Study on the mechanism of the anti-tumor effects of matrine via regulation of lipid metabolism mediated by SREBP signaling pathway

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Abstract

Objective: To investigate the therapeutic effects of matrine on CT26 tumor-bearing mice and its effect on the sterol regulatory element binding protein (SREBP) signaling pathway.

Methods: A CT26 tumor-bearing mouse model was established using CT26 cells. Different doses of matrine were orally administered to mice, and the tumor size and weight in each group of mice before and after administration were measured to calculate the tumor inhibition rate of matrine. Subsequently, tumor tissues were subjected to hematoxylin and eosin (HE) staining to observe morphological changes in tumor tissue, and quantitative polymerase chain reaction (qPCR) was performed to detect the expression of the genes of the lipid metabolism-related enzymes sterol regulatory element binding transcription factor 1 (Srebf1), ATP citrate lyase (Acly), acetyl-Coenzyme A carboxylase alpha (Acc), and fatty acid synthase (Fasn) in tumor tissues before and after matrine intervention. Results: Compared to those in the model group, tumor-bearing mice in both the low- and high-dose matrine groups showed significantly reduced tumor weights. HE staining showed that matrine significantly inhibited tumor cell proliferation in both the low- and high-dose groups. The qPCR results showed that, compared with the model group, the expression levels of the genes of lipid metabolism-related enzymes Srebf1, Acly, Acc, and Fasn in tumor tissues were significantly downregulated in both the low- and high-dose matrine groups. Conclusion: Matrine modulates the lipid metabolism pathway, affects tumor cell lipid metabolism, and exerts antitumor effects.

Keywords: Matrine; CT26; tumor; lipid metabolism; SREBP.
**Introduction**

Global malignancy incidence and mortality rates are increasing every year, and according to the American Cancer Society, there were approximately 18.1 million new cases of cancer and 9.6 million cancer deaths worldwide in 2018 [1]. According to the latest data from the National Cancer Center of China, there were 3.929 million new cases of malignant tumors and 2.338 million deaths in China in 2015 [2].

Current conventional treatments for malignant tumors include surgery, chemotherapy, and radiation therapy. Surgery is suitable for patients with early, intermediate, and localized tumors; however, in most patients with malignant tumors, the disease would have progressed to an advanced stage at the time of diagnosis, and thus the optimal surgical timing is often missed. Although chemotherapy and radiotherapy can suppress disease progression to a certain extent, several problems remain, such as recurrence, metastasis, and reduced quality of life for patients. Therefore, the development of new antitumor drugs and elucidation of their mechanisms of action have important clinical significance.

Research has shown that many traditional Chinese medicines with antitumor effects exert their effects by regulating lipid metabolism. The active ingredients of Polygonum cuspidatum, such as emodin and other anthraquinones, can downregulate the gene and protein expression levels of Sterol regulatory element binding protein 1 (SREBP1) in liver cancer cells, thereby inhibiting liver cancer cell proliferation and exerting antitumor effects [3]. Additionally, rhein inhibits Sterol regulatory element binding protein (SREBP) transcription and downregulates the expression of SREBP and its target genes, thereby reducing the triglyceride content in liver cancer cells and alleviating hepatocyte steatosis [4].

Studies have shown that tumor cells must uptake and synthesize large amounts of lipid substances to meet their energy requirements as well as for cell membrane synthesis [5, 6]. Studies have shown that tumor cells have defects in glycolipid metabolism [7, 8]. Specifically, tumor cells frequently show increased glucose uptake and enhanced glycolytic activity and lactate accumulation, a phenomenon referred to as the Warburg effect [9], which provides a large amount of intermediate products for the de novo synthesis of fatty acids. Moreover, tumor cells exhibit rapid lipid synthesis, and the activities of glucose transporter proteins and enzymes involved in regulating fatty acid synthesis, such as sterol regulatory element binding transcription factor 1 (Srebf1), ATP citrate lyase (Acly), acetyl-Coenzyme A carboxylase alpha (Acc), and fatty acid synthase (Fasn), are significantly increased.

In this study, we established a CT26 tumor-bearing mouse model and administered different doses of matrine via gavage. We measured the tumor weight and tumor inhibition rate in each group of mice after drug administration to explore the therapeutic effect of matrine on CT26 tumor-bearing mice. Additionally, we performed hematoxylin and eosin (HE) staining on the tumor tissues of each group of mice to examine the pathological changes in the tumor tissue. Subsequently, we performed quantitative polymerase chain reaction (qPCR) to detect the expression of lipid metabolism-related genes in tumor tissues to investigate the effect of matrine on lipid metabolism in the tumor tissues of tumor-bearing mice.

The animal experiments conducted in this study were approved by the Committee on Clinical Scientific Research and Ethics of Experimental Animals of the First Hospital of Fuzhou City, Fujian Medical University (approval number: 202110015). All procedures involving animals were performed in accordance with internationally recognized principles and guidelines for the use and care of laboratory animals, as outlined in the Guide for the Care and Use of Laboratory Animals: Eighth Edition, published by The National Academies Press in Washington, DC.

**Main reagents**

CT26 cells (colon cancer cells) were purchased from BeNa Culture Collection. Matrine (CAS:519-02-8) was purchased from Sichuan WeiKeqi Biological Technology Co. Ltd. Cyclophosphamide (D025770) was purchased from the national standard substance resource platform. A total RNA extraction kit (DP419-02), cDNA First Strand Synthesis kit (KR116-02), and SYBR Green Real-Time RT-PCR kits were purchased from Tiangen Biotechnology Co.

**CT26 Tumor-bearing mouse model establishment and grouping**

Forty 8-week-old healthy male C57 BL/6 mice weighing 20 ± 2 g were purchased from Beijing Huafu Kang Biotechnology Co. The mice were reared at 25 ± 2°C under a relative humidity of 50 ± 15% and light and dark cycles of 12 h, and provided free access to water and food. The study was approved by the First Hospital of Fuzhou City, Fujian Medical University (approval number: 2021110015).

After one week of adaptive feeding, a CT26 cell suspension (0.2 mL, 1 × 10⁶ cells/mouse) was injected into the armpit of each mouse and tumor growth was observed every 3 days. The model was established approximately one week later. The mice were randomly divided into four groups: model, positive control, saline low-dose (Matrine-L), and saline high-dose (Matrine-H) (n = 10 in each group). The model group received 0.2 mL of normal saline daily by gavage. The positive control group received 25 mg/kg cyclophosphamide daily by gavage. The Matrine-L group received 25 mg/kg matrine by gavage daily after modeling. The Matrine-H group received 50 mg/kg matrine by gavage daily after modeling. All treatments were continuously administered by gavage for 14 days.

**Tumor tissue growth**

On day 14, mice were euthanized 2 h after drug administration. The tumor tissues were washed slightly in physiological saline to remove residual blood, gently dried with absorbent paper to remove excess water, weighed, and the data were recorded to calculate the tumor inhibition rate using the following formula: tumor inhibition rate = (average tumor weight of model group - average tumor weight of experimental group) / average tumor weight of model group × 100%.

**Pathological staining**

The pathological tissues fixed with 4% paraformaldehyde were cut into paraffin sections, stained with HE, and observed under a light microscope for pathological changes in the tumor tissues.

**qPCR assay**

Total RNA was extracted from the tumor tissues of each group of mice using the RNA extraction kit, and the purity and concentration of the extracted RNA were determined. Subsequently, the cDNA was reverse-transcribed to obtain cDNA. The mRNA expression levels of Srebf1, Acly, Acc, and Fasn were measured in the tumor tissues of each group of mice using cDNA as a template and Actb as the internal reference gene. Primer sequences are listed in Table 1, in 2⁻ΔΔCT method to calculate mRNA relative expression was calculated.

**Statistical methods**

The experimental results were analyzed using SPSS Statistics 20.0 statistical software, and the measurement data were expressed as X±S. The t-test and one-way analysis of variance were used for comparison of means between multiple groups. Statistical significance was set at p < 0.05.

**Results**

Effect of matrine on tumor volume and tumor suppression rate in tumor-bearing mice
At the end of the administration period, we observed that the tumor volume was the highest in the model group. Compared with the model group, the positive control \((p<0.01)\), Matrine-L \((p<0.01)\), and Matrine-H \((p<0.01)\) groups showed varying degrees of tumor reduction (Figure 1). As shown in Table 2, the positive control group exhibited the most significant tumor-suppressive effect. After matrine treatment, the high-dose group showed the best effect.

**Effect of matrine on histopathological changes of tumors in tumor-bearing mice**

HE staining showed that the tumor cells in the model group were neatly arranged without edema or breakage and were closely spaced. Following drug intervention, tumor cells showed varying degrees of change, with a disorganized tumor cell arrangement, increased cell spacing, and large amounts of vacuole-like degeneration and tumor cell necrosis (Figure 2).

### Table 1 Primer sequences

<table>
<thead>
<tr>
<th>Genes</th>
<th>Full names</th>
<th>Sequence (5'-&gt;3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actb</td>
<td>Actin, beta</td>
<td>F: CCCCTGAACCCTAAGGCCA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: ATGGCTAGTGATCTGGGCTGG</td>
</tr>
<tr>
<td>Srebf1</td>
<td>Sterol regulatory element binding transcription factor 1</td>
<td>F: ACTGGACACACGGGTCTTTGA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: CTCAGGAGATTCGGCACCCTG</td>
</tr>
<tr>
<td>Acly</td>
<td>ATP citrate lyase</td>
<td>F: CAGCTATGCCCCAAGGAAGGAAGA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: CGTCTCAGGACACACGCTAG</td>
</tr>
<tr>
<td>Fasn</td>
<td>Fatty acid synthase</td>
<td>F: GAGGCGAGGATAGACACGCT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GCCTCAGGAGGATTTGGCCTG</td>
</tr>
<tr>
<td>Acc</td>
<td>A6cetyl-Coenzyme A carboxylase alpha</td>
<td>F: AGATCTACCCAGCCACAT</td>
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</table>

### Table 2 Tumor inhibition rate in each group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tumor weight (g)</th>
<th>Tumor suppression rate (%)</th>
</tr>
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<tbody>
<tr>
<td>Model</td>
<td>1.69±0.08</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1.20±0.16 **</td>
<td>29.06%</td>
</tr>
<tr>
<td>control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matrine-L</td>
<td>1.50±0.11 **</td>
<td>11.29%</td>
</tr>
<tr>
<td>Matrine-H</td>
<td>1.26±0.07**</td>
<td>25.60%</td>
</tr>
</tbody>
</table>

Note:** compare with Model group \(P<0.01\).
Tumor tissues were harvested from each group of mice, and the mRNA expression levels of the genes Srebf1, Acly, Acc, and Fasn, which are involved in fatty acid synthesis, were analyzed in the tumor tissues. The results showed that, compared with the model group, gene expression levels of Srebf1 (p < 0.01), Acly (p < 0.01), Acc (p < 0.01), and Fasn (p < 0.01) in the positive control group were significantly downregulated in tumor tissues; gene expression levels of Acly (p < 0.05) and Fasn (p < 0.05) were significantly downregulated in the tumor tissues of tumor-bearing mice in the Matrine-L group; and gene expression levels of Srebf1 (p < 0.01), Acly (p < 0.01), Acc (p < 0.01), and Fasn (p < 0.01) were significantly downregulated in the tumor tissues of tumor-bearing mice in the Matrine-H group (Figure 3).

Discussion

Matrine is an alkaloid present in Sophora flavescens, a plant used in traditional Chinese medicine, and is often used in clinical practice as a matrine/oxymatrine injection to treat digestive system tumors [10]. Clinical studies have shown that matrine combined with chemotherapy significantly improves the efficacy of advanced colon cancer treatment and reduces the toxic side effects of chemotherapy [11]. Compared with monotherapy, the use of compound matrine injections combined with chemotherapy drugs can improve the short-term efficacy of advanced colorectal cancer treatment and improve the quality of life of patients [12]. Matrine has been reported to inhibit the proliferation of human colon cancer cell lines in vitro, and its mechanism may be associated with cell cycle arrest and apoptosis induction [13]. Furthermore, matrine inhibits the growth of K-ras mutant colon cancer cells by inhibiting the p38 signaling pathway to suppress the proliferation and invasion of colon cancer cells [14, 15]. Additionally, matrine has been reported to significantly inhibit the growth of H22 tumor cells and improve the survival of H22 sarcoma mice [16].

This study investigated the therapeutic effects of matrine in colon cancer-bearing mice and its effect on pathological changes in tumor
tissues. The results showed that matrine effectively inhibited the growth of tumor tissue in colon cancer-bearing mice, with tumor inhibition rates of 11.29% and 25.61% in the low- and high-dose matrine groups, respectively. Furthermore, HE staining showed that the degree of cell necrosis in the tumor tissues of both the low- and high-dose matrine groups was more severe, suggesting that matrine can inhibit the proliferation of tumor cells and alter the pathological characteristics of tumor tissues in colon cancer-bearing mice.

qPCR was performed to examine the changes in the mRNA expression levels of lipid metabolism-related genes in the tumor tissue of colon cancer-bearing mice in each group and to explore the effect of matrine on the SREBP signaling pathway in tumor tissue. Numerous studies have shown that the SREBP signaling pathway is closely associated with lipid metabolism disorders in tumor cells [17]. SREBPs are a class of transcription factors that regulate de novo fatty acid synthesis and cholesterol uptake in mammalian cells [18]. SREBP1c in mammalian cells include SREBP1-a, SREBP1-c, and SREBP-2, which are encoded by the SREBP1 and SREBP2 genes. Srebf1 encodes SREBP1-a and SREBP1-c by selectively transcribing the start site [19, 20], whereas Srebf2 encodes SREBP-2 [21]. Studies have shown that SREBP-related gene knockout can inhibit fatty acid synthesis in colon cancer cells, and compared to normal tumor-bearing mice, tumor growth is significantly reduced in tumor-bearing SREBP1c gene knockout mice, suggesting that inhibiting SREBP can inhibit fatty acid synthesis in tumor cells and thus inhibit tumor development [22]. The results of this study show that matrine can downregulate the mRNA expression levels of de novo fatty acid synthesis-related genes, including Srebf1, Acly, Acc, and Fasn, in the tumor tissue of colon cancer-bearing mice.

In summary, we conclude that matrine exerts its antitumor effects by modulating the SREBP signaling pathway and affecting tumor cell lipid metabolism. However, this study has some limitations. In future studies, Oil Red O staining and western blotting should be used to further evaluate lipid accumulation and lipid metabolism-related protein expression in tumor tissues after matrine treatment.

References


