RETRACTED ARTICLE: Anti-gastric cancer effect of Salidroside through elevating miR-99a expression

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ABSTRACT
Salidroside is an active ingredient extracted from *Rhodiola rosea* that has anti-tumor activities. The current paper attempted to assess the impact of Salidroside on gastric cancer (GC) and explore the potential mechanism. GC cell lines (SNU-216 and MGC803) and gastric epithelial cell line GES-1 were treated with Salidroside. CCK-8 assay, colony formation assay, flow cytometry and Transwell assay were respectively performed to evaluate GC cells phenotype. qRT-PCR and western blot were conducted to reveal the downstream genes and signaling of Salidroside. We found that 800 μM Salidroside was capable of reducing GC cells viability, while has no such impacts on GES-1 cells. Salidroside inhibited GC cells proliferation, migration, invasion and promoted apoptosis, which coupled with the down-regulation of p21, Bcl-2, MMP2, RhoA, p-ROCK1, Vimentin and the up-regulations of CyclinD1, Bax, cleaved caspases. miR-99a was found to be highly expressed in response to Salidroside treatment. Besides, the inhibition of MAPK/ERK and PI3K/AKT signaling induced by Salidroside was attenuated by miR-99a silence and in this process, IGF1R worked as a target of miR-99a. The anti-GC effect of Salidroside was also confirmed in a mouse model of GC. The promoting effect of Salidroside on miR-99a expression was also verified in vivo. Furthermore, Salidroside promoted the cisplatin-sensitivity of SGC7901/DDP cells. In conclusion, this study demonstrated that Salidroside possessed anti-GC effects through regulating miR-99a/IGF1R axis and inhibiting MAPK/ERK and PI3K/AKT pathways.

Introduction

Gastric cancer (GC) continues to be the leading cause of cancer-related death that frequently occurs in the gastric mucosa and has brought a great burden to people’s daily lives [1]. Currently, it is well-known that the risk factors of GC are mainly related with the environment of daily lives, dietary habits, factors related to genetic and immune systems, and other chronic gastric lesions [2]. Currently, the efficacy still remains very limited due to a lack of effective early diagnostic biomarkers and the side-effects of systemic therapies [3]. So, the development of novel and effective drugs to prevent and treat this cancer is of great significance.

It has been reported that many natural herbal compounds share the chemo-preventive and chemo-therapeutic properties including anti-tumor effects [4–6]. Salidroside, an active ingredient isolated from dried roots, rhizomes or whole plants of *Rhodiola rosea*, has been identified to display a wide range of pharmacological activities, like anti-aging, anti-hypoxia, anti-inflammation, neuroprotection activities, potentiating the immune function, enhancing the body’s resistance to fatigue, protecting body from irradiation injury and anti-tumor [7–12]. Regarding its anti-tumor properties, a preliminary study has reported that Salidroside suppressed the proliferation of various human cancer cells such as human mammary adenocarcinoma (MCF-7), human hepatocellular carcinoma (HHCC), human lung adenocarcinoma (A549), and human malignant glioma (BT-325) cells [13]. Meanwhile, it has been reported that Salidroside exerted its beneficial effects via regulating MAPK/ERK and PI3K/AKT [14], two signaling implicated in determining GC cells fate [15,16]. However, the regulatory role and the deep mechanisms of Salidroside on human GC cells have not been fully elucidated yet.

This study made efforts to preliminary decode the effect of Salidroside on the growth and migration of GC cell lines, to evaluate the anti-tumor potential of Salidroside against GC. Besides, considering the anti-cancer mechanisms of Chinese medicinal herbs targeting microRNAs (miRNAs) [17], we herein studied the potential mechanisms underlined Salidroside’s anti-tumor property by focusing on miRNAs. miR-99a is one of the most studied miRNAs in the field of human cancers. In regard of GC, miR-99a was observed to be high-expressed in GC tissues, and the up-regulated miR-99a was correlated with the survival of GC patients [18]. Another in vitro study uncovered the anti-tumor role of miR-99a in GC cells [19]. Based on these reasons, miR-99a, rather than other miRNAs, was selected for further investigation. The findings of this study will provide new clues for the therapy strategies for GC.

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Materials and methods

Cell treatment

Human GC cell lines SNU-216 and MGC-803 were, respectively, brought from the Korean Cell Line Bank (Seoul, Korea) and National Infrastructure of Cell Line Resource (Beijing, China). Human gastric epithelial cell line GES-1 was obtained from the Beijing Institute for Cancer Research (Beijing, China). SNU-216 and GES-1 cells were cultivated in Roswell Parker Memorial Institute (RPMI)-1640 medium (Gibco, Grand Island, NY) and MGC-803 cells were cultivated in Dulbecco's Modified Eagle's Medium (DMEM; Gibco). The complete culture medium was complemented with 10% fetal bovine serum (FBS; Gibco). The cells were cultured at 37 °C in a humidified 5% CO2 incubator.

Cisplatin (DDP)-resistant SGC7901 cells were brought from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). Those cells were cultivated in RPMI-1640 medium (Gibco) complemented with 10% FBS (Gibco). Also, cells were maintained at 37 °C in the incubator. Cisplatin (Solarbio, Beijing, China) with concentrations ranging from 0.3 to 20 μg/mL was applied to treat DDP-resistant SGC7901 cells for 12 h.

The Salidroside used to treat cells for 24 h was purchased from Sigma-Aldrich (SMB00072) and the treated concentrations of Salidroside ranged from 0.8 to 8000 μM.

Cell viability

Cell Counting Kit-8 (CCK-8; Beyotime, Shanghai, China) was employed to assess cell viability. 5000 cells were planted in each well of 96-well plate and incubated for 24 h before treatment. Cells were stimulated with various concentrations of Salidroside (0.8–8000 μM) for 24 h and the culture medium was added with CCK-8 (10 μL), followed by incubation for another 1 h at 37 °C. Finally, the absorbance was tested at 450 nm by a Microplate Reader (Bio-Rad, Hercules, CA).

Colony formation assay

Briefly, 500 cells were seeded onto each well of 6-well plates and allowed to be cultured for 24 h. Cells were treated with or without Salidroside (800 μM) for 24 h. Afterwards, the culture medium containing Salidroside was discarded and the cells were carefully rinsed with phosphate-buffered saline (PBS) for twice. The rinsed cells continued to be incubated in fresh complete culture medium for an additional 10 days. Subsequently, the cell colonies were fixed with precooled methanol for 20 min at room temperature. Then, the fixed cells were carefully rinsed with PBS twice, followed by staining with crystal violet (Sigma-Aldrich) for 30 min. The number of visible colonies was finally counted.

Cell-cycle progression

The analysis of cell-cycle progression was carried out by using Cell Cycle Analysis Kit (4A Biotech, Beijing, China). In brief, cells were harvested and incubated in 70% ethanol at 4 °C overnight. Later, the cells were stained by 50 μg/mL propidium iodide (PI). After incubating away from light for 30 min, the samples were analyzed by a FACS can (Beckman Coulter, Fullerton, CA). Data were analyzed by the ModFit software (Verity Software House, Topsham, ME).

Apoptosis assay

FITC-Annexin V/PI Apoptosis Detection Kit (Jiancheng, Nanjing, China) was applied in this experimental process. Cells were harvested and resuspended into a single-cell solution at a density of 1 × 106 cell/mL with binding buffer. The resuspended cells were stained with 5 μL Annexin V-FITC and 10 μL PI for 30 min at room temperature away from light. Finally, the samples were immediately detected by the FACS can and the data were analyzed using FlowJo (TreeStar, Inc., Ashland, OR).

Transwell assay

The migratory ability of cells was tested by a modified two-chamber Transwell system (Corning, Lowell, MA). Briefly, 1 × 105 cells suspended in 0.2 ml serum-free medium were plated on the upper compartment, and the lower chamber was added with 0.6 ml of culture medium containing 10% FBS. To test cell invasion, the chamber was pre-coated with 20 μg of Matrigel (BD Biosciences, Bedford, MA) at 37 °C for 6 h. After incubation for 24 h, the migrated and invaded cells in the lower chamber were fixed with 100% methanol for 30 min and then stained with 1% crystal violet (Sigma-Aldrich) for 20 min. The stained cells were counted in five random fields.

Quantitative reverse transcription PCR (qRT-PCR)

TRIzol reagent (Life Technologies Corporation, Carlsbad, CA) was utilized to extract total RNA from cultured cells. For detecting the mRNA expression of CyclinD1, 500 ng total RNA was reversely transcribed to cDNA by the First Strand cDNA Synthesis Kit (Roche, Basel, Switzerland) and then qRT-PCR was conducted using SYBR® Premix Ex TaqTM II (TaKaRa, Dalian, China). For the detection of miR-99a levels, total RNA was transcribed to cDNA by using Taqman MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA), and qRT-PCR was performed by Taqman Universal Master Mix II (Applied Biosystems) according to the manufacturer's protocols. The mRNA level of CyclinD1 was normalized to b-actin and the expression of miR-99a was normalized to U6, with 2− ΔΔCt method [20]. The primary sequences were provided in Table 1.

miRNA transfection

miR-99a inhibitor, the negative control (NC), miR-99a mimic and the mimic NC were brought from GenePharma (Shanghai, China). Cells were transfected with oligonucleotides by lipofectamine 3000 (Life Technologies Corporation). Transfection was conducted for 48 h.
Table 1. The primary sequences used in qRT-PCR.

| Gene name | Sequences                     |
|-----------|-------------------------------|
| CyclinD1  | 5'-GACATCCTCCCCCTGACGCGGAG-3' (F) |
| β-actin   | 5'-ACACCTTCTACATAGCTG-3' (F)   |
| hsa-miR-99a| 5'-GCTGATACAGTGCAGGTCGCTGTCGATTTCCGC ACTGGACAAAG-3' (F) |
| U6        | 5'-GAATGAGCTTCCAGAATTTG-3' (R) |
| ACTGGACAAG-3' (F) |

Western blot assay

RIPA lysis buffer (Beyotime) was used to extract the protein from cultured cells for western blot analysis. The quantification of proteins samples was confirmed by the Micro BCA™ Protein Assay Kit (Thermo Fisher Scientific, Waltham). Equal amount of protein samples (30 μg) were loaded and separated by Bio-Rad Bis-Tris Gel system and then transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA) according to the specific molecular weight (kDa) of proteins. All the antibodies were used as follows: Bcl-2 (#4223), Bax (#5023), caspase-3 (#9662), caspase-9 (#9502), MMP2 (#87809), RhoA (#2117), ROCK1 (#4035), Vimentin (#5741), MAPK (#9212), p-MAPK (#9211), ERK (#9102), p-ERK (#9101), CyclinD1 (#2978), p21 (#2947), β-actin (#4970; Cell Signaling Technology, Beverly, MA), p-ROCK1 (ab203273), IGF1R (ab182408), p-AKT (ab8933), AKT (ab8805), p-Pi3K (ab182651) and Pi3K (ab182651; Abcam, Cambridge, UK). Detection was performed using an enhanced chemiluminescence (ECL) method. The signals intensity of each band was quantified by using Image Lab™ (Bio-Rad).

Luciferase reporter assay

The 3'UTR of IGF1R which contains the predicted miR-99a-binding sequence was constructed into pmirGLO Reporter Vector (Promega, Madison, WI) to make a reporter vector (IGF1R-wt). The predicted binding site was placed by non-targeting sequences and then formed a control vector (IGF1R-mut). The vectors were transfected into HEK-293T cells along with miR-99a mimic or mimic NC. Dual-Luciferase Reporter Assay System (Promega) was utilized for the management of luciferase activity.

Tumor xenograft model

Sixty male nude mice (6 weeks old) were brought from Vital River Laboratories (Beijing, China). The animal experiments were approved by the Animal Ethics Committee of the Affiliated Hospital of Qingdao University. The mice were housed under specific pathogen-free (SPF) grade experimental system. 1 week later, 2 × 10^6 SNU-216 and MGC803 cells were injected into the right flank of the mice. When the tumor xenograft model was grown to 0.1 cm³, the mice were divided into two groups (n = 15). Mice in Salidroside group were intraperitoneally injected with Salidroside (50 mg/kg body weight) on an alternate day for 3 weeks. Control mice received an injection of the same volume of PBS. Tumor volume was measured at 1–5 weeks by using vernier calipers. In the end, the mice were sacrificed by decapitation, and the tumor weight and miR-99a expression in tumor tissues were measured.

Statistical analysis

The results are shown as mean ± standard deviation (SD). Statistical analyses were conducted using GraphPad Prism 6 (GraphPad Software, San Diego, CA). The p values were counted by ANOVA test or Student t-test. p values of < .05 were considered significant.

Results

Salidroside inhibited proliferation of GC cells

Firstly, GES-1, SNU-216 and MGC803 cells were stimulated with Salidroside at a range concentration from 0.8 μM to 8000 μM for 24 h, and cell viability was checked. Figure 1(A–C) showed that Salidroside treatment with concentrations lower than 8000 μM had no impacts on GES-1 cells viability (p > .05), while could significantly lower SNU-216 and MGC803 cells viability (p < .05, p < .01 or p < .001). Besides, Salidroside reduced GC cells viability in both dose- and time-dependent fashion (Supplementary Figure 1). We next assessed the effect of Salidroside on growth-related factors expression. As seen in Figure 1(D,E), the protein level of CyclinD1 was significantly repressed while p21 was up-regulated by Salidroside treatment in SNU-216 cells (p < .001, Figure 1(F)) . Same trends were also observed in MGC803 cells (Figure 1(G)). Colony formation assay revealed that Salidroside significantly declined the survival fractions (p < .01 or p < .001, Figure 2(A,B)). The S-phase cell percentage was clearly declined by Salidroside treatment (Figure 2(C,D)). These data uncovered that Salidroside suppressed proliferation of GC cells.

Salidroside promoted apoptosis of GC cells

We then checked the impacts of Salidroside on apoptosis of SNU-216 and MGC803 cells and found that Salidroside increased the apoptotic cell rates of these two cell lines in a dose-dependent way (p < .05 or p < .01, Figure 3(A,B)). Additionally, Salidroside notably suppressed the protein level of Bcl-2, while promoted the levels of Bax, cleaved-caspase 3 and cleaved-caspase 9 in a dose-dependent way (Figure 3(C)). These data suggested that Salidroside facilitated GC cells apoptosis.

Salidroside suppressed the migration and invasion of GC cells

Next, we checked the impacts of Salidroside on GC cells migration and invasion. We observed that Salidroside treatment significantly reduced both SNU-216 and MGC803 cells
migration ($p < .01$ or $p < .001$, Figure 4(A,B)) which was accompanied by MMP2, RhoA and p-ROCK1 down-regulations ($p < .001$, Figure 4(C,D)). Consistently, Solidroside weakened the invasion of SNU-216 and MGC803 cells, as invasive cells were declined ($p < .05$ or $p < .01$, Figure 4(E,F)) and the Vimentin expression was down-regulated ($p < .05$ or $p < .01$, Figure 4(G,H)). The data illustrated the negative impacts of Solidroside on the migration and invasion of GC cells.

**Solidroside inhibited MAPK/ERK and PI3K/AKT signaling**

To further explore the deep mechanism, MAPK/ERK and PI3K/AKT signaling were assessed. As seen in Figure 5(A,B), Solidroside treatment significantly inhibited MAPK/ERK and PI3K/AKT signaling, as the inhibited phosphorylation of MAPK, ERK, PI3K and AKT ($p < .01$ or $p < .001$). Those data illustrated that Solidroside inhibited the activation of MAPK/ERK and PI3K/AKT signaling in SNU-216 cells.

**Solidroside suppressed MAPK/ERK and PI3K/AKT signaling through regulating miR-99a/IGF1R axis**

As shown in Figure 6(A), we found that Solidroside led to miR-99a up-regulation in SNU-216 cells ($p < .01$). We then explored whether miR-99a mediated the regulatory effect of Solidroside on MAPK/ERK and PI3K/AKT pathways. miR-99a
Figure 2. Salidroside inhibited the survival of GC cells. SNU-216 and MGC803 cells were stimulated with 800 μM Salidroside for 24 h. (A,B) Colony formation, and (C,D) cell-cycle progression were measured by colony formation assay and flow cytometry. **, *** indicate p < .01 and .001.

Figure 3. Salidroside promoted apoptosis of GC cells. (A) SNU-216 and (B) MGC803 cells were stimulated with Salidroside (0.8–8000 μM) for 24 h. Flow cytometry detection was carried out for testing apoptotic cell rate. (C) Apoptosis-associated protein levels were checked by western blot. *, ** indicate p < .05 and .01.
expression was altered by transfection and the efficiency was identified utilizing qRT-PCR (p<.01 or p<.001, Figure 6(B)). Interestingly, miR-99a inhibitor abolished the modulatory effect of Salidroside on pathways. Indeed, miR-99a silence elevated the expression of p-MAPK, p-ERK, p-PI3K and p-AKT in Salidroside-treated SNU-216 cells (p<.01 or p<.001, Figure 6(C,D)). In line with the findings reported elsewhere [21], IGF1R was found as a target of miR-99a. As related to co-transfection with IGF1R-wt and mimic NC, the luciferase activity was inhibited by co-transfection with IGF1R-wt and miR-99a mimic (p<.05, Figure 6(E)). Additionally, Salidroside treatment repressed IGF1R expression, while miR-99a silence recovered its expression (Figure 6(F)). Overall, it appeared that Salidroside might inhibit MAPK/ERK and PI3K/AKT signaling through regulating miR-99a/IGF1R axis.

**Salidroside inhibited the tumor growth of xenograft animal model**

The *in vivo* anti-tumor property of Salidroside was verified in a xenograft animal model. To this end, SNU-216 and MGC803
cells were injected into nude mice and the tumor volume and weight were recorded. Figure 7(A,B) shows that tumor volume was increased in a time-dependent fashion. More than that, treatment of Salidroside could slow down the growth of tumor volume ($p < .05$, $p < .01$ or $p < .001$). Same trends were observed in Figure 7(C,D) that tumor weight was significantly lowered by Salidroside ($p < .05$). Additionally, a high level of miR-99a in tumor tissues was observed after treating with Salidroside ($p < .01$, Figure 7(E,F)). These data suggested the in vivo anti-GC function of Salidroside and confirmed the negative effects of Salidroside on miR-99a expression.

**Salidroside enhanced the cisplatin-sensitivity of SGC7901/DDP cells**

Finally, the impacts of Salidroside on cisplatin-resistant SGC7901/DDP cells were explored. As seen in Figure 8(A), miR-99a level in SGC7901/DDP cells was declined by treating with 200 $\mu$g/mL cisplatin ($p < .01$). Additionally, the viability of SGC7901/DDP cells was repressed by Salidroside treatment ($p < .01$, Figure 8(B)). And transfection of cells with miR-99a inhibitor remarkably attenuated Salidroside-induced viability loss in SGC7901/DDP cells ($p < .05$). It seems that Salidroside treatment could make SGC7901/DDP cells more sensitive to cisplatin.

**Discussion**

Accumulating evidences have demonstrated that traditional Chinese medicines or natural products exhibit advantages in prevention and treatment for diseases, including various cancers [3, 22–24]. Salidroside is a major active component of *Rhodiola rosea*, which has been widely used as a tonic herb to enhance the fatigue resistance of the body and to extend human longevity due to its anti-oxidative, anti-aging, anti-inflammatory and other biological properties [25–27]. Herein, we attempted to investigate the impact of Salidroside on GC cells growth, migration and invasion. Our results showed that Salidroside significantly reduced SNU-216 and MGC803 cells viability in both dose- and time-dependent fashion. However, Salidroside with concentrations lower than 8000 $\mu$M had no cytotoxicity in normal gastric epithelial GES-1 cells. This result indicated the specific killing effects of Salidroside on GC cells. Meanwhile, we found that Salidroside at 800 $\mu$M dramatically reduced GC cells proliferation, migration and invasion, while enhanced apoptosis. The anti-tumor properties of Salidroside were also verified in an animal model of GC. Our results were similar with those previous studies regarding the effect of...
Salidroside on other types of tumors. As selected examples, Salidroside significantly reduced cell viability of bladder cancer cell lines [28]. Sun et al. demonstrated that Salidroside efficiently inhibited cell proliferation, migration and invasion, as well as inhibited the MMP2 expression in human fibrosarcoma HT1080 cells [29]. Our results indicated the anti-GC properties of Salidroside, which may provide novel clues for GC management.

The effects of Salidroside on cisplatin-resistant SGC7901/DDP cells were also studied. Results in the current paper...
revealed that Salidroside was capable of enhancing the cisplatin-sensitivity of SGC7901/DDP cells, indicating its potential usage as a neoadjuvant drug for chemotherapy.

MAPK/ERK and PI3K/AKT pathways have been considered as the major signaling in the progression of various cancers [30–32]. Meanwhile, it has been reported that the activation of these two pathways would contribute to the proliferation and metastasis of GC cells, such as SGC7901 and BGC823 cells [33]. In addition, Salidroside could suppress PI3K/AKT phosphorylation, which might mediate its anti-tumor effect on bladder cancer cells [28]. Also, Chang et al. reported that Salidroside attenuated ethanol-induced acute gastric ulcer and hydrogen peroxide (H₂O₂)-induced gastric epithelial cell damage through regulating the MAPK pathway [34]. In terms of PI3K/AKT signaling cascade, Salidroside has been reported to enhance cell apoptosis and autophagy via suppressing PI3K/AKT pathway in human colorectal cancer cells [35]. Moreover, previous studies revealed that Salidroside possessed a protective effect on oxidative stress or other injured factors induced cardiac or brain damages through modulating PI3K/AKT pathway [36,37]. In our present study, Salidroside was found to suppress MAPK/ERK and PI3K/AKT pathways in SNU-216 cells. Salidroside treatment significantly reduced the protein levels of p-MAPK, p-ERK, p-PI3K and p-AKT, which might be associated with its anti-tumor effects on GC. These reports implied that Salidroside might also exhibit a regulatory effect on MAPK/ERK and PI3K/AKT signaling cascades in GC cells.

miR-99a has been reported to function as an anti-tumor miRNA in human cancers, such as breast [38], lung [39], and cervical cancers [40]. Z miR-99a was less expressed in GC tissues from 30 pairs of GC patients' samples, and miR-99a
up-regulation led to a significant loss of cell viability and induction of apoptosis [41]. Thus, we explored whether Salidroside could regulate miR-99a expression in SNU-216 cells. Interestingly, the addition of Salidroside significantly up-regulated miR-99a expression, implying miR-99a might be implicated in the regulation of Salidroside on the growth and migration of GC cells.

Moreover, miR-99a could inhibit the activity of PI3K/AKT signaling, thus resulted in the inhibition of cell growth in human glioblastoma in vitro and in vivo [42]. Meanwhile, miR-99a has been revealed to suppress cell growth and migration through directly modulating IGF1R [21], while IGF1R was a previously reported upstream factor of PI3K/AKT and MAPK/ERK pathways [21]. This finding was also observed in this study, that IGF1R functioned as a target of miR-99a. Thus, it seems that miR-99a regulated PI3K/AKT and MAPK/ERK signaling via an indirect way. miR-99a inhibits the gene expression of IGF1R via base binding effect [43]. So, Salidroside inhibited PI3K/AKT and MAPK/ERK pathways possibly through regulation of miR-99a/IGF1R axis.

In conclusion, the current study demonstrated the anti-GC properties of Salidroside. Furthermore, Salidroside dramatically augmented miR-99a expression, which targeted IGF1R and further inhibited MAPK/ERK and PI3K/AKT signaling. Our preliminary results might provide the experimental basis for exploring pharmacologic effects of Salidroside and also provide new clues for the treatment of GC.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**References**

[1] Oh DY, Doi T, Shirako K, Lee KW, Park SR, Chen Y, Yang L, Valota O, Bang YJ: Phase I Study of Axitinib in Combination with Cisplatin and Capecitabine in Patients with Previously Untreated Advanced Gastric Cancer. *Cancer research and treatment : official journal of Korean Cancer Association* 2015, 47(4):687–696.

[2] Demirel BE, Akkas BE, Vural GU: Clinical factors related with *Helicobacter pylori* infection—Is there an association with gastric cancer history in first-degree family members? *Asian Pacific Journal of Cancer Prevention Apcp* 2013, 14(3):1797–1802.

[3] Chiang JH, Yang JS, Ma CY, Yang MD, Huang HY, Hsia TC, Kuo HM, Wu PP, Lee TH, Chung JG: Danthron, an anthraquinone derivative, induces DNA damage and caspase cascades-mediated apoptosis in SNU-1 human gastric cancer cells through mitochondrial permeability transition pores and Bax-triggered pathways. *Chemical research in toxicology* 2011, 24(1):20–29.

[4] Ko JK, Leung WC, Ho WK, Chiu P: Herbal diterpenoids induce growth arrest and apoptosis in colon cancer cells with increased expression of the nonsteroidal anti-inflammatory drug-activated gene. *European Journal of Pharmacology* 2007, 559(1):1–13.

[5] Z H, JD L, KV C, CS Y: Effects of tea polyphenols on signal transduction pathways related to cancer chemoprevention. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 2004, 555(1–2):3–19.

[6] Sparreboom A, Cox MC, Acharya MR, Figg WD: Herbal remedies in the United States: potential adverse interactions with anticancer agents. *Journal of Clinical Oncology Official Journal of the American Society of Clinical Oncology* 2004, 22(12):2489–2503.

[7] Chen X, Liu J, Gu X, Ding F: Salidroside attenuates glutamate-induced apoptotic cell death in primary cultured hippocampal neurons of rats. *Brain Research* 2008, 1238(C):189–198.

[8] Huang X, Zou L, Yu X, Chen M, Rui G, Hui C, Dan Y, Xu Y, Chen Y, Cheng D: Salidroside attenuates chronic hypoxia-induced pulmonary hypertension via adenosine A2a receptor related mitochondrial-dependent apoptosis pathway. *Journal of Molecular & Cellular Cardiology* 2015, 82:153–166.

[9] Guan S, Feng H, Song B, Guo W, Xiong Y, Huang G, Zhong W, Huo M, Chen N, Lu J: Salidroside attenuates LPS-induced pro-inflammatory cytokine responses and improves survival in murine endotoxemia. *International Immunopharmacology* 2011, 11(12):2194–2199.

[10] Qu ZQ, Zhou Y, Zeng YS, Lin YK, Li Y, Zhong ZQ, Chan WY: Protective effects of a Rhodiola crenulata extract and salidroside on hippocampal neurogenesis against streptozotocin-induced neural injury in the rat. *PLoS One* 2012, 7(1):e29641.
[11] Darbinyan V, Kteyan A, Panossian A, Gabrielian E, Wikman G, Wagner H: Rhodiola rosea in stress induced fatigue—A double blind cross-over study of a standardized extract SHR-5 with a repeated low-dose regimen on the mental performance of healthy physicians during night duty. Phytomedicine: international journal of phytotherapy and phytopharmacology 2000, 7(5):365–371.

[12] Kanupriya, Prasad D, Sai Ram M, Kumar R, Sawhney RC, Sharma SK, Ilavazhagan G, Kumar D, Banerjee PK: Cytoprotective and anti-oxidant activity of Rhodiola imbricata against tert-butyl hydroperoxide induced oxidative injury in U-937 human macrophages. Mol Cell Biochem 2005, 275(1-2):1–6.

[13] Hu X, Lin S, Yu D, Qiu S, Zhang X, Mei R: A preliminary study: the anti-proliferation effect of salidroside on different human cancer cells. Cell Biology & Toxicology 2014, 30(4):1159–1164.

[14] Qu J, Wu Y, Zhao M, et al: Gastric cancer exosomes promote tumour cell proliferation through PI3K/Akt and MAPK/ERK activation. Digest Liver Dis. 2009, 41:875–880.

[15] He Y, Ge Y, Jiang M, et al: miR-592 promotes gastric cancer proliferation, migration, and invasion through the PI3K/AKT and MAPK/ERK signalling pathways by targeting Spry2. Cell Physiol Biochem. 2018, 47:1465–1468.

[16] Hong M, Wang N, Tan HY, et al: MicroRNAs and Chinese medicinal herbs: new possibilities in cancer therapy. Cancers. 2015, 7:1643–1657.

[17] Zhang C, Zhang DM, Ma MH, et al: Three-microRNA signature identified by bioinformatics analysis predicts prognosis of gastric cancer patients. WJG. 2018, 24:1206–1215.

[18] Zhang Y, Xu W, Ni P, et al: MiR-99a and MiR-491 regulate esplatin resistance in human gastric cancer cells by targeting CAPNS1. Int J Biol Sci. 2016, 12:1437–1447.

[19] Livak KJ, Schmittgen TD: Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2–ΔΔCT Method. Methods 2001, 25(4):402–408.

[20] Si C, Yu Q, Yao Y: Effect of miR-146a-5p on proliferation and metastasis of triple-negative breast cancer via regulation of SOX5. Exp Ther Med. 2018, 15:4515–4521.

[21] Lund T, Stokke T, Olsen OE, Fedstad O. Garlic arrests MDA-MB-435 cancer cells in mitosis, phosphorylates the proapoptotic BH3-only protein BimEL and induces apoptosis. Br J Cancer 2005;92(9):1773–1781.

[22] Cragg GM, Grothaus PG, Newman DJ: Impact of natural products on developing new anti-cancer agents. Chemical reviews 2009, 109(7):3021–3043.

[23] Tan W, Lu J, Huang M, Li Y, Chen M, Wu G, Gong J, Zhong Z, Xu Z, Dong Y, et al: Anti-cancer natural products isolated from chinese medicinal herbs. Chin Med 2011, 6(1):27.

[24] Amsterdam JD, Panossian AG: Rhodiola rosea L as a putative botanical antidepressant. Phytomedicine: international journal of phytotherapy and phytopharmacology. 2016 Jun 15;23(7):770-83. doi: 10.1016/j.phyto.2016.02.009. PubMed PMID: 27013349; eng.

[25] Nabavi SF, Braidy N, Orhan IE, et al. Rhodiola rosea and Alzheimer’s Disease: From Farm to Pharmacy. Phytotherapy research : PTR. 2016 Apr;30(4):532-9. doi: 10.1002/ptr.5569. PubMed PMID: 27059687; eng.

[26] Crompton M, Banks AP, Boyle J. The Effects of Rhodiola rosea L. Extract on Anxiety, Stress, Cognition and Other Mood Symptoms. Phytotherapy research : PTR. 2015 Dec;29(12):1934-9. doi: 10.1002/ptr.5486. PubMed PMID: 26502953; eng.

[27] Li T, Xu K, Liu Y. Anticancer effect of salidroside reduces viability through autophagy/PI3K/Akt and MMP-9 signaling pathways in human bladder cancer cells. Oncology letters. 2018;16(3):3162-3168. doi: 10.3892/ol.2018.8982. PubMed PMID: 30127910; PubMed Central PMCID: PMCPmc6096056. eng.

[28] Sun C, Wang Z, Zheng Q, Zhang H: Salidroside inhibits migration and invasion of human fibrosarcoma HT1080 cells. Phytomedicine : international journal of phytotherapy and phytopharmacology 2012, 19(3):355–363.

[29] Chin YR, Toker A: Function of Akt/PKB signaling to cell motility, invasion and the tumor stroma in cancer. Cell Signalling 2009, 21(4):470–476.

[30] Meng Q, Xia C, Fang J, Rojanasakul Y, Jiang BH: Role of PI3K and AKT specific isoforms in ovarian cancer cell migration, invasion and proliferation through the p70S6K1 pathway. Cell Signal 2006, 18(12):2262–2271.

[31] Amiri A, Noei F, Jeganathan S, Kulkarni G, Pinke DE, Lee JM: eEF1A2 activates Akt and stimulates Akt-dependent actin remodeling, invasion and migration. Oncogene 2007, 26(21):3027–3040.

[32] Qu J, Lu J, Zhao MF, Teng YE, Zhang Y, Hou KJ, Zhang YH, Yang XH, Liu YP: Gastric cancer exosomes promote tumour cell proliferation through PI3K/AKT and MAPK/ERK activation. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2009, 41(12):375–380.

[33] Chang X, Luo F, Jiang W, Zhu L, Gao J, He H, Wei T, Gong S, Yan P: Protective activity of salidroside against ethanol-induced gastric ulcer via the MAPK/NF-kappaB pathway in vivo and in vitro. Int Immunopharmacol 2015, 28(1):604–615.

[34] Fan XJ, Wang Y, Wang L, Zhu M: Salidroside induces apoptosis and autophagy in human colorectal cancer cells through inhibition of PI3K/Akt/mTOR pathway. Oncol Rep 2016, 36(6):3559–3567.

[35] Xu MC, Shi HM, Gao XF, Wang H: Salidroside attenuates myocardial ischemia-reperfusion injury via PI3K/Akt signaling pathway. Journal of Asian Natural Products Research 2013, 15(3):244–252.

[36] Zhu Y, Shi YP, Wu D, Ji YJ, Wang X, Chen HL, Hu SS, Huang DJ, Jiang W: Salidroside protects against hydrogen peroxide-induced injury in cardiac H9c2 cells via PI3K-Akt dependent pathway. DNA and cell biology 2011, 30(10):809–819.

[37] Hu Y, Zhu Q, Tang L: MiR-99a Antitumor Activity in Human Breast Cancer Cells through Targeting of mTOR Expression. PLoS One 2014, 9(3):e92099.

[38] Yu SH, Zhang CL, Dong FS, Zhang YM: miR-99a suppresses the metastasis of human non-small cell lung cancer cells by targeting AKT1 signaling pathway. J Cell Biochem 2015, 116(2):268–276.

[39] Zhang Y, Chang L, Li Z, Gao Q, Cai D, Tian Y, Zeng L, Li M: miR-99a and -98b inhibit cervical cancer cell proliferation and invasion by targeting mTOR signaling pathway. Medical Oncology 2014, 31(5):1–8.

[40] Zhang E-b, Kong R, Yin D-d, You L-h, Sun M, Han L, Xu T-p, Xia R, Yang J-s, De W et al: Long noncoding RNA ANRIL indicates a poor prognosis of gastric cancer and promotes tumor growth by epigenetically silencing of miR-99a/miR-449a. Oncotarget 2014, 5(8):2276-2282.

[41] Chakraborti M, Banik NL, Ray SK: Photofrin based photodynamic therapy and miR-99a transfection inhibited FGFR3 and PI3K/Akt signaling mechanisms to control growth of human glioblastoma in vitro and in vivo. PLoS One 2013, 8(2):e55652.

[42] Gusnott S, Jenkins CE, Lam SH, et al. IGFR1 derived PI3K/AKT signaling maintains growth in a subset of human T-cell acute lymphoblastic leukemias. PLoS One. 2016;11:e0161158.