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Original Research

Sesamolin exerts anti-proliferative and apoptotic effect on human colorectal cancer cells via inhibition of JAK2/STAT3 signaling pathway

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Abstract: Colorectal cancer (CRC) is a common malignant tumor that seriously threatens human health and quality of life. At present, the search for safe and more effective treatment for CRC has become necessary. The present study investigated the anti-proliferative and apoptotic effects of sesamolin on human colorectal cancer (HCT116) cells, and the underlying mechanism. Cell proliferation was determined using MTT assay, while the expressions of JAK2, STAT3 and p-STA3 were determined using Western blotting. The levels of expression of matrix metalloproteinases-1, 2 and 9 (MMP1, MMP2 and MMP9) were determined using real-time quantitative polymerase chain reaction (qRT-PCR). The degree of migration and invasion of the cells was assessed using wound healing assay. The results of MTT assay showed that sesamolin significantly and time- and dose-dependently inhibited the proliferation of HCT116 cells ($p < 0.05$). Treatment of HCT116 cells with sesamolin significantly inhibited their migratory ability ($p < 0.05$). The expressions of p-JAK2 and p-STAT3 were significantly down-regulated 48 h after 20 µM of JAK2 specific inhibitor (AG490) was added to HCT116 cells ($p < 0.05$). The expression of p-STAT3 was also significantly and dose-dependently down-regulated 6 h after treatment of HCT116 cells with sesamolin ($p < 0.05$). Sesamolin and AG490 had synergistic effect and their combination significantly down-regulated the expression of p-STAT3, when compared with sesamolin alone ($p < 0.05$). Treatment of HCT116 cells with sesamolin significantly and dose-dependently reduced the levels of IL-6-induced expressions of MMP-1, MMP-2 and MMP-9 ($p < 0.05$). These results suggest that sesamolin induces apoptosis in HCT116 cells and prevents cell invasion via inhibition of the JAK2/STAT3 signaling pathway.

Key words: Colorectal cancer; Sesamolin; JAK2/STAT3 pathway; Metastasis; Expression.

Introduction

Colorectal cancer (CRC) is a common malignant tumor of the digestive tract, and its pathogenesis is linked to several factors (1). The incidence and mortality of CRC are on the increase due to changing lifestyle, preference for Western-type diet, and deterioration of the environment (2). Colorectal cancer (CRC) metastasizes easily. The main cause of death in CRC patients is distant metastasis rather than the primary tumor itself (3-4). Surgery, radiotherapy and chemotherapy are the common treatments for CRC. Postoperative patients are usually faced with poor quality of life, high recurrence and metastasis, drug resistance and other toxic side effects. Treatment of CRC with Traditional Chinese Medicine (TCM) effectively improves the immunity and quality of life of patients, and reduces pain and toxicity (5).

Sesamum indicum L., an important oil crop, is one of the oldest oil crops known (6). Although the plant is widely cultivated in temperate regions of the world, China remains the major producer of sesame. The plant is rich in fat, protein, carbohydrates, calcium, magnesium, iron, phosphorus and dietary fiber (1, 2). Sesamolin is a bioactive compound present in sesame, and it exerts very potent antioxidant properties (7). The relative abundance values of sesamolin in sesame extract and oil are 0.1 - 0.3 % and 0.2 - 0.6 %, respectively (8). Sesamolin is an important antioxidant lignan with varied pharmacological activities such as anti-hypercholesterolemic, antihypertensive and antitumor effects (9). However, not much is known about its antitumor activity against human colon cancer (HCT-116) cells. Signal transduction and activator of transcription 3 (STAT3) is highly expressed in cancer cells, and the over-activation of JAK2/STAT3 signaling pathway is linked to malignant transformation of various tumors (10). The pharmacological effect of sesamolin on JAK2/STAT3 signaling pathway in HCT116 cells has not been fully elucidated. The present study investigated the anti-proliferative and apoptotic effects of sesamolin on HCT116 cells, and the underlying mechanism.

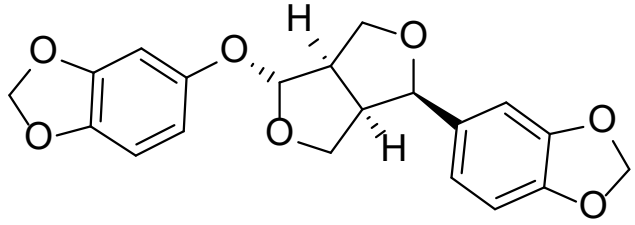

Figure 1. Chemical structure of sesamolin.

Materials and Methods

Drugs and main reagents

Sesamolin was purchased from Nagara Science (Japan); AG490 was obtained from Tocris Bioscience Company, and interleukin 6 (IL-6) was purchased from Sigma (USA). Bicinchoninic acid (BCA) kit was a product of Biyuntian Biotechnology Institute. Rabbit anti-human JAK2, STAT3, p-STAT3, β-actin and horseradish peroxidase-labeled goat anti-rabbit IgG were purchased from Cell Signaling Technology Co., Ltd. Human colorectal cancer cell line (HCT116) was obtained from American Type Culture Collection (ATCC, USA), while RPMI 1640 medium, FBS and Applied Biosystems 7300 RT- PCR machine were products of Life Technologies (USA). Trizol reagent was obtained from Favorgen Biotech Co., (Taiwan), and ReverTra Ace Q PCR RT Master Mix kit was a product of Toyobo (Japan). The PCR primers were purchased from Qiagen (USA), while SYBR Green PCR mixture was obtained from Applied Biological Material (Canada).

Cell culture

The HCT116 cells were cultured in RPMI 1640 medium supplemented with 10 % FBS and 1 % penicillin/streptomycin solution at 37 °C and 10 % CO_2. The resultant adherent cells were trypsinized and centrifuged at 1000 rpm for 10 min, and the cell mass was then reduced to single cell suspension which was inoculated in a 10 cm culture dish at a density of 5 x 10^8 cells/L and incubated. When the cells attained 60 - 70 % confluency, they were treated with serum-free medium and varied concentrations of sesamolin for 24 h. Normal cell culture without sesamolin served as control group. Appropriate concentrations of sesamolin and AG490 were combined and used to treat a separate group of HCT116 cells.

MTT assay

This was performed to determine the proliferative activity of HCT116 cells. The cells (2 × 10^8 cells/well) were seeded into 96-well plates and cultured in DMEM for 24 h. Sesamolin (0 - 40 μM) was added to the cells and incubated for 3 days. At the end of the third day, 20 μl of 5 g/L MTT solution was added to the wells, followed by incubation for another 4 h. The medium was finally replaced with 150 mL of 0.1 % dimethyl sulfoxide (DMSO) solution, agitated at 50 oscillations/min for 10 min, and absorbance of the samples was read in a microplate reader at 540 nm (11). The degree of proliferation was determined at different time points: 24 h, 36 h and 48 h.

Wound healing assay

Cells in logarithmic growth phase (2 x 10^8 cells/well) were seeded into 6-well plates until they attained 80 % confluency, and scratches were made on cell monolayers. After washing twice with phosphate-buffered saline (PBS), the cells were treated with varied concentrations of sesamolin and incubated for 24 h, after which they were observed and photographed. The photomicrographs were analyzed using Image J software.

Western blotting

The HCT116 cells were treated with varied doses of sesamolin for 6 h, and 5 μM AG490 for 48 h. Changes in expression levels of JAK2, STAT3 and p-STA3 were determined using Western blotting. The cells were inoculated in a 10 cm culture dish at a density of 5 x 10^8/L. Cells in logarithmic growth phase were collected and randomly assigned to five groups: normal control group, positive control group (5 μM AG490), 5 μM sesamolin group, 20 μM sesamolin group, and combined drug (5 μM AG490 + 20 μM sesamolin) treatment group. Before collecting the cells, each group was treated with 25 μg/L IL-6 for 15 min. The cells were washed with PBS and ice-cold radio-immunoprecipitation assay buffer (RIPA) containing protease inhibitor was used to lyse them. The resultant lysate was centrifuged at 12, 000 rpm for 10 min at 4 °C, and the protein concentration of the supernatant was determined using BCA assay kit. A portion of total cell protein (30 μg) from each sample was separated on a 12 % sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis and transferred to a fixed polyvinylidene fluoride membrane at 110 V and 90 ° C for 120 min. Subsequently, non-fat milk powder (3 %) in Tris-buffered saline containing 0.2 % Tween-20 (TBS-T) was added with gentle shaking at 37 °C and incubated to block non-specific binding of the blot. Incubation of the blots was performed overnight at 4 °C with primary antibodies of JAK2, p-JAK2, STAT3, p-STAT3 and β-actin at a dilution of 1 to 1000. Then, the membrane was washed thrice with TBS-T and further incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody for 1.5 h at room temperature. The blot was developed using an X-ray film. Grayscale analysis of the bands was performed using Bio-rad gel imaging system Respective protein expression levels were normalized to that of β-actin which was used as a standard.

qRT-PCR

The cells were treated with varied doses of sesamolin for 48 h, and the expression levels of MMP1, MMP2 and MMP9 in HCT116 cells were measured using qRT-PCR. Trizol RNA extraction reagent was used to extract total RNA from cells of each group, while cDNA synthesis kit was used to perform cDNA synthesis reaction according to the instructions of the manufacturer. Light Cycler 1536 RT-PCR detection system was used for the estimation of the mRNA expressions of MMP1, MMP2 and MMP9. Variation in the cDNA content was normalized using glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The PCR reaction mixture (20 μl) consisted of 6.4 μl of dH_2O, 1.6 μl of gene-specific primer (10 μM), 2 μl of synthesized cDNA and 10 μl of SYBR Premix Ex Taq™ II. The Ct value of U6 was taken as the internal parameter and $2^{-\Delta\Delta Ct}$ was used to calculate the relative expression levels of the proteins.

Statistical analysis

Data are expressed as mean ± SD, and the statistical analysis was performed using GraphPad Prism (7.0). Groups were compared using Student *t*-test. Values of $p < 0.05$ were considered statistically significant.

Results

Effect of sesamolin on the proliferation of HCT116 cells

The results of MTT assay (Figure 2) showed that sesamolin significantly and time- and dose-dependently inhibited the proliferation of HCT116 cells ($p < 0.05$).

Effect of sesamolin on migration of HCT116 cells

Treatment of HCT116 cells with sesamolin significantly inhibited their migratory ability ($p < 0.05$). The wound healing ability of the cells treated with sesamolin was significantly and dose-dependently decreased ($p < 0.05$). These results are shown in Figure 3.

Effect of sesamolin on the expressions of JAK2/STAT3 proteins in HCT-116 cells

The expressions of p-JAK2 and p-STAT3 were significantly down-regulated 48 h after AG490 (20 μM) was added to HCT116 cells ($p < 0.05$). The expression of p-STAT3 was also significantly and dose-dependently down-regulated 6 h after treatment of HCT116 cells with sesamolin ($p < 0.05$). Sesamolin and AG490 had synergistic effect and their combination significantly down-regulated the expression of p-STAT3, when compared with sesamolin alone (Figure 4).

Effect of sesamolin on IL-6-induced MMPs expression

As shown in Figure 5, treatment of HCT116 cells with sesamolin significantly and dose-dependently reduced the IL-6-induced expressions of MMP-1, MMP-2 and MMP-9 ($p < 0.05$).

Discussion

Colorectal cancer (CRC), one of the most common malignant tumors of the gastrointestinal tract (GIT), is ranked second in the hierarchy of tumors in terms of prevalence. The predilection sites are rectum, and the junction of rectum and sigmoid colon. Most cases of CRC occur in adults aged 40 years and above, and the ratio of male to female is 2:1 (12). Surgery is the main treatment for CRC. Due to the fact that the disease has a quiet onset which prevents early diagnosis and treatment, it usually enters middle and advanced stage (13, 14). Therefore, the search for new drugs that can effectively prevent and treat CRC has become necessary.

Sesamolin, an antioxidant molecule found in sesame, regulates fatty acid metabolism (15, 16). It has anti-hypercholesterolemic, antihypertensive, and anti-tumor activities. However, reports on its effect on CRC are scanty. This study investigated the anti-proliferative and apoptotic effects of sesamolin on HCT116 cells, and the underlying mechanism.

The results of MTT assay showed that sesamolin significantly and time- and dose-dependently inhibited the proliferation of HCT116 cells. Signal transduction and activator of transcription 3 (STAT3) is a proto-oncogene that has been implicated in many malignant tumors. The STAT3 pathway is the major pathway of cancer-related inflammation, which induces the release of a variety of inflammation-related molecules (17). The activation and overexpression of STAT3 are linked to initiation of tumorigenesis (18, 19). Janus protein tyrosine kinase (JAK)/(STAT) pathway is a signal transduction pathway which transmits signals under the stimulation of cytokines *in vivo* (20). At present, JAK2/STAT3 signaling pathway has been reported to regulate the proliferation, differentiation and apoptosis of cancer cells, especially CRC (21, 22). During the activation of JAK/STAT pathway, IL-6 binds to its receptor (IL-6Ralpha), and activates JAK in the cytoplasm. The resultant phosphorylation of STAT activates JAK2/STAT3 pathway,

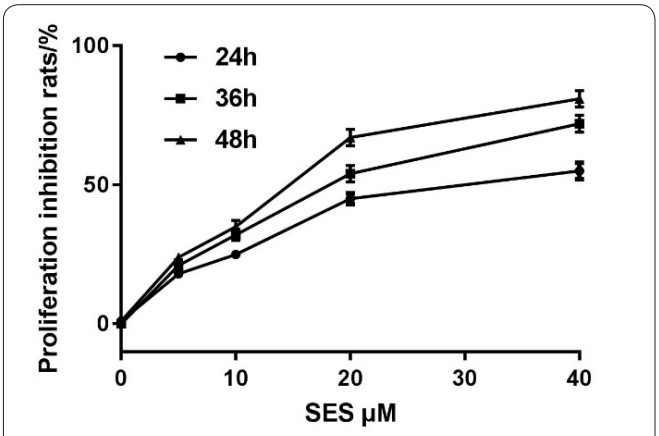

Figure 2. Effect of sesamolin on HCT116 cell proliferation.

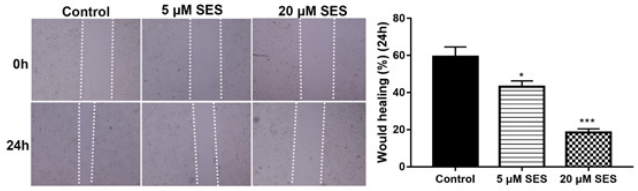

Figure 3. Effect of sesamolin on the migration of HCT116 cells $^{*}p < 0.05$ & $^{***}p < 0.001$, when compared with normal control group.

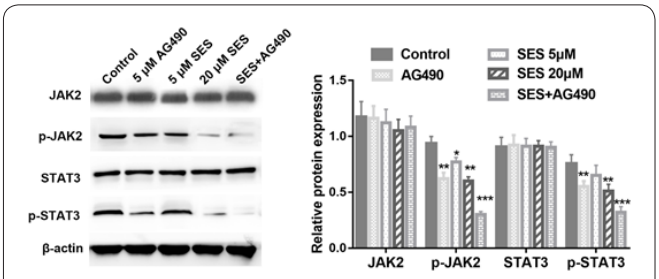

Figure 4. Effect of sesamolin on JAK2, STAT3 and p-STAT3 expressions in HCT116 cells. $^{*}p < 0.05$, $^{**}p < 0.01$ & $^{***}p < 0.001$, when compared with normal control group.

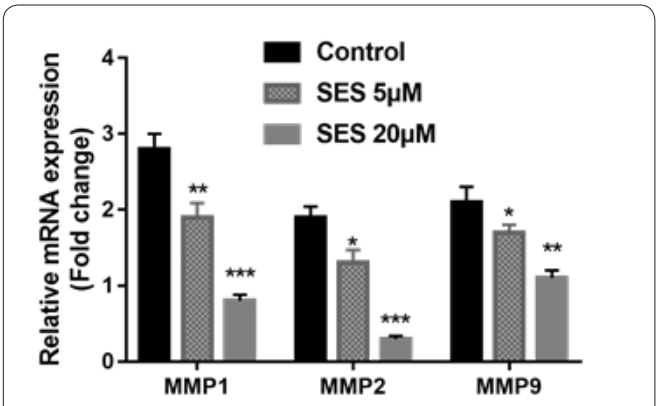

Figure 5. Effect of sesamolin on the expressions of MMP-1, MMP-2 and MMP-9 in HCT116 cells $^{*}p < 0.05$, $^{**}p < 0.01$ & $^{***}p < 0.001$, when compared with normal control group.

which in turn regulates tumor invasion and migration (23, 24). It has been reported that early phosphorylation of STAT3 occurs 6 h after drug induction (25). The results of this study showed that sesamolin significantly reduced the expression of p-STAT3 in HCT-116 cells.

In this study, the activation of JAK2/STAT3 pathway was blocked in HCT116 cells treated with AG490. In addition, sesamolin treatment significantly and dose-dependently inhibited the expression of p-STAT3 in HCT116 cells, when compared with AG490 alone group. Sesamolin and AG490 had synergistic effect and their combination significantly down-regulated the expression of p-STAT3, when compared with treatment with sesamolin alone. These results suggest that sesamolin may inhibit the activation of JAK2/STAT3 pathway in HCT116 cells *in vitro.*

Cancer metastasis is a complex process, and cell invasion is a prerequisite for metastasis (26). When tumor cells invade and migrate, they recruit MMPs in the degradation of adjacent extracellular matrix (27). The expressions of MMP-2 and MMP-9 are upregulated in cancer cells, and are positively correlated with the degree of tumor metastasis. These proteins are the most closely related members of the MMPs family. The results of this study suggest that sesamolin may effectively inhibit the invasion of CRC cells via inhibition of IL-6-induced expression of MMPs. It is likely that sesamolin blocks the activation of JAK2/STAT3 pathway, inhibits proliferation and induces apoptosis in HCT116 cells.

Sesamolin induces apoptosis in HCT116 cells and prevents cell invasion via inhibition of the JAK2/STAT3 signaling pathway.

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Conflict of Interest
There are no conflicts of interest in this study.

Author's contribution
All work was done by the authors named in this article and the authors accept all liabilities resulting from claims which relate to this article and its contents. The study was conceived and designed by Wei Zhang. Di Wu, Xin-Ping Wang, Wei Zhang collected and analyzed the data. Di Wu wrote the manuscript. All authors read and approved the manuscript prior to publication.

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