of tumor suppressor genes to promote the proliferation of cancer cells.

The quantitative ultrasonography parameters indicate that the blood supply in the liver cancer lesion is rich and shows the pattern of fast in and fast out; the changes of blood flow characteristics are closely related to cell proliferation and angiogenesis, and the quantitative ultrasonography parameters can evaluate cell proliferation and angiogenesis.

References


