Effect of cordycepin on non-small cell lung cancer cell line H358 proliferation and related gene expression

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ARTICLE INFO

Objective: To study the effect of cordycepin on non-small cell lung cancer cell line H358 proliferation and related gene expression. Methods: C57BL/6 mice were selected as experimental animals, and the mouse model with transplanted tumor was established after non-small cell lung cancer cell line H358 was cultured; the mice with transplanted tumor were divided into the intervention group who received Corbrin Capsule therapy and the model group who received no drug therapy. 30 d after treatment, the expression of proliferation activity indexes, proliferation genes and invasion genes in transplanted tumor tissue were determined. Results: 30 d after treatment, the mRNA expression and protein expression of PCNA and Ki-67 in transplanted tumor lesion of intervention group were significantly lower than those of model group, and Notch-1, Bmi-1, MIF, Rap2a, NGAL and SIRT1 mRNA expression in transplanted tumor lesion were significantly lower than those of model group while PAQR3, LATS1, E-cadherin, ZO-1 and TES mRNA expression were significantly higher than those of model group. Conclusion: Cordycepin can inhibit the proliferation and invasive growth of non-small cell lung cancer cell line H358 in transplanted tumor tissue.

1. Introduction

Non-small cell lung cancer is the most common pathological type of lung cancer with a high incidence and low early diagnosis rate. After diagnosis, it has mostly developed to mid-advanced stage and missed the chance of surgical resection. Chemotherapy drugs, targeted drugs and traditional Chinese medicine preparations are commonly used in the treatment of advanced lung cancer, and the value of TCM preparations for adjuvant treatment of lung cancer has received more and more attention. Cordycepin is the main active ingredient in *Cordyceps sinensis*, its chemical essence is purine alkaloid, and it has the biological effects such as anti-tumor, immune regulation and free radical scavenging. Modern medical research has confirmed that cordycepin can inhibit the proliferation and invasion of the lung cancer cell lines cultured in vitro[1,2]. Corbrin Capsule is the capsule preparation containing Cordyceps Fungus Powder, the main active ingredient is cordycepin, it is used in the treatment of a variety of lung diseases, but the value of Corbrin Capsule for lung cancer treatment is unclear. In the following study, we cultured lung cancer cell lines H358, established the animal models with transplanted tumor and specifically analyzed the effect of cordycepin on lung cancer cell proliferation and related gene expression in transplanted tumor lesions.

2. Experimental materials and methods

2.1 Experimental materials

Non-small cell lung cancer cell line H358 was purchased from the ATCC Cell Company, and the culture medium and serum for cell culture were purchased in Gibco Company; Corbrin Capsule was purchased from East China Pharmaceutical Co., Ltd; the enzyme-linked immunosorbent assay kit was purchased from Shanghai Westang Company; RNA extraction and RT-PCR kits were purchased from Promega Company.
2.2 Experimental animals

C57BL/6 mice with body mass of 18-22 g were selected as experimental animals and purchased in Beijing Vital River Laboratory Animal Technology Co., Ltd., and the animal license number was SCXK (Beijing) 2006-0009; animal experiments passed the ethical review of the hospital, the procedures were followed for animal experiments and treatment after death. A total of 20 mice were randomly divided into model group and intervention group, 10 in each group.

2.3 Experimental methods

2.3.1 Model establishing and medication

H358 cell lines were cultured and sub-cultured, the sub-cultured amplified cells were collected, the density was adjusted to $10^7 / \text{mL}$, 100 μL tumor cell suspension was injected into the armpit of right forelimb of the mice, and the intervention started when the transplantation tumor lesion was formed after 14 d. Intervention group were given intragastric administration of 5 g/kg Corbrin Capsule, the solution was configured with saline, and the treatment was conducted 1 time a day for 30 consecutive days; model group were given intragastric administration of same dosage of saline, 1 time per day for 30 consecutive days.

2.3.2 Gene mRNA expression detecting

30 d after medication, the mice with transplanted tumor were put to death and anatomicized to get transplanted tumor tissue and add it in Trizol lysate to extract the RNA in the tissue, then RT-PCR kits were used to amplify PCNA, Ki-67, Notch-1, Bmi-1, MIF, PAQR3, LATS1, Rap2a, NGAL, SIRT1, E-cadherin, ZO-1 and TES, and the mRNA expression was calculated according to the amplification curve.

2.3.3 Gene protein expression detecting

Transplanted tumor tissue after 30 d of medication was taken and added in RIPA lysate to extract the protein in the tissue and then centrifuge it to separate the supernatant liquid, and enzyme-linked immunosorbent assay kit was used to detect the protein expression of PCNA and Ki-67.

2.4 Statistical methods

SPSS 18.0 software was used for t test of data between two groups and $P<0.05$ indicated statistical significance in differences in test results.

3. Results

3.1 Proliferation activity index expression in transplanted tumor lesion

Analysis of the mRNA expression and protein expression of proliferation activity indexes PCNA and Ki-67 in transplanted tumor lesion between intervention group and model group was as follows: the mRNA expression and protein expression of PCNA and Ki-67 in transplanted tumor lesion of intervention group were significantly lower than those of model group. Differences in the mRNA expression and protein expression of PCNA and Ki-67 in transplanted tumor lesion were statistically significant between intervention group and model group ($P<0.05$).

3.2 Proliferation gene expression in transplanted tumor lesion

Analysis of proliferation genes Notch-1, Bmi-1, MIF, PAQR3 and LATS1 mRNA expression in transplanted tumor lesion between intervention group and model group was as follows: Notch-1, Bmi-1 and MIF mRNA expression in transplanted tumor lesion of intervention group were significantly lower than those of model group while PAQR3 and LATS1 mRNA expression were significantly higher than those of model group. Differences in Notch-1, Bmi-1, MIF, PAQR3 and LATS1 mRNA expression in transplanted tumor lesion were statistically significant between intervention group and model group ($P<0.05$).

Table 1. Proliferation activity index expression in transplanted tumor lesion.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>mRNA expression</th>
<th>Protein expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PCNA</td>
<td>Ki-67</td>
</tr>
<tr>
<td>Intervention group</td>
<td>10</td>
<td>0.44±0.07</td>
<td>0.35±0.06</td>
</tr>
<tr>
<td>Model group</td>
<td>10</td>
<td>1.04±0.12</td>
<td>0.98±0.16</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>13.218</td>
<td>15.687</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 2. Proliferation gene expression in transplanted tumor lesion.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Notch-1</th>
<th>Bmi-1</th>
<th>MIF</th>
<th>PAQR3</th>
<th>LATS1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention group</td>
<td>10</td>
<td>0.28±0.05</td>
<td>0.46±0.07</td>
<td>0.37±0.06</td>
<td>2.49±0.35</td>
<td>1.77±0.23</td>
</tr>
<tr>
<td>Model group</td>
<td>10</td>
<td>1.03±0.15</td>
<td>0.99±0.11</td>
<td>1.01±0.14</td>
<td>1.06±0.18</td>
<td>0.95±0.13</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>27.687</td>
<td>13.219</td>
<td>18.592</td>
<td>13.485</td>
<td>8.704</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>
Ki-67 are markers of cell proliferation activity. Both are involved in the growth of non-small cell lung cancer. PCNA and Kir8.1 were selected as the study objects, and Corbrin Capsule intervention was provided to clarify the effect of Corbrin Capsule on the growth of non-small cell lung cancer. In the study, the mice with transplanted non-small cell lung cancer were statistically significant between intervention group and model group (P<0.05).

3.3 Invasion gene expression in transplanted tumor lesion

Analysis of invasion genes Rap2a, NGAL, SIRT1, E-cadherin, ZO-1 and TES mRNA expression in transplanted tumor lesion between intervention group and model group was as follows: Rap2a, NGAL and SIRT1 mRNA expression in transplanted tumor lesion of intervention group were significantly lower than those of model group while E-cadherin, ZO-1 and TES mRNA expression were significantly higher than those of model group. Differences in Rap2a, NGAL, SIRT1, E-cadherin, ZO-1 and TES mRNA expression in transplanted tumor lesion were statistically significant between intervention group and model group (P<0.05).

4. Discussion

Non-small cell lung cancer is one of the most common malignant tumors in our country, its early diagnosis rate is low, and the majority of patients have developed the middle-advanced tumor when diagnosed, and needs chemotherapy and targeted therapy to kill cancer cells and prolong survival time. The value of TCM drugs for lung cancer has received more and more attention, and the Cordyceps Fungus Powder, cordycepin is the main active ingredient in Cordyceps Fungus Powder, cordycepin can inhibit cell proliferation by the change of HIPPO/YAP pathway function and anchor the intracellular free Raf kinase in dictyosome, and its biological function is to anchor the intracellular free Raf kinase in dictyosome and suppress the activation of downstream MEK to inhibit cell proliferation activity; the products encoded by LATS1 gene have negative regulating effect on c-myc, which can accelerate the development of downstream MEK to inhibit cell proliferation activity; the products encoded by LATS1 gene have negative regulating effect on HIPPO/YAP signaling pathway, which can inhibit cell proliferation by the change of HIPPO/YAP pathway function.[13,14]. In the study, analysis of the above proliferation-related gene expression within the transplantation tumor lesions showed that Notch-1, Bmi-1 and MIF mRNA expression in transplanted tumor lesion of intervention group were significantly lower than those of model group while PAQR3 and LATS1 mRNA expression were significantly higher than those of model group. It means that Corbrin Capsule can adjust the proliferation-related gene expression in transplanted non-small cell lung cancer lesions, which is characterized by inhibiting the pro-proliferation gene expression and increasing the anti-proliferation gene expression.

On the basis of proliferation, the cancer cells in non-small cell lung cancer lesions will constantly infiltrate towards the surrounding tissue, invasive growth is the biological basis of tumor lesion infiltration, and the abnormal expression of a variety of pro-invasion genes and anti-invasion genes is associated with the aggressive growth of cancer cells. Rap2a is a kind of GTP-binding protein involved in the regulation of cell differentiation, intracellular transport and cytoskeleton form, and the molecule can promote cell invasion by downstream PI3K pathway and ERK pathway in the process of the malignant tumor formation.[15]; NGAL is a kind of glycoprotein in lipocalin family[16], SIRT1 is an important signal transduction molecule in cells[17,18], and both are involved in regulating cellular epithelial mesenchymal transition, and can proliferation in transplanted non-small cell lung cancer lesions, and it also confirms that cordycepin can inhibit the growth of transplanted non-small cell lung cancer lesions.

The proliferation of non-small cell lung cancer is related to the abnormal expression of many pro-proliferation genes and anti-proliferation genes. Notch-1, Bmi-1, MIF are genes that promote proliferation. The products encoded by Notch-1 gene have synergistic effect on c-myc, which can accelerate the development of cell cycle and promote cell proliferation[7,8]; Bmi-1 can on the one hand, regulate telomerase activity to maintain cell proliferation activity, and on the other hand, inhibit tumor suppressor gene function and prolong cell life[9,10]; the pro-proliferation effect of MIF, on the one hand, depends on the inhibition on tumor suppressor gene p53, and on the other hand, relies on the enhancement on the ERK proliferation signaling pathway[11,12]. PAQR3 is the PAQR gene family member, the products encoded by it are the seven-span transmembrane proteins locating in dictyosome, and its biological function is to anchor the intracellular free Raf kinase in dictyosome and suppress the activation of downstream MEK to inhibit cell proliferation activity; the products encoded by LATS1 gene have negative regulating effect on HIPPO/YAP signaling pathway, which can inhibit cell proliferation by the change of HIPPO/YAP pathway function[13,14]. In the study, analysis of the above proliferation-related gene expression within the transplantation tumor lesions showed that Notch-1, Bmi-1 and MIF mRNA expression in transplanted tumor lesion of intervention group were significantly lower than those of model group while PAQR3 and LATS1 mRNA expression were significantly higher than those of model group. It means that Corbrin Capsule can adjust the proliferation-related gene expression in transplanted non-small cell lung cancer lesions, which is characterized by inhibiting the pro-proliferation gene expression and increasing the anti-proliferation gene expression.
regulate the expression of E-cadherin and ZO-1 and induce epithelial phenotype transition to mesenchymal phenotype to promote cell invasion; E-cadherin and ZO-1 are the important molecules that maintain close epithelial intercellular tight junction, and they are also markers of epithelial phenotype and can promote intercellular adhesion and inhibit cell invasion.19,20 TES is a tumor suppressor gene that inhibit invasion and migration, the carboxyl terminal of its encoded products has three tandem LIM structural domains, and the molecule can inhibit cell invasion by LIM structural domain21.

In the study, analysis of the changes in above invasion-related gene expression within the transplantation tumor lesions showed that Rap2a, NGAL and SIRT1 mRNA expression in transplanted tumor lesion of intervention group were significantly lower than those of model group while E-cadherin, ZO-1 and TES mRNA expression were significantly higher than those of model group. It means that Corbrin Capsule can adjust the invasion-related gene expression in transplanted non-small cell lung transplantation lesions, which is specifically characterized by inhibiting the pro-invasion gene expression and increasing the anti-invasion gene expression.

In the study, Corbrin Capsule was selected as the drug delivery system of cordycepin and the mice models with transplanted tumor were selected as the research objects to specifically analyze the cordycepin influence on non-small cell lung cancer cell line growth, and the preliminary conclusions are as follows: cordycepin has inhibiting effect on the transplanted tumor tissue of non-small cell lung cancer cell lines H358, which is specifically characterized by inhibiting cancer cell proliferation and invasion.

References
