Effect of luteolin on HepG2 liver cancer cell proliferation in vitro and angiogenesis activity

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ABSTRACT

Objective: To study the effect of luteolin on HepG2 liver cancer cell proliferation in vitro and angiogenesis activity. Methods: HepG2 liver cancer cell lines were cultured and divided into the luteolin group that were treated with serum-free medium containing 100 μmol/L luteolin and the normal control group that treated with serum-free medium containing drugs. After 24 h of treatment, MTT kit was used to detect the cell proliferation activity, and fluorescence quantitative PCR kit was used to detect the mRNA expression of pro-proliferation genes, pro-apoptosis genes and angiogenesis molecules. Results: After 24 h of treatment, the cell proliferation activity of luteolin group was significantly lower than that of normal control group; LETM1, URG11, PICK1, CyclinD1, Uba2, VEGF, Fra-1, HIF-1α and Rac1 mRNA expression in luteolin group were significantly lower than those in normal control group while Merlin, AlkBH8, ARID1A and Panx1 mRNA expression were significantly higher than those in normal control group. Conclusion: Luteolin has inhibiting effect on the HepG2 liver cancer cell proliferation in vitro and angiogenesis activity.

1. Introduction

Liver cancer is one of the most common malignant tumors in the digestive system in China. The malignancy of the tumor is high, the proliferation of cancer cells is vigorous and the number of new blood vessels in the lesion increases significantly. In recent years, with the constant development of surgical resection, radiofrequency ablation, targeted drugs and other therapies, the prognosis of patients with liver cancer has been improved to certain extent, but the overall 5-year survival rate is still not ideal, and the recurrence rate and mortality after treatment are high[1,2]. In recent years, the value of traditional Chinese medicinal materials for malignant tumors has attracted more and more attention, and various active components in Chinese medicinal materials have anti-tumor value. Luteolin is a polyphenol flavonoid that is extracted from the Chinese medicinal materials such as honeysuckle and selfheal, and has various biological activities such as anti-inflammation, anti-oxidation and anti-tumor[3,4]. In order to define the luteolin value for liver cancer, we specifically analyzed the effect of luteolin on the HepG2 liver cancer cell proliferation in vitro and angiogenesis activity.

2. Experimental materials and methods

2.1 Experimental materials

HepG2 liver cancer cell lines were bought from ATCC cell company, luteolin was bought Sinopharm Chemical Reagent Co., Ltd., cell culture medium, fetal bovine serum and trypsin were purchased from Gibco company, and Trizol lysate, reverse transcription kit and fluorescence quantitative PCR kit were bought from Invitrogen company.

2.2 Experimental methods

2.2.1 Cell culture and treatment

HepG2 liver cancer cells were cultured within the culture bottle with culture medium containing 5% fetal bovine serum, digested
with trypsin after the cell density reached 80%–90% and then inoculated within the culture bottle for amplification and subculture; the cells after 3-4 times of culture were inoculated in the culture plate and treated, control group were treated with serum-free medium containing no drug, and LUT group were treated with serum-free medium containing 100 μmol/L luteolin.

2.2.2 Cell proliferation activity detecting
Cells were inoculated in 96-well culture plate and treated with different conditions for 24 h, then MTT cell viability test liquid was added in the culture medium, the cells were continuously cultured for 4 h, and the absorbance value at 450 nm wavelength was measured in microplate reader and used as cell proliferation activity value.

2.2.3 Gene expression detecting
Cells were inoculated in 12-well culture plate and treated with different conditions for 24 h, the culture medium was abandoned, the cells were kept and broken with Trizol lysate from Invitrogen company, trichloromethane and isopropanol were used in turn for extraction and centrifuge to get RNA, and cDNA synthesis kit was used to synthesize the RNA into cDNA by reverse transcription. The primers for LETM1, URG11, PICK1, CyclinD1, Merlin, AlkBH8, ARID1A, Panx1, Uba2, VEGF, Fra-1, HIF-1α and Rac1, and fluorescence quantitative PCR kit was used to amplify cDNA, and the mRNA expression was calculated according to amplification curve.

2.3 Statistical methods
SPSS 19.0 software was used for t test of the differences in measurement data between two groups and \( P < 0.05 \) indicated statistical significance in differences in test results.

3. Results

3.1 HepG2 cell proliferation activity
The cell proliferation activity of luteolin group was \( (0.62±0.09) \) and the cell proliferation activity of routine control group was \( (1.27±0.22) \). Analysis of the differences in cell proliferation activity between luteolin group and routine control group by t test showed that the cell proliferation activity of luteolin group was significantly lower than that of normal control group, and the differences in cell proliferation activity were statistically significant between the two groups (\( P < 0.05 \)).

Table 1.
Comparison of pro-proliferation gene expression between luteolin group and routine control group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>LETM1</th>
<th>URG11</th>
<th>PICK1</th>
<th>CyclinD1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luteolin group</td>
<td>6</td>
<td>0.37±0.08</td>
<td>0.22±0.06</td>
<td>0.56±0.09</td>
<td>0.33±0.08</td>
</tr>
<tr>
<td>Control group</td>
<td>6</td>
<td>1.04±0.16</td>
<td>1.01±0.18</td>
<td>0.97±0.14</td>
<td>1.06±0.20</td>
</tr>
<tr>
<td>( t )</td>
<td></td>
<td>18.798</td>
<td>25.685</td>
<td>9.180</td>
<td>21.257</td>
</tr>
<tr>
<td>( P )</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

3.2 Pro-proliferation gene expression in cells
Analysis of pro-proliferation genes LETM1, URG11, PICK1 and CyclinD1 mRNA expression between luteolin group and routine control group was as follows: LETM1, URG11, PICK1 and CyclinD1 mRNA expression in luteolin group were significantly lower than those in normal control group. Differences in LETM1, URG11, PICK1 and CyclinD1 mRNA expression were statistically significant between the two groups (\( P < 0.05 \)).

3.3 Pro-apoptosis gene expression in cells
Analysis of pro-apoptosis genes Merlin, AlkBH8, ARID1A and Panx1 mRNA expression between luteolin group and routine control group was as follows: Merlin, AlkBH8, ARID1A and Panx1 mRNA expression in luteolin group were significantly higher than those in normal control group. Differences in Merlin, AlkBH8, ARID1A and Panx1 mRNA expression were statistically significant between the two groups (\( P < 0.05 \)).

Table 2.
Comparison of pro-apoptosis gene expression between luteolin group and routine control group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Merlin</th>
<th>AlkBH8</th>
<th>ARID1A</th>
<th>Panx1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luteolin group</td>
<td>6</td>
<td>2.74±0.42</td>
<td>3.61±0.56</td>
<td>2.84±0.33</td>
<td>2.02±0.34</td>
</tr>
<tr>
<td>Control group</td>
<td>6</td>
<td>1.04±0.17</td>
<td>0.95±0.13</td>
<td>1.02±0.16</td>
<td>1.07±0.19</td>
</tr>
<tr>
<td>( t )</td>
<td></td>
<td>17.698</td>
<td>24.286</td>
<td>18.3986</td>
<td>12.103</td>
</tr>
<tr>
<td>( P )</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

3.4 Angiogenesis molecule expression
Analysis of angiogenesis molecules Uba2, VEGF, Fra-1, HIF-1α and Rac1 mRNA expression between luteolin group and routine control group was as follows: Uba2, VEGF, Fra-1, HIF-1α and Rac1 mRNA expression in luteolin group were significantly lower than those in normal control group. Differences in Uba2, VEGF, Fra-1, HIF-1α and Rac1 mRNA expression were statistically significant between the two groups (\( P < 0.05 \)).

Table 3.
Comparison of angiogenesis molecule expression between luteolin group and routine control group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Uba2</th>
<th>VEGF</th>
<th>Fra-1</th>
<th>HIF-1α</th>
<th>Rac1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luteolin group</td>
<td>6</td>
<td>0.29±0.06</td>
<td>0.20±0.08</td>
<td>0.58±0.07</td>
<td>0.46±0.09</td>
<td>0.41±0.06</td>
</tr>
<tr>
<td>Control group</td>
<td>6</td>
<td>1.03±0.16</td>
<td>0.98±0.17</td>
<td>1.01±0.15</td>
<td>0.95±0.14</td>
<td>1.05±0.18</td>
</tr>
<tr>
<td>( t )</td>
<td></td>
<td>29.395</td>
<td>34.572</td>
<td>8.586</td>
<td>11.286</td>
<td>13.507</td>
</tr>
<tr>
<td>( P )</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
4. Discussion

The blood supply of hepatocellular carcinoma is abundant and the proliferation activity of cancer cells in the lesion is vigorous, cancer cells are prone to distant metastasis, so the overall prognosis of the disease is not good, and both metastasis rate and mortality are higher. In recent years, the anti-tumor effect of TCM drugs has received more and more attention, and many Chinese medicinal materials and their active components have been proven to have anti-tumor activity. The luteolin is the polyphenol flavonoid extracted from Chinese medicinal herbs such as honeysuckle and selfheal, which has various biological activities such as anti-inflammation, anti-oxidation and anti-tumor. In the study of cytology about solid tumors such as lung cancer, ovarian cancer and prostate cancer, luteolin has been proven to be able to inhibit the proliferation, migration and invasion of many malignant tumor cells[5–7]. In the study, in order to define the luteolin effect on liver cancer cell growth, the liver cancer cell proliferation activity was analyzed after luteolin treatment in the study, and the results showed that the cell proliferation activity of luteolin group was significantly lower than that of normal control group. This indicates that the luteolin has inhibitory effect on the proliferation activity of liver cancer cells.

The high expression of proliferation genes is an important pathological link that causes the enhancement of hepatocellular carcinoma cell proliferation activity. LETM1, URG11 and PICK1 are currently known to be closely related to the proliferation of hepatocellular carcinoma cells. LETM1 is a transmembrane protein with leucine zipper structure in the mitochondria and can adjust the biosynthesis and translation system in mitochondria, which on the one hand, increases the generation of ATP and provides energy for cell proliferation, and on the other hand, activates PI3K/AKT pathway and accelerates cell growth[8]; URG11 is the cell cycle regulator associated with hepatitis B virus X protein, which can promote the development of cell cycle from G1 phase to S phase, and then accelerate cell proliferation; PICK1 is a pro-proliferation molecule with PDZ domain, which can increase the expression of CyclinD1 by downstream signaling molecules such as Notch and Jagged, thus accelerate cell cycle and enhance cell proliferation activity[9]. In the study, analysis of luteolin effect on the pro-proliferation gene expression in liver cancer cells showed that LETM1, URG11, PICK1 and CyclinD1 mRNA expression in luteolin group were significantly lower than those in normal control group. This indicates that the luteolin can inhibit the expression of proliferation genes in hepatocellular carcinoma cells and inhibit cell proliferation.

In addition to the high expression of proliferation genes, the low expression of pro-apoptotic tumor suppressor genes also plays an important role in the abnormal proliferation process of hepatocellular carcinoma cells. Merlin is a tumor suppressor gene that regulates PKA signaling pathway and Wnt/β-catenin signaling pathway, which can inactivate the corresponding signaling pathways, inhibit cell proliferation and promote apoptosis[10,11]; AlkBH8 is the homologous protein of AlkB family that can adjust the methylation levels of various RNA and influence the RNA translation into corresponding protein, and the high expression of AlkBH8 in liver cancer cells can make the cell proliferation activity inhibited[12]; ARID1A is a non-catalytic subunit in the SWI/SNF chromatin remodeling complex, which can recruit transcription factors and promote the expression of tumor suppressor genes, such as p53 and p21 so as to induce apoptosis[13,14]; Panx1 is one of the subtypes of the Pannexin gene, which inhibits the migration and proliferation of cancer cells by affecting intercellular gap junctions[15]. In the study, analysis of luteolin effect on the pro-apoptosis gene expression in liver cancer cells showed that Merlin, AlkBH8, ARID1A and Panx1 mRNA expression in luteolin group were significantly higher than those in normal control group. This indicates that luteolin can increase the expression of apoptosis genes in hepatocellular carcinoma cells and induce apoptosis.

Hepatocellular carcinoma receives blood supply from both portal vein and hepatic artery, and is with abundant blood flow; at the same time, the cancer cells themselves have a strong activity in promoting angiogenesis, and can increase the number of new blood vessels in the lesion. The abundant blood supply of tumor lesions can on the one hand, help enhance the proliferation activity of cancer cells, and on the other hand, also provide a pathway for the metastasis of cancer cells. Uba2 is part of the SUMO activating enzyme, which can promote the SUMO activation, participate in posttranslational regulation of proteins, help stabilize the protein properties of pro-angiogenesis molecules such as VEGF, and ensure that VEGF continuously exerts biological effect of promoting angiogenesis in local lesions[16,17]; Fra-1 and HIF-1α are the transcription factors that regulate angiogenesis, which can promote the formation of new blood vessels by regulating the proliferation, migration and adhesion of endothelial cells[18,19]; Rac1 is a member of the small G protein Rho family, which can facilitate the formation of vascular structures by regulating the formation of the cell filopodia[20,21]. In the study, analysis of luteolin effect on the angiogenesis molecule expression in liver cancer cells indicated that Uba2, VEGF, Fra-1, HIF-1α and Rac1 mRNA expression in luteolin group were significantly lower than those in normal control group. This indicates that the luteolin can inhibit the expression of angiogenesis molecules in hepatocellular carcinoma cells and inhibit the angiogenesis activity of the cells.

Above all, it can be concluded in above cell research that luteolin
can adjust the expression of pro-proliferation and pro-apoptosis genes as well as angiogenesis molecules in hepatocellular carcinoma HepG2 cells and then inhibit hepatocellular carcinoma cell proliferation in vitro and angiogenesis activity.

References


