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Original Article

Anticancer effect of salidroside on colon cancer through inhibiting JAK2/STAT3 signaling pathway

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Abstract: Salidroside is considered to have anti-tumor properties. We investigate its effects on colon carcinoma SW1116 cells. Cell viability was assessed by CCK-8. Propidium iodide (PI) staining was used to determine the cell cycle by flow cytometry. The migration and invasion were detected by Transwell. Western blot was used to detect the expression of STAT3 signal related proteins. As the result, high concentrations of salidroside (10, 20. 50 μg/ml) significantly inhibited proliferation of SW1116 cells in a parallelly, cell cycle arrest was increased at the G0/G1 phase after salidroside treatment. Furthermore, salidroside inhibited migration and invasion of SW1116 cells. Salidroside treatment decreased proteins expression of phosphorylation levels in JAK2/STAT3 signaling, while MMP-2 and MMP-9 proteins levels were decreased and protein expression of VEGF and VEGFR-2 were down-regulated. In Conclusion, salidroside inhibited proliferation, decreased the migration and invasion of SW1116 cells in JAK2/STAT3-dependent pathway, the specific mechanisms need further study.

Keywords: Colon carcinoma, salidroside, apoptosis, invasion, JAK2/STAT3

Introduction

Colorectal cancer (CRC) is the most common digestive malignant and most devastating primary tumor. It is a worldwide health problem and the second most cause of cancer-related death [1, 2]. Despite very aggressive treatment including surgery and combined radio and chemotherapy, colon cancer remains a ruinous disease with invariable manifestation of tumor recurrence. And it has been the leading cause of deaths and rising the mortality every year [3]. So the therapeutic strategies aimed at preventing and delaying the disease might be a reasonable choice treatment.

In recent years, the natural medicine was widely used in clinical and medical research because of its low toxicity and their high biological activity. Rhodiola rosea has long been used as an adaptogen traditional Chinese medicine [4]. Salidroside, a phenylpropanoid glycoside extracted from Rhodiola rosea. It has been reported to possess various pharmacological properties including vessel protection, neuroprotective, anti-inflammation, immune regulatory and strong antioxidative activities [5-9]. In addition, the existing evidences indicated that salidroside has significant antitumor effects, such as inhibit the cell proliferation, arrest the cell cycle, and promote apoptosis of the human bladder, breast, lung or liver cancer cells [10, 11]. Salidroside also can play an antitumor role by inhibiting tumor metastasis and reducing new angiogenesis [12]. The performance been found that can inhibit tumor metastasis to reduce new angiogenesis in fibrosarcoma cells [13]. At present, the mechanism action of salidroside against colon cancer is unclear, and there is seldom research report evidence of salidroside effect on colon cancer.

Signal transducer and activator of the activator of transcription 3 (STAT3) is a pleiotropic transcription factor involved in JAK2/STAT3 signaling pathway. STAT3 is indispensable for many different tumor cells and often as a prime target for therapeutic intervention in various neoplasms, including colon cancers. And it was first identified as a DNA-binding factor for interleukin-6 (IL-6) responsive element [14]. The biological functions of STAT3 are very broad, such

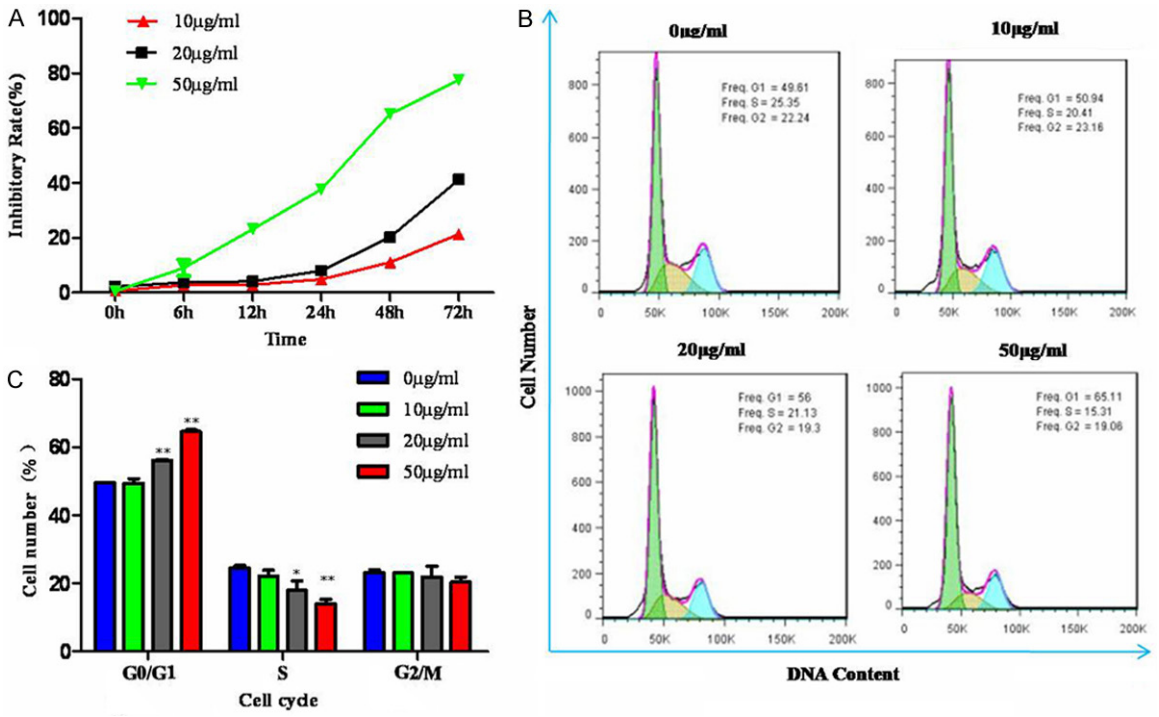

Figure 1. Effects of salidroside on SW1116 cells. A. The inhibition ratio on cell proliferation was determined by the CCK-8 assay after 24 h of incubation. B. Cell cycle was determined by using PI staining in the G0/G1, S and G2/M phases. Salidroside treatment increased the percentage of G0/G1 phase cells in a concentration-dependent manner. Images from three experiments are shown. C. Cell cycle was analyzed in SW1116 cells treated with various concentrations of salidroside (0, 10, 20 and 50 μg/ml) for 24 h. Data are presented as the means ± SD, from three independent experiments. $*P < 0.05$, $**P < 0.01$ vs. control group.

as in the regulation of cell proliferation, apoptosis, survival and differentiation. Phosphorylation and activation of STAT3 is a nuclear factor in regulation of tumors metastases [15]. We have shown that STAT3 activity promotes in vivo angiogenesis, in part by inducing the vascular endothelial growth factor (VEGF) [16], and stimulates invasion and metastasis by inducing matrix metalloproteinase (MMP) [17].

The aim of the current study was to investigate the anticancer effects of salidroside on cells proliferation, apoptosis, migration and invasion in the colon cancer SW1116 cell line. In addition, SMAT3 and related proteins expression were detected, and their association of SW1119 cells treated with salidroside was explored.

Materials and methods

Cell culture

The human colon carcinoma cell line SW1116 was purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in RPMI1640 medium containing 10% fetal bovine serum, 100 U/ml penicillin, and 100 mg/ml streptomycin. Cell line was kept at 37°C in a humidified atmosphere composed of 5% CO_2 and 95% air.

Cell proliferation assay

Cell viability was measured by Cell Counting Kit 8 (CCK-8, Boster, Wuhan, China). After treatment with various concentrations of salidroside (0, 10, 20, 50 μg/ml) for 24 h, cells (5×10^5 cells/ml) were loaded in 96-well plates and treated with DMSO for the indicated times. CCK-8 (10 μl) was added to each well with treated cells and incubated at 37°C for another 4 h. The absorbance was read at 450 nm using a microplate reader. All assays were performed in quintuplicate and repeated at least three times.

Cell cycle analysis

SW1116 cells at the logarithmic growth phase were randomly seeded at a density of 5×10^5

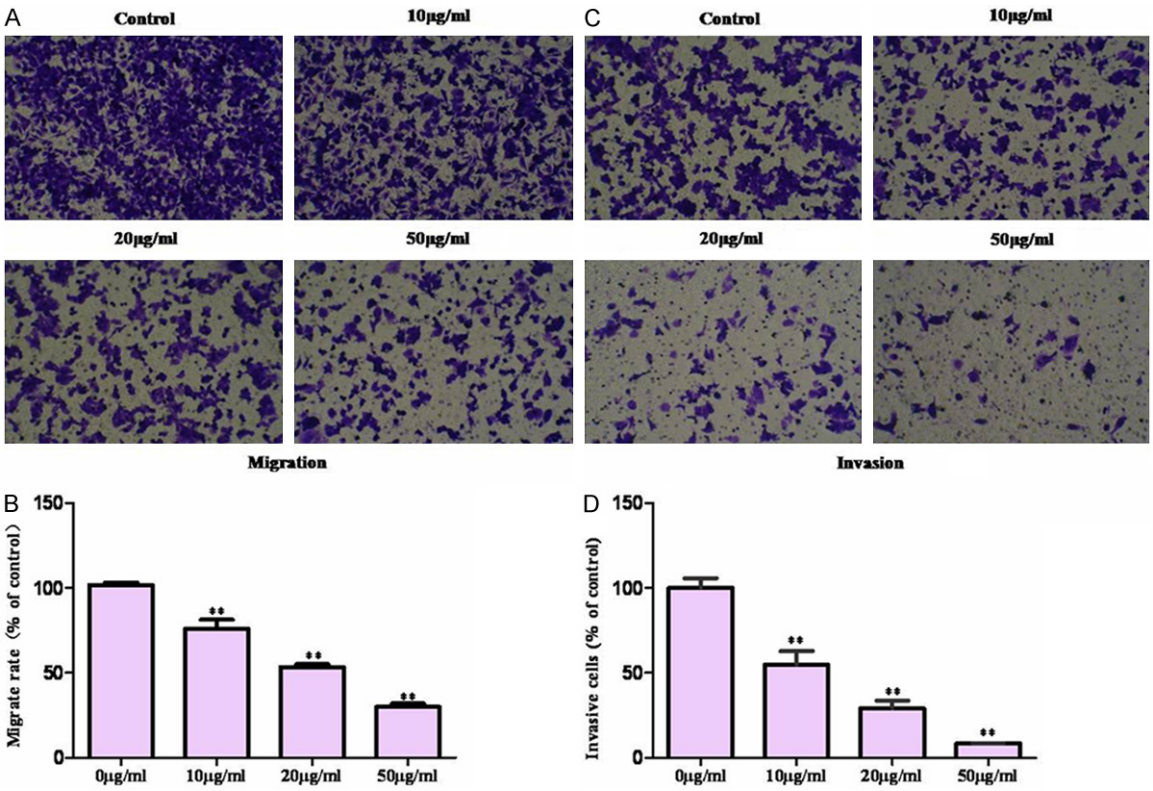

Figure 2. Salidroside inhibited the migration and invasion of SW1116 cells in vitro. A, B. The migration of SW1116 cells was quantified by measuring transwell. The migration cells were effectively reduced in salidroside treated groups. C, D. The invasion of SW1116 cells was measured by Matrigel-coated transwell inserts. Photographs indicate the invaded cells by microscope after different concentrations salidroside treatment. All data are presented as means ± SD, from three independent experiments. $^{**}P < 0.01$ vs. control group.

cells per well in 6-well plates, cultured overnight and then treated with various concentrations of salidroside (0, 10, 20, 50 μg/ml) in complete medium. Then cells were harvested. Finally, 1 ml propidium iodide (PI) stain solution was added to samples, which were analyzed on a FACScan instrument (Becton Dickinson, Franklin Lakes, USA).

Cell migration assay

Cell migration assay was carried out in 24-well tissue culture plates with Transwell filter membrane. Cells untreated or treated with salidroside. The wells were resuspended in 100 μl serum-free DMEM media at 24 h. SW1116 cells were harvested and re-suspended in serum-free DMEM containing 1% FBS; then, cells were added (1×10^5/well) to each well and incubated at 37°C for 48 h. The wells were washed twice with PBS to remove the unattached cells, and the attached cells were then stained with GIMSA for 10 min. Once stained, the cells were observed by using the optical microscope (Olympus, Japan).

Matrigel invasion assay

Invasion of tumor cells was evaluated using Transwell cell culture chambers which were pre-coated with 80 μl of Matrigel. Briefly, 2.5×10^5 cells untreated or treated with salidroside were seeded into the upper well of the chamber containing serum-free culture medium. After 24 h incubation at 37°C, no invasive cells were gently removed from the top of the matrigel with a cotton-tipped swab. Invasive cells at the bottom of the matrigel were fixed in methanol, stained with 1% crystal violet and counted under a microscope. Results were averaged from three independent experiments.

Western blot assay

Proteins from cell simples were separated by SDS-PAGE, followed by electro-transfer to a

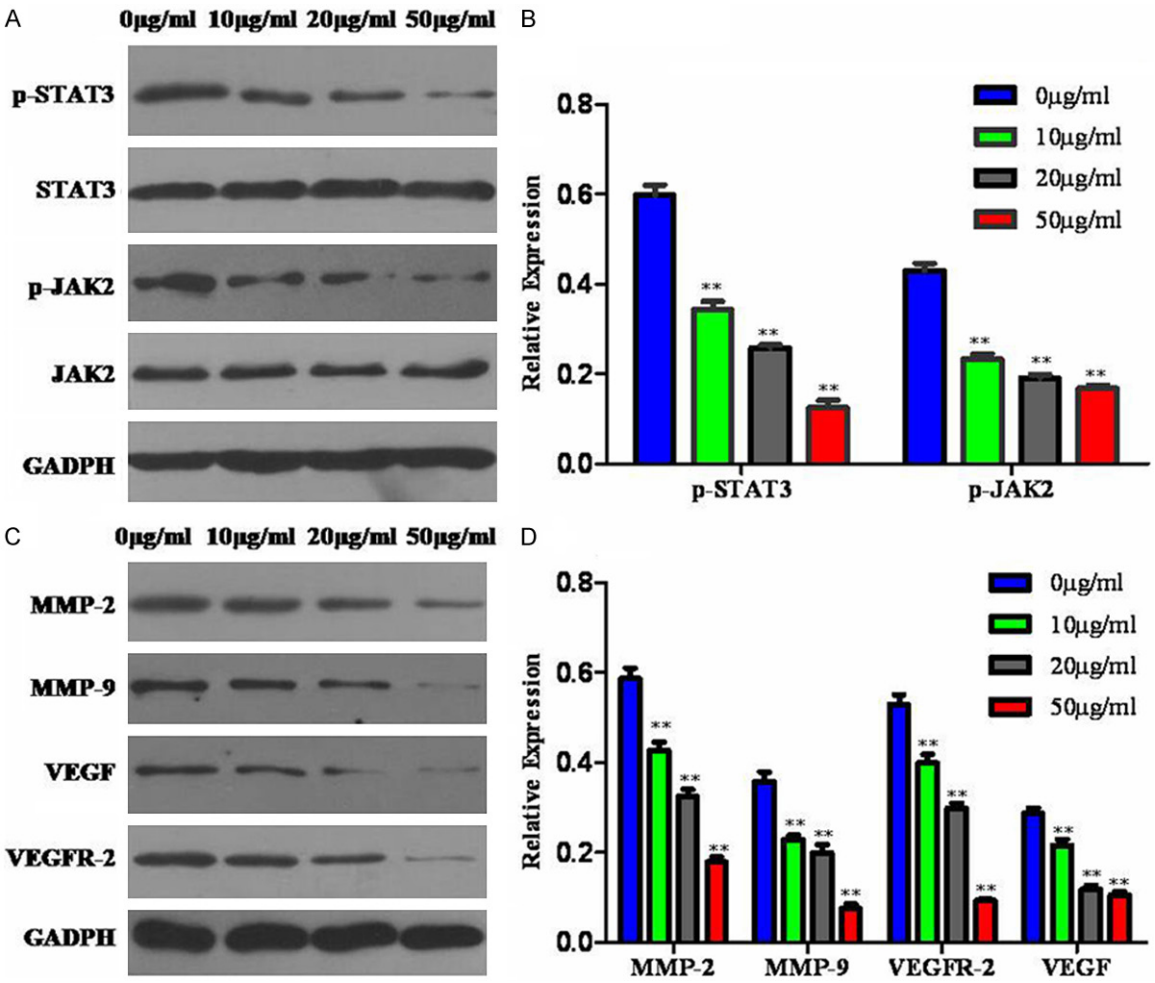

Figure 3. Effects of salidroside on the activation of JAK2/STAT3. SW1116 cells were treated with various concentrations (10, 20, and 50 μg/ml) of salidroside for 24 h. A, B. The phosphorylation of JAK2 and STAT3 was analyzed by western blotting method. GADPH was used as a loading control. C, D. The related proteins MMP and VEGF expression and activity. Data are presented as means ± SD based on three independent experiments. Asterisks indicate means that are significantly different compared with controls.

nitrocellulose membrane by means of a transfer cell (Bio-Rad, Hercules, CA, USA). The proteins were then transferred nitrocellulose membrane and incubated overnight at 4°C with the following antibodies: anti-MMP2, anti-MMP9, anti-VEGF, anti-VEGFR2 (Abcam Biotech, Shanghai, China), anti-p-JAK2, anti-JAK and anti-GADPH (Cell Signaling Technology, Beverly, MA, USA), Immuno-reactive bands were detected by reaction with the ECL detection system reagents (Amersham, Arlington Heights, IL, USA) and exposure to X-ray film, which was the developed and photographed.

Statistical analysis

Statistical differences were evaluated by GraphPad Prism 5.0 (SanDiego, CA, USA). Differences were considered statistically significant when P values were < 0.05. All data are expressed as mean ± SD of three independent experiments.

Results

Salidroside inhibited the proliferation of human colon cancer SW1116 cells

The roles of salidroside on the viability of SW116 cells for 6, 12, 24, 48, 72 h were assessed by the CCK-8 after 24 h of exposure. As shown in **Figure 1A**, compared to the control group, high concentrations of salidroside (10, 20, 50 μg/ml) significantly inhibited proliferation of SW116 cells.

Salidroside induced G0/G1 phase cell cycle arrest in SW1116 cells

To investigate the detailed mechanism of the underlying anti-proliferative activity of salidroside, flow cytometry was used to determine cell cycle distribution. Followed by treatment with various concentrations of salidroside (0, 10, 20 and 50 μg/ml) for 24 h. The result was found to significantly increase the percentage of cells in the G0/G1 phase in a dose-dependent manner ($P < 0.01$) (**Figure 1B**, **1C**). This assay indicated that salidroside inhibited the proliferation of SW1116 cells by inducing G0/G1 phase arrest.

Salidroside inhibited migration and invasion of SW1116 cells

To elucidate whether salidroside can impede the migratory potential of malignant colon cancer cells, an in-vitro transwell assay was used. Cell migration cells was captured under the microscope and normalized to untreated controls (**Figure 2A**). In the salidroside treated experimental group, the SW1116 cell line had the highest migratory potential (**Figure 2B**).

We further investigated whether pharmacological of salidroside would affect the invasion of colon cancer cells (**Figure 2C**). As shown in **Figure 2D**, after treatment with 10, 20, 50 μg/ml salidroside for 24 h, invasion of SW1116 cell lines investigated through the matrigel-coated pores was significantly reduced compared with controls. These findings suggest that salidroside is, indeed, involved in migration/invasion of human colon cancer cells.

Salidroside inhibited the phosphorylation JAK2 and STAT3

To further confirm the ability of salidroside to inhibit metastasis of SW116 cells in which signaling. The expression of JAK2 and STAT3 were measured. The resulted demonstrated that the expression of p-JAK2 and p-STAT3 in SW1116 cells were decreased after 10, 20 and 50 μg/ml salidroside treatment respectively (**Figure 3A**, **3B**).

Salidroside down-regulated the MMP and VEGF expression in SW1116 cells

Since MMPs and VEGF play a pivotal role in tumor cell invasiveness, we detected the effect of salidroside on MMP-2 and MMP-9 proteins expression level by western blot. The resulted showed that salidroside treatment significantly suppressed the MMP-2 and MMP-9 expression of SW1116 cells. Meanwhile VEGF and VEGFR2 levels decreased obviously (**Figure 3C**, **3D**).

Discussion

Salidroside has been reported to have many kinds of pharmacological effects in both in vivo and in vitro experimental models. In the present study, salidroside was found to show anticancer effects on colon cancer SW1116 cells. These effects were demonstrated by suppressed cell proliferation, decreased tumor migration and invasion, arrested cell cycle. The underlying mechanisms may be associated with the inhibition of JAK2/STAT3 signaling.

Cell proliferation is an essential process to cells survive, it is also an important biological characteristic of tumor formation. Therefore, inhibition of tumor proliferation is one of the aims for tumor treatment. Salidroside was found to reduce viable cells in a dose-dependent manner and the detailed mechanism lies in cell cycle arrest. Following salidroside treatment, the percentage of cells in the G0/G1 phase was significantly increased. The results are consistent with those of previous study that salidroside caused G1- or G2- phase arrest in various cells [10].

Antitumor effects of salidroside involves in a variety of molecules or signal pathways, including cyclinD1, mTOR, HIF-1α and VEGF pathway [10, 18, 19], but the specific mechanism still remains puzzling. JAK/STAT pathway is a pivotal role in many signal transductions in vivo. JAK family proteins include JAK1, JAK2, JAK3 and tyrosine kinase2. The JAK activation has important role in cell proliferation, differentiation, migration and apoptosis. Structure activation of JAKs can make phosphorylation of some important materials, including STAT family. STAT3 is a member of STATs, which associates with numerous tumor signaling pathways. The activation of STAT3 is closely related to the survival and growth of solid tumors cells. Novel synthetic derivatives of the natural substances berbamine was a inhibitor of the JAK2/STAT3 signaling and induce apoptosis of human melanoma cells [20]. Pterostilbene has shown to have potent antitumor activity to against human osteosarcoma cells by inhibiting JAK2/STAT3

signaling [21]. Structurally modified curcumin analogs inhibited JAK2/STAT3 signal and promoted cells apoptosis of human renal carcinoma and melanoma [22]. Therefore, we further study whether the JAK2/STAT3 signaling pathway could be affected by salidroside. We found that salidroside can directly inhibit the phosphorylation of JAK2 and the downstream STAT3 phosphorylation. In addition, salidroside also down-regulated the STAT3 target proteins MMP and VEGF expression.

Tumor invasion is a more complex and multistage process. Tumor cells in the process of transfer must first to penetrate the basement membrane and extracellular matrix (ECM). Matrix metalloproteinases (MMPs) are mainly enzymes that involved ECM degradation. Now, MMP-2, MMP-9 are considered the most direct and important MMP in the process of tumor invasion and metastasis. STAT3 is known to directly regulate the expression of molecules, such as, MMP-2, MMP-9, which play a key role in tumor cells invasion, migration [23]. In the research of melanoma, the p-STAT3 and MMP-2 expression were positively correlated [24]. The mammary epithelial cells were also demonstrated that a positive correlation between activated STAT3 and MMP-9 protein expression levels [25]. In our study, the potency of invasiveness was reduced in salidroside-mediated, and the expression of MMP-2, MMP-9 were also down-regulated, which had the consistent results of p-STAT3 expression.

In the colon cancer process of growth, invasion and metastasis, tumor angiogenesis plays an important role. VEGF and its receptor VEGFR is the most important regulatory factors for angiogenesis. There are studies had reported that its expression was regulated by STAT3 in lung, breast and colerector tumors [26-28]. The expression of VEGF and VEGFR-2 were down-regulated, the result could infer to be associated with the STAT3 signaling.

The present study investigated the anticancer effects of salidroside on colon cancer cells, indicating a novel strategy for colon cancer treatment. In conclusion, salidroside can inhibit proliferation, arrest cell cycle, decrease the migration and invasion of the colon cancer SW1116 cells. While the JAK-2/STAT3 signaling related proteins expression were inhibited. It can be predicted, with the deepening of the research, salidroside will be the used as a potential clinical antitumor auxiliary drug, the roles in anticancer will be supported by the deeper molecular mechanism.

Disclosure of conflict of interest

None.

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