Effect of radiofrequency-assisted hepatectomy treatment of primary liver cancer on cancer cell apoptosis and normal liver tissue damage

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Abstract

Objective: To study the effect of radiofrequency-assisted hepatectomy treatment of primary liver cancer on cancer cell apoptosis and normal liver tissue damage. Methods: 50 patients with primary liver cancer who received surgery in our hospital between May 2011 and August 2015 were collected, their operation methods and test results were reviewed, and then they were divided into the control group \((n=30)\) who received hepatectomy treatment alone and the observation group \((n=20)\) who received radiofrequency-assisted hepatectomy treatment. Intraoperative tumor tissue was collected to detect the proliferation and apoptosis gene mRNA expression by fluorescent quantitative PCR; 3 d after operation, serum was collected to detect serum liver enzyme index levels by immune scatter turbidimetry. Results: IFITM3, NCX1, CXCR2, Cep55 and Bcl-2 mRNA expression in liver cancer tissue of observation group were lower than those of control group while Caspase-3 and Cyt-C mRNA expression were higher than those of control group \((P<0.05)\); 3 d after operation, serum liver enzyme indexes ALT, AKP and GGT levels of observation group were not significantly different from those of control group \((P>0.05)\). Conclusion: Radiofrequency-assisted hepatectomy can effectively induce liver cancer cell apoptosis without increasing the normal liver tissue damage.

1. Introduction

Primary liver cancer is a tumor disease with extremely high malignant degree, and patients without distant metastasis should early accept radical operation. Current studies have found that intrahepatic metastases may be left behind in patients with surgical treatment after operation and lead to early postoperative tumor recurrence\(^{[1,2]}\). At present, many scholars have recommended preoperative adjuvant treatment to kill intrahepatic local metastases, reduce the malignant degree of tumor cells and provide favorable conditions for the operation, but the choice of specific auxiliary treatment is controversial. Radiofrequency ablation belongs to physical thermal coagulation, it was mostly used for the conservative treatment of middle-advanced malignant tumors in the past, and it is currently believed that it is also extremely valuable for patients with early and middle malignant tumors who accept surgical treatment, and can create favorable conditions for radical tumor resection\(^{[3,4]}\). In the following study, the effect of radiofrequency-assisted hepatectomy treatment of primary liver cancer on cancer cell apoptosis and normal liver tissue damage was analyzed.

2. Materials and methods

2.1. General information

50 patients with primary liver cancer who received surgery in our hospital between May 2011 and August 2015 were selected, and patients themselves signed the informed consent. The operation methods and test results were reviewed, and then they were divided into the control group \((n=30)\) who received hepatectomy treatment alone and the observation group \((n=20)\) who received radiofrequency-assisted hepatectomy treatment. Control group included 16 male cases and 14 female cases, they were 43–72 years old, and the maximum tumor diameter was 2–7 cm and \((3.82\pm0.49)\) cm.
cm in average; observation group included 11 male cases and 9 female cases, they were 41–73 years old, and the maximum tumor diameter was 1–7 cm and (3.53±0.69) cm in average. Two groups of patients were not statistically different in the distribution of gender, age and maximum tumor (P>0.05), and the research was approved by the hospital ethics committee.

2.2. Inclusion and exclusion criteria

Inclusion criteria: (1) with mass detected by abdominal ultrasound and MRI, and diagnosed with primary liver cancer by pathological examination; (2) with tumor staging Ia–IIb stage and without distant metastasis; (3) with cardiac function I–II grade; (4) participating in the whole study and with complete clinical data. Exclusion criteria: (1) associated with primary malignant tumor of other tissues and organs; (2) complicated with hepatic failure; (3) associated with systemic infectious diseases; (4) associated with autoimmune diseases.

2.3. Treatment methods

Control group of patients received conventional hepatectomy, and the method was the same as that in the study of Liu and Guo[5]. Observation group of patients received radiofrequency-assisted hepatectomy, which was as follows: ultrasound was used to identify tumor size and location, and scratch line was pre-marked at least 1 cm from the tumor. Before dividing the liver parenchyma, RF electrode needle was inserted into the liver parenchyma along the excision line to achieve bloodless hepatectomy effect, and high-frequency electrotome combined with vessel clamp was used at last to divide the liver parenchyma.

2.4. Observation indexes

2.4.1. Proliferation and apoptosis gene expression

Tumor tissue blocks were kept during operation, added in Trizol reagent (Hangzhou Simgen Biological Reagents Development Co., LTD., the article number 5301100) and 0.2 mL chloroform (Shanghai Kang Lang Biological Technology Co., LTD., the article number A0757) and then centrifuged for 15 min at 4℃ under high speed, and supernatant was collected, added in same volume of isopropyl alcohol (Anhui Hongzhe Biotech Co., LTD., the article number I811932) to precipitate total RNA. 75% ethanol (Jiangsu KeyGEN Biotech Co., LTD., the article number KGDN6) was used to clean RNA precipitation, the reverse transcription kit (Beijing Kit-guide Bo High Biological Technology Co., LTD., the article number K1622) instructions were followed to synthesize sample cDNA, and the fluorescence quantitative PCR kit (Beijing Biolab Technology Co., LTD., the article number BTN90408) instructions were followed for the mRNA amplification of proliferation genes IFITM3, NCX1, CXCR2 and Cep55 as well as invasion genes Bcl-2, Caspase-3 and Cyt-C.

2.4.2. Liver enzyme indexes

3 d after operation, 1.5–2.0 mL of fasting venous blood was extracted from two groups of patients, let stand at room temperature and centrifuged at low speed to get supernatant, and immune scatter turbidimetry was used to detect serum liver enzyme indexes alanine transaminase (ALT), γ-glutamyl transpeptidase (GGT) and alkaline phosphatase (AKP) levels.

2.5. Statistical methods

Data in the study was input in software SPSS15.0, measurement data was in terms of \( \bar{x} \pm s \), comparison between groups was by group \( t \) test and \( P<0.05 \) indicated statistical significance in differences.

3. Results

3.1. Proliferation gene mRNA expression

Comparison of proliferation genes IFITM3, NCX1, CXCR2 and Cep55 mRNA expression in liver cancer tissue between two groups of patients was as follows: proliferation genes IFITM3, NCX1, CXCR2 and Cep55 mRNA expression in liver cancer tissue of observation group were lower than those of control group. Differences in proliferation genes IFITM3, NCX1, CXCR2 and Cep55 mRNA expression in liver cancer tissue between two groups of patients (\( P<0.05 \)), shown in Table 1.

3.2. Apoptosis gene mRNA expression

Comparison of apoptosis genes Bcl-2, Caspase-3 and Cyt-C mRNA expression in liver cancer tissue between two groups of

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>IFITM3</th>
<th>NCX1</th>
<th>CXCR2</th>
<th>Cep55</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>20</td>
<td>71.42±7.89</td>
<td>75.38±8.15</td>
<td>69.26±7.23</td>
<td>72.18±7.49</td>
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<tr>
<td>Control group</td>
<td>30</td>
<td>98.23±9.12</td>
<td>97.46±10.05</td>
<td>100.57±12.61</td>
<td>98.59±9.63</td>
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<tr>
<td>( t )</td>
<td></td>
<td>8.293</td>
<td>8.493</td>
<td>11.172</td>
<td>9.023</td>
</tr>
<tr>
<td>( P )</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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</tr>
</tbody>
</table>
patients was as follows: apoptosis gene Bcl-2 mRNA expression in liver cancer tissue of observation group was lower than that of control group while Caspase-3 and Cyt-C mRNA expression were higher than those of control group. Differences in apoptosis genes Bcl-2, Caspase-3 and Cyt-C mRNA expression in liver cancer tissue between two groups of patients ($P<0.05$), shown in Table 2.

### 3.3. Liver enzyme indexes

3 d after operation, comparison of serum liver enzyme indexes ALT, AKP and GGT levels between two groups of patients was as follows: serum liver enzyme indexes ALT, AKP and GGT levels of observation group were not significantly different from those of control group. Differences in serum liver enzyme indexes ALT, AKP and GGT levels were not statistically significant between two groups of patients 3d after operation ($P>0.05$), shown in Table 3.

### 4. Discussion

Primary liver cancer is with high malignant degree and fast progression, and for patients in early and middle stage, radical resection of tumor and local metastases is the most reliable way to prolong patients’ survival time and improve the quality of life. Study shows that early postoperative intrahepatic recurrence may occur in patients with primary liver cancer, which is mainly directly related to the local metastase residue, high tumor cell proliferation activity and so on. The basic principle of radiofrequency ablation is to use the electromagnetic waves from the electrode needle to generate heat, local tumor temperature rises after absorbing heat, and it produces irreversible damage to cancer cells[6]. Radiofrequency ablation technology, as a minimally invasive physical therapy technology, is mostly used in the treatment of middle-advanced cancer patients with distant metastasis at present, and many scholars have currently recommended it as an adjuvant means before radical resection of tumor in order to improve the overall treatment effect[7].

Liver cancer is directly related to cancer cell proliferation/apoptosis imbalance, it is specifically characterized by the enhanced proliferation and suppressed apoptosis, observation group of patients in the study received radiofrequency ablation treatment before operation, and tumor tissue blocks were collected during operation to determine the proliferation and apoptosis gene expression changes. IFITM3, NCX1, CXCR2 and Cep55 are the commonly studied proliferation genes, and IFITM3 is highly expressed in primary liver cancer and can control matrix metalloproteinase-9 (MMP-9) expression to promote liver cancer cell proliferation[8]. Activated NCX1 can increase the content of calcium ion in tumor cells, and current research has confirmed that NCX1 gene is highly expressed in breast cancer, gastric cancer and other tumor tissues[9], CXCR2 is a chemokine receptor, it is highly expressed in prostate cancer, pancreatic cancer, colorectal cancer and other malignant tumor cells, and it can promote the tumor cell proliferation and metastasis[10]. Cep55 is a newly discovered gene that is involved in spindle formation, it can regulate cell proliferation, and the study of Zhang et al[11] shows that targeted down-regulation of Cep55 gene expression can inhibit the liver cancer cell proliferation. In the study, the above gene expression levels in liver cancer tissue were detected, and it was found that compared with the control group, the observation group were with lower proliferation genes IFITM3, NCX1, CXCR2 and Cep55 mRNA expression in liver cancer tissue, confirming that radiofrequency ablation can effectively restrain the liver cancer cell proliferation activity.

Apoptosis is a programmed death process, the cell proliferation and apoptosis process are in dynamic balance under physiological state, and after tumor progression, cell apoptosis process is destroyed and apoptosis gene expression is abnormal, eventually leading to the apoptosis disorder and infinite proliferation of tumor cells[12,13]. Apoptosis gene expression can objectively reflect the malignant degree of tumor cells, the Bcl-2 has anti-apoptotic effect and can prevent the cell death process caused by cytotoxity, and its excessive expression can enhance the tumor cell resistance to DNA damage factors. The study of Xie et al[14] confirms that Caspase-3

### Table 2
Comparison of apoptosis gene mRNA expression in liver cancer tissue ($\overline{x} \pm s$).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Bcl-2</th>
<th>Caspase-3</th>
<th>Cyt-C</th>
</tr>
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<tbody>
<tr>
<td>Observation</td>
<td>20</td>
<td>75.28±8.12</td>
<td>120.47±13.66</td>
<td>124.15±13.85</td>
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<tr>
<td>Control group</td>
<td>30</td>
<td>98.22±10.19</td>
<td>97.28±10.59</td>
<td>101.25±13.18</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>8.192</td>
<td>9.737</td>
<td>9.672</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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</tr>
</tbody>
</table>

### Table 3
Comparison of serum liver enzyme index levels ($\overline{x} \pm s$).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>ALT (IU/L)</th>
<th>AKP (U/L)</th>
<th>GGT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>20</td>
<td>45.38±5.09</td>
<td>57.21±6.09</td>
<td>78.43±8.34</td>
</tr>
<tr>
<td>Control group</td>
<td>30</td>
<td>46.12±5.89</td>
<td>58.68±6.21</td>
<td>76.18±8.43</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>0.172</td>
<td>0.217</td>
<td>0.152</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>
activity is directly related to cell apoptosis rate, and the increased Caspase-3 expression can promote tumor cell apoptosis. Cyt-C is a pro-apoptotic molecule, and it can activate the downstream effector molecule Caspase-9 step by step and exert apoptotic effect[15]. In the study, the expression levels of above apoptosis molecule in tumor tissue were detected, and it was found that compared with the control group, the observation group were with lower Bcl-2 mRNA expression, and higher Caspase-3 and Cyt-C mRNA expression in liver cancer tissue, showing that radiofrequency ablation can effectively promote the tumor cell apoptosis and help to improve the completeness of surgical resection of liver cancer and reduce the risk of postoperative recurrence.

The efficiency of preoperative radiofrequency has confirmed in the study, but its treatment safety is still in doubt. In radiofrequency ablation, the electrode needle is inserted in the liver parenchyma 1 cm from the tumor edge, and it is the major concern of clinical scholars whether the technology will damage the normal liver tissue and cause liver injury when it results in tumor tissue coagulation and necrosis[16]. There are a large number of enzymes in liver cells, their levels in circulating blood are little content under physiological conditions, a large number of enzymes enter into the peripheral blood from kidney cells and are detected when they are damaged, and they can objectively reflect the degree of liver cell damage[17]. ALT, AKP and GGT all belong to liver-specific enzymes, their serum levels were detected in the study, and it was found that compared with the control group, serum ALT, AKP and GGT levels of observation group were not significantly different from those of control group, confirming that the adjuvant radiofrequency ablation will not cause significant damage to normal liver tissue, will not increase the risk of postoperative liver dysfunction and has good treatment safety.

To sum up, it is concluded that radiofrequency-assisted hepatectomy can inhibit liver cancer cell proliferation and promote liver cancer cell apoptosis without causing significant damage to normal liver tissue, and it has advantages in both treatment efficiency and safety.

References


