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***Basic Study***

**Paeoniflorin inhibits human** [**gastric**](app:ds:gastric)[**carcinoma**](app:ds:carcinoma) **cell proliferation through up-regulation microRNA-124 and suppression of PI3K/Akt and STAT3 signaling**

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**Abstract**

**AIM:** To examine the potential anti-tumor activity of paeoniflorin on the human gastric carcinoma cell line MGC-803.

**METHODS:** Cell viability and cell cytotoxic of MGC-803 cells were analyzed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and lactate dehydrogenase assay, respectively. Cell apoptotic of MGC-803 cells was measured using both flow cytometry, DAPI staining assay and caspase-3 activity assays. Quantitative reverse transcription-polymerase chain reaction (RT-PCR) was used to measure the expression of microRNA-124 (miR-124) in response to paeoniflorin. The expression of phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt), phospho-Akt (p-Akt) and phospho-signal transducer and activator of transcription 3 (p-STAT3) were also measured by quantitative RT-PCR and Western blot analysis in normal, miR-124 and anti-miR-124 over-expressing MGC-803 cells, treated with paeoniflorin.

**RESULTS:** Paeoniflorin was found to inhibit MGC-803 cell viability in a dose-dependent manner. Paeoniflorin treatment was associated with the induction of apoptosis and caspase-3 activity in MGC-803 cells. Paeoniflorin treatment significantly increased miR-124 levels and inhibited the expression of PI3K, Akt, p-Akt and p-STAT3 in MGC-803 cells. Interestingly, the over-expression of miR-124 inhibits PI3K/Akt and phospho-STAT3 expressions in MGC-803 cells. PI3K agonist (IGF-1, 1 μg/10 μL) or over-expression of STAT3 reversed the effect of paeoniflorin on the cell proliferation of MGC-803 cells. Over-expression of anti-miR-124 in MGC-803 cells reversed paeoniflorin-induced up-regulation.

**CONCLUSION:** In summary, the *in vitro* data suggests that paeoniflorinis a potential novel therapeutic agent against gastric carcinoma, which inhibits cell viability and induces apoptosis through the up-regulation of miR-124 and suppression of PI3K/Akt and STAT3 signaling.

**Key words:**Gastric cancer; Paeoniflorin; MicroRNA-124; Phosphatidylinositol 3-kinase; Akt; Signal transducer and activator of transcription 3

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**Core tip:** The *in vitro* data suggests that paeoniflorinis is a potential novel therapeutic agent against gastric carcinoma, which inhibits cell viability and induces apoptosis through the up-regulation of microRNA-124 and suppression of phosphatidylinositol 3-kinase/Akt signaling.

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**INTRODUCTION**

Gastric cancer is one of the most common malignant tumors in China. According to the International Agency for Research on Cancer, there are approximately 989000 new cases of gastric cancer worldwide in 2008. Of this population, the number of new cases in China was 463000, accounting for 46.8% of the global incidence of gastric cancer[[1](#_ENREF_1),[2](#_ENREF_2)]. Similarly, the number of deaths due to gastric cancer worldwide was approximately 737000. China accounted for 47.8% of global gastric cancer deaths. Gastric cancer ranked third in cancer deaths, after lung cancer and liver cancer, in 2004-2005. Despite this, the mortality of gastric cancer appears to be declining from its leading position as the number one cause of cancer deaths in China in 1973-1975 and 1990-1992[[3](#_ENREF_3),[4](#_ENREF_4)].

Micro-RNA is a small non-coding sequence of RNA that may function as an oncogene or tumor-suppressor gene, as well as regulate cell proliferation, apoptosis and differentiation[[5](#_ENREF_5)]. Micro-RNA may also play an important role within tumorigenesis and development[[6](#_ENREF_6),[7](#_ENREF_7)]. Studies show that expression of microRNA-124 (miR-124) is down-regulated in liver and cervical cancer[[8](#_ENREF_8),[9](#_ENREF_9)]. In gastric cancer, the expression of miR-124 is down-regulated and is associated with the clinical disease stage, the degree of differentiation and lymph node metastasis. MiR-124 can also inhibit the cell growth and invasion of medulloblastoma cells by targeting CDK6. As a result, targeting miR-124 expression may be a novel therapeutic strategy against gastric cancer[[10](#_ENREF_10),[11](#_ENREF_11)].

Embryo prototype mutations of phosphatase and tension homolog (PTEN) are prevalent many diseases. A substrate for PTEN is a lipid, generated by phosphatidylinositol 3-kinase (PI3K), and is necessary for the activation of protein kinase B (Akt). PTEN regulates the activity of Akt *via* activated phosphatidyl inositol triphosphate (PIP3). Therefore, a mutation of PTEN uncouples Akt regulation, resulting in unchecked cell proliferation and tumorigenesis. PTEN can dephosphorylate-PIP3, and reduce the concentration of PIP3 within the cells and inhibits the activation of Akt[[5](#_ENREF_5)]. Protein phosphatase activity is also closely associated with tumors. Yu *et al*[[13](#_ENREF_13)] reported that the induction of apoptosis was accompanied by the inactivation of the PI3K/Akt signaling pathway[[12](#_ENREF_12)]. Torkinib suppresses cell proliferation of gastric cancer through inhibition of the PI3K/Akt pathway.

STAT3 is an important member of signal transducers and activators of transcription (STAT), defined as oncogene currently[[14](#_ENREF_14)]. STAT3 plays an important role in promoting tumor cell proliferation, inhibiting tumor cell apoptosis, and promoting tumor invasion and metastasis as well as immune escape, and the continuous activation of its signal transduction pathway is closely related to the occurrence and development of tumor[[15](#_ENREF_15)].

Paeoniflorin is the main active monomer ingredient of paeonialactiflo and is reported to have anti-inflammatory, immunomodulatory, liverand nerve protective activity[[16-18](#_ENREF_16)]. Recent *in vitro* studies report and confirm that the anti-cancer effect of paeoniflorin inhibits human lung cancer cells proliferation [[19](#_ENREF_19), [20](#_ENREF_20)], [human hepatocellular carcinoma cells](http://www.ncbi.nlm.nih.gov/pubmed/25077366)[[7](#_ENREF_7)], [colorectal carcinoma](http://www.ncbi.nlm.nih.gov/pubmed/22326807)[[8](#_ENREF_8)] and so on. However, the mechanisms underlying the anti-cancer effects of paeoniflorin on gastric cancer are still indeterminate. Therefore, we aim to investigate the potential anti-proliferative effects of paeoniflorin to gastric cancer. We also explored the associated changes in the miR-124, PI3K/Akt, STAT3 signaling axis with paeoniflorin-mediated antitumor activity.

**MATERIALS AND METHODS**

***Materials***

Paeoniflorin (Sigma, with a purity > 97%) was dissolved in physiological saline according to the manufacturer's instructions and the chemical structure of it was indicated in Figure 1. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) was from Gibco (Grand Island, NY, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was purchased from Sigma (Japan). The Annexin V fluorescein isothiocyanate (FITC)-propidium Iodide (PI) apoptosis kit and Bicinchoninic Acid (BCA) Protein Assay kit were purchased from Sangon Biotech (Shanghai, China). Caspase-3 colorimetric assay kit and a Nuclear and Cytoplasmic Protein Extraction Kit were purchased from Beyotime (Nanjing, China). Trizol was purchase from Tiangen (Beijing, China). High Capacity cDNA Reverse Transcription kit and ABI 7500 were purchase from TAKARA (Japan).

***Cell culture***

Human [gastric](app:ds:gastric) [carcinoma](app:ds:carcinoma) cell MGC-803 cells and Human normal gastric mucosa cell lines GES-1 cells were obtained from Academy of Military Medical Sciences (Beijing, China) and cultured in DMEM supplemented with 10% FBS with 100 U/mL penicillin and 100 mg/mL streptomycin under a humidified atmosphere of 5% CO2 and 95% air at 37 °C. MGC-803 cells were treated with paeoniflorin in complete DMEM medium.

***Cell proliferation assay***

MGC-803 cells (5000 cells per well) and GES-1 cells (5000 cells per well) were seeded in 96-well. Cell proliferation of MGC-803 cells and GES-1 cells were analyzed using MTT (Sigma, Japan) assay after the treatment of paeoniflorin (0, 5, 10 and 20 μM) for 0, 24, 48 and 72 h. Then, 10 μL MTT (5 mg/mL) was added into every well and incubated for 4 h under a humidified atmosphere of 5% CO2 and 95% air at 37 °C. [Afterwards](app:ds:afterwards), 150 μL dimethyl sulfoxide was added to each well and shaken for 10-20 min in [table](app:ds:table) [concentrator](app:ds:concentrator). The absorbance was determined with an ELISA reader at 450 nm.

***Lactate dehydrogenase assay***

MGC-803 cells (5000 cells per well) were seeded in 96-well. Cell cytotoxic of MGC-803 cells was measured using LDH assay after the treatment of paeoniflorin (0, 5, 10 and 20 μM) for 0, 24, 48 and 72 h. Then, 100 μL LDH solution was added to each well and incubated for 30 min at room temperature. The absorbance was read at 490nm using a multiwell spectrophotometer (BioTek, XL-828, USA).

***Flow cytometry analysis of cell apoptosis***

MGC-803 cells (1 × 105 cells per well) were seeded in 6-well. Cell apoptosis was detected using Annexin V FITC-PI kit assay after the treatment of paeoniflorin (0, 5, 10 and 20 μM) for 48 h. The cells were washed and collected with twice PBS according to the manufacturer’s instructions (Sangon Biotech, Shanghai, China). The cells were added with 5 μL VFITC and incubated for 30 min at darkness. 10 μL PI was added into cell at darkness. The stained cells were detected using FACSCalibur flow cytometry (BD Biosciences, San Jose, CA) [at](app:ds:at) [once](app:ds:once) and analyzed with Cell-Quest software.

***DAPI staining assay***

MGC-803 cells (1 × 105 cells per well) were seeded in 6-well. MGC-803 cells were washed twice with PBS. Then, 0.5 ml (4 %) paraformaldehyde was added to each well and incubated for 30 min at 4 °C. MGC-803 cells were washed twice with PBS, added with Sodium citrate (0.1%) containing 0.1% Triton X-100 and incubated for 5 min at 4 ˚C. Then, DAPI was added to each well and incubated for 20 min at 4 ˚C in the dark. Apoptotic cells were excitated by Ultraviolet. MGC-803 cells was observed and photographed under florescence microscopy at 340 nm.

***Caspase-3 activity assays***

MGC-803 cells (5000 cells per well) were seeded in 96-well. The activity of caspase-3 was analyzed using the caspase-3 colorimetric assay kit (Beyotime, Nanjing, China) assay according to the manufacturer’s instructions, after the treatment of paeoniflorin (0, 5, 10 and 20 μM) for 48 h. Cells were collected and washed twice with ice-cold PBS. Then, cells (1 × 105 cells/50 mL) were resuspended with BD Cytofix/Cytoperm solution. Next, cells were incubated for 30 min on ice and then washed twice using Perm/Wash buffer at room temperature. The resuspended cells (1 × 106 cells/ 20 mL) were incubated for 30–60 min at room temperature. Finally, each test was washed with 1.0 ml Perm/Wash buffer and then resuspended with 0.5 mL Perm/Wash buffer. The caspase-3 activity was analyzed at the wavelength of 405 nm.

***Real-time quantitative for*** ***miR-124***

Total RNA was extracted from MGC-803 cells using Trizol (Tiangen, Beijing, China). MiR-124 was performed using the High Capacity cDNA Reverse Transcription kit (TAKARA, Japan) with ABI 7500 (TAKARA, Japan) quantitative PCR system according to the manufactures instructions. All primers used are purchased from Sangon Biotech (Shanghai, China). The MiR-124-forward: 5′-GCGGCCGTGTTCACAGCGGACC-3′ and miR-124- reverse: 5′-GTGCAGGGTCCGAGGT-3′. U6-forward: 5'-CGCTTCGGCAGCA CATATACTA-3'; and U6- reverse: 5'-CGCTTCACGAATTTGCGTGTCA.

***Western blot analysis for*** ***PI3K******, Akt and******STAT3***

MGC-803 cells (1 × 105 cells per well) were seeded in 6-well. The expressions of PI3K and Akt protein were detected using Western blot assay after the treatment of paeoniflorin (0, 5, 10 and 20 μM) for 48 h. Cells were collected and washed twice with ice-cold PBS. In according with the protocol described by the manufacturer (Beyotime, Haimen, China), cells were lysed using a Nuclear and Cytoplasmic Protein Extraction Kit. [Smudge](app:ds:smudge) [cells](app:ds:cells) were centrifuged at 15000 × *g* for 10 min at 4 °C. Then, protein concentrations were determined with the Bicinchoninic Acid (BCA) Protein Assay kit (Sangon Biotech, Shanghai, China). Protein samples were analyzed with SDS-polyacrylamide gel electrophoresis (PAGE) and then transferred to PVDF membrane (Millipore, Bedford, MA). After blocking with 5% (v/v) nonfat milk for 2 h in TBST buffer overnight at 4 °C. Membranes were incubated with anti-PI3K, anti-phospho-Akt (p-Akt), anti-Akt, anti-STAT3, anti-phospho-STAT3 (p-STAT3) and anti-β-actin from Abcam (Boster Biological, Wuhan, China) overnight at 4 °C. Then, membranes incubated with the peroxidase-linked secondary antibodies for 2 h at room temperature. Proteins were developed using the enhanced chemilumin-escence detection system (Amersham Biosciences, Piscataway, NJ, USA).

***MiR-124 and anti-miR124*** ***plasmids transfection***

MiR-124, anti-miR124 and ***STAT3*** plasmids were designed and structured by Gene Pharma (Shanghai, China). MGC-803 cells (1 × 105 cells per well) were seeded in 6-well. when the MGC-803 cells reached 70-80% confluence, miR-124 and Anti-miR124 plasmids were transfected into cells using lipofectamine 2000 (Invitrogen, USA) according to the manufacturer’s protocol. After 6 h, [culture](app:ds:culture) [medium](app:ds:medium) was removed, change [complete](app:ds:complete) [medium](app:ds:medium) and cultured under a humidified atmosphere of 5% CO2 and 95% air at 37 °C.

***Statistical analysis***

The data were analyzed statistically using SPSS software 17 and shown as mean ± SD. Analysis was performed using one-way ANOVA followed by Dunnett’s post-hoc test. The *P* ≤ 0.05 were considered indicative of statistical significance.

**RESULTS**

***Paeoniflorin*** ***inhibites*** ***cell proliferation***

Cell viability was evaluated using MTT assays. Figure 2A showed a dose- and time-dependent decrease in cell viability; paeoniflorin (20 μM) could observably refrained cell viability for 48 and 72 h, and paeoniflorin (10 μM) could also effectively refrained cell viability for 72 h. Hence, 20 μM was determined as the optimal dose for the effect paeoniflorin on MGC-803 cells. Then, we checked that the effect of paeoniflorin (20 μM) influenced normal gastric mucosa cell lines (GES-1). Figure 2B showed that increased cell proliferation of GES-1 was increased after the treatment with paeoniflorin (20 μM) for 48 h, compared to the MGC-803 cells + paeoniflorin (20 μM) group.

***Paeoniflorin inhibites*** ***cell cytotoxic***

Cell cytotoxic of MGC-803 cells was measured using LDH assay. Figure 3 showed that paeoniflorin increased cell cytotoxic in a dose-dependent. There are a significant elevate cell cytotoxic after the treatment with paeoniflorin (20 μM) for 48 h (Figure 3).

***Paeoniflorin promotes apoptosis***

To investigate whether paeoniflorin protects against apoptosis, MGC-803 cells were incubated with paeoniflorin (10 and 20 μM) for 48 h. Cells were then stained with Annexin VFITC/PI, and the apoptosis of MGC-803 cells was determined with flow cytometry. Figure 4A–4B showed a significant increase in the apoptosis rate when the MGC-803 cells were exposed to 20 μM paeoniflorin for 48 h. Moreover, the results of DAPI staining show that different concentrations of paeoniflorin (5, 10 and 20 μM) markedly increased the apoptosis of MGC-803 cells (Figure 4C).

***Paeoniflorin*** ***induces caspase-3 activity***

To further research whether paeoniflorin induces caspase-3 activity, MGC-803 cells were incubated with paeoniflorin (10 and 20 μM) for 48 h. When the MGC-803 cells were treated with different concentrations of paeoniflorin (5, 10 and 20 μM), the caspase-3 activity of MGC-803 cells was dose-dependent. When 20 μM paeoniflorin could effectively increase the activity of caspase-3 in MGC-803 cells (Figure 5).

***Paeoniflorin activates the expression of miR-124***

To determine whether paeoniflorin activates the expression of miR-124 in MGC-803 cells, the miR-124 expression of MGC-803 cells was detected by QPCR. Figure 5 showed treatment with paeoniflorin (5, 10 and 20 μM) resulted in a dose-dependent inhibition of the miR-124 expression (Figure 6). However, the levels of miR-124 were significantly increased in the paeoniflorin (20 μM)-treated cells for 48 h.

***Paeoniflorin restrains*** ***the expression of*** ***PI3K/Akt***

To clarify the anticancer mechanisms of paeoniflorin on MGC-803 cells, we examined the expression levels of the PI3K, p-Akt and Akt expression in MGC-803 cells. Figure 7A and 7B showed treatment of MGC-803 cells with different concentration of paeoniflorin (5, 10 and 20 μM) for 48 h induced an decreased in the expressions of PI3K, p-Akt and Akt protein. Certainly, paeoniflorin (20 μM) could observably restrain the expression of PI3K, p-Akt and Akt in MGC-803 cells (Figure 7A and 7B).

***Paeoniflorin restrains the expression of*** ***STAT3***

To explore the anticancer mechanisms of paeoniflorin on MGC-803 cells, p-STAT3 and STAT3 protein expression levels were checked in MGC-803 cells. Figure 8A and 8B showed that p-STAT3 protein expression levels were elevated by paeoniflorin administration (Figure 8A and 8B).

***PI3K agonist*** ***inhibites the effect of paeoniflorin on cell proliferation***

To further research whether PI3K agonist inhibites the effect of paeoniflorin on cell proliferation, the expression of PI3K/Akt and the cell proliferation of MGC-803 cells were detected. First, when the treatment with paeoniflorin (20 μM) for 72 h, the expressions of PI3K, p-Akt and Akt proteins in MGC-803 cells were measured. We observed that the expressions of PI3K, p-Akt and Akt proteins in paeoniflorin treatment group were remarkably increased (Figure 9A). But, PI3K agonist (IGF-1, 1 μg/10 μL) inhibited the effect of paeoniflorin on the PI3K, p-Akt and Akt proteins expressions in MGC-803 cells (Figure 9A). Meanwhile, IGF-1 reversed the effect of paeoniflorin on the cell proliferation of MGC-803 cells (Figure 9B).

***Overexpression of*** ***STAT3 inhibites the effect of paeoniflorin on cell proliferation***

To further detect whether overexpression of STAT3 inhibites the effect of paeoniflorin on cell proliferation, p-STAT3 protein expression levels and the cell proliferation of MGC-803 cells were ascertain. p-STAT3 protein expression levels were evidently boosted (Figure 10A). Certainly, overexpression of STAT3 reversed the effect of paeoniflorin on the cell proliferation of MGC-803 cells (Figure 10B).

***Overexpression of*** ***miR-124 and*** ***the expression of PI3K/Akt and p-STAT3***

To determine whether miR-124 is a crucial transcription factor for regulating the expression of PI3K, p-Akt, Akt and p-STAT3, miR-124 plasmid was transfection into MGC-803 cells. Figure 11A showed that miR-124 plasmid could effectively augment the expression of miR-124 level in MGC-803 cells. Meanwhile, overexpression of miR-124 could also control the PI3K, p-Akt, Akt (Figure 11B), p-STAT3 protein expression levels (Figure 11C) in MGC-803 cells

***Anti-miR-124 and the effect of paeoniflorin***

To further investigate the correlations of miR-124 expression level with the effect of paeoniflorin, the effect of paeoniflorin (20 μM) on MGC-803 cells was performed for 72 h. Our results showed that transfection of anti-miR-124 plasmids were transfected into MGC-803 cells and significantly reduced the expression of miR-124 in MGC-803 cells (Figure 12A). Meanwhile, anti-miR-124 plasmids could significantly reduce the effect of paeoniflorin on cell proliferation (Figure 12B) and apoptotic effect of MGC-803 cells (Figure 12C). Meanwhile, anti-miR-124 plasmids could promote the levels of PI3K, p-Akt, Akt and p-STAT3 in MGC-803 cells (Figure 12D).

**DISCUSSION**

Age-specific incidence and mortality curves show that gastric cancer is an important malignant tumor in the elderly population, and the regional difference of morbidity and mortality indicates in China that pathogenic factors and susceptibility to gastric cancer is varied[[21](#_ENREF_21)]. The incidence of gastric cancer in urban areas of China shows no clear upward trend in 2009, while in rural areas, incidence of gastric cancer seems to increase with time[[22](#_ENREF_22)]. However, the change in standardized rate has stabilized; change in mortality of gastric cancer is similar to the incidence in the same period. Our most significant finding was that paeoniflorin could markedly inhibit the cell viability of MGC-803 gastric carcinoma cells[[23](#_ENREF_23)]. Other studies have shown paeoniflorin could attenuate the viability, and increases apoptosis in Aβ25-35 in SH-SY5Y cells. In this study, paeoniflorin effectively suppressed cell proliferation, increased cell cytotoxic, induced apoptotic and accelerated the activity of caspase-3 in MGC-803 cells. Wang *et al*[[24](#_ENREF_24)]. reported that paeoniflorin significantly ameliorated glutamate-induced reduction of cell viability, and facilitated apoptotic alteration in PC12 cells Paeoniflrin was also found to have a pro-apoptotic and anti-proliferative effect in human colorectal carcinoma HT-29 cells, as shown by the activation of caspase-3 and caspase-9 *in vitro* and *in vivo*[[8](#_ENREF_8)].

Oncogenic microRNA promotes cell proliferation, inhibits apoptosis, inhibits immune cell development and regulates cell cycle. Conversely, tumor suppressive microRNA inhibits cell growth and promotes apoptosis[[25](#_ENREF_25)]. Several microRNA genes are reported to show oncogenic or tumor suppressive activity related to tumor development. Studies show highly-expressed miR-124 influences the proliferation of MKN-45 cell[[26](#_ENREF_26)]. The results show that the invasion speed of MKN-45 cells, transfected with miR-124, declines significantly after 24 hours compared with that of scramble sequence control. This indicates that miR-124 can inhibit the invasion of gastric cancer cells motility, which may play an important role in the gastric tumorigenesis and development[[27](#_ENREF_27)]. In our study, paeoniflorin treatment could significantly increase the levels of miR-124 in MGC-803 gastric carcinoma cells.

The expressions variance of PI3K and Akt was not statistically significant in cancer-adjacent tissues and normal gastric mucosa. This indicates that appearance of PI3K and Akt in gastric cancer may be a late molecular event[[28](#_ENREF_28)]. Mammary epithelial cells, from mice, inhibit AKT1 expression, delay epithelial cell differentiation and accelerate apoptosis and recovery of cells during pregnancy, lactation and recovery periods. The inhibition of AKT2 results in the opposite effect, suggesting that different subtypes of AKT have different functions[[29](#_ENREF_29),[30](#_ENREF_30)].

Our results suggest that paeoniflorin could observably inhibit the expression of PI3K, Akt and phospho-Aktin MGC-803 gastric carcinoma cells. Xu *et al*[[31](#_ENREF_31)] reported that paeoniflorin attenuates lipopolysaccharide-induced permeability of endothelial cells through the suppression of the phosphorylations of PI3K/Akt. Wu *et al*[[32](#_ENREF_32)] demonstrated that paeoniflorin-mediated neural stem/progenitor cells protection from hydrogen peroxide injury is dependent on the activation of the PI3K/Akt-1 pathway. In our study, we found that miR-124 regulates and controls the expression of PI3K, Akt and phospho-Akt in MGC-803 cells.

STAT3 regulates target gene expression of downstream to accelerate tumor cell proliferation, prevent apoptosis and promote tumor angiogenesis. Vivo blocking or inhibiting STAT3 signaling pathway of certain tumor cells can inhibit cell proliferation and survival, and induce apoptosis[[33](#_ENREF_33)]. Therefore, the application of negative regulatory proteins to interfere with STAT3 signaling pathway has become an important strategy for cancer intervention, and STAT3 is considered to be a new target for cancer therapy. The results show that the expression of STAT3 protein in gastric carcinoma tissues is significantly higher than that in normal gastric tissues, and its expression is not associated with patient gender and tissue type, but only with lymph node metastasis, differentiation degree and clinical stage[[34](#_ENREF_34)]. The activation of STAT3 signaling pathway, promotes cell proliferation and inhibits apoptosis, and this relationship may lead to malignant cell transformation and abnormal proliferation, thus promoting the occurrence and development of gastric cancer[[35](#_ENREF_35)]. In this study, the results of our study show for first time that paeoniflorin could suppress p-STAT3 protein expression in MGC-803 cells. Meanwhile, we found that overexpression of STAT3 inhibited the effect of paeoniflorin on cell proliferation of MGC-803 cells. Recent studies show that miR-124 functions as a tumor suppressor suppressed p-STAT3 in the human endometrial carcinoma cell line HEC-1B[[36](#_ENREF_36)] and colorectal cancer[[37](#_ENREF_37)].

In summary, this study demonstrates that paeoniflorin possesses antitumor activity on gastric cancer cells by inhibiting cell proliferation and stimulating apoptosis *via* the up-regulation miR-124, and suppression of PI3K/Akt signaling. Follow up studies are required to evaluate the efficacy of this drug within cancer-bearing animal models.

**COMMENTS**

***Background***

Gastric cancer is one of the most common malignant tumors in China. According to the International Agency for Research on Cancer, there are approximately 989000 new cases of gastric cancer worldwide in 2008. The mechanisms underlying the anti-cancer effects of paeoniflorin on gastric cancer are still indeterminate.

***Research frontiers***

The authors aim to investigate the potential anti-proliferative effects of paeoniflorin to gastric cancer. The authors also explored the associated changes in the miR-124, PI3K/Akt, STAT3 signaling axis with paeoniflorin-mediated antitumor activity.

***Applications***

The authors found that *in vitro* data suggests that paeoniflorinis a potential novel therapeutic agent against gastric carcinoma, which inhibits cell viability and induces apoptosis through the up-regulation of miR-124 and suppression of PI3K/Akt and STAT3 signaling.

***Peer-review***

In this work authors presented data suggesting that paeoniflorin is a potential novel therapeutic agent against gastric carcinoma, which inhibits cell viability and induces apoptosis through the up-regulation of miR-124 and suppression of PI3K/Akt signaling. They carried out in vitro assays with the gastric carcinoma cell line MGC-803. It is an interesting study, with a lot of work.

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**Figure 1 The chemical structure of paeoniflorin.**

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**Figure 2** **Paeoniflorin inhibites cell proliferation****.** Paeoniflorin inhibites cell proliferation of MGC-803 cells after the treatment with paeoniflorin (20 μM) for 72 h (A) and cell proliferation of GES-1 after the treatment with paeoniflorin (20 μM) for 48 h (B). a*P <* 0.01 *vs* 0 μM paeoniflorin treatment group.

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**Figure 3 Paeoniflorin inhibites cell cytotoxic.**

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**Figure 4 Paeoniflorin promotes apoptosis.** Flow-cytometric analysis for detecting cellular apoptosis (A), statistical analysis of cellular apoptosis level (B) and DAPI staining for detecting cellular apoptosis (C). a*P <* 0.01 *vs* 0 μM paeoniflorin treatment group.

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**Figure 5 Paeoniflorin induces caspase-3 activity.** a*P <* 0.01 *vs* 0 μM paeoniflorin treatment group.

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**Figure 6 Paeoniflorin activates the expression of** **miR-124.** a*P <* 0.01 *vs* 0 μM paeoniflorin treatment group.

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**Figure 7 Paeoniflorin restrains the expression of PI3K/Akt.** Indicated representative western blotting analysis of PI3K, p-Akt and Akt protein levels (A) and statistical analysis of PI3K, p-Akt and Akt protein level (B-D). a*P <* 0.01 *vs* control treatment group.

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**Figure 8 Paeoniflorin restrains the expression of****STAT3.** Indicated representative western blotting analysis of p-STAT3 protein levels (A) and statistical analysis of p-STAT3 protein level (B). a*P <* 0.01 *vs* control treatment group.

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**Figure 9 PI3K agonist inhibites the effect of paeoniflorin on cell proliferation.**Indicated representative western blotting analysis of PI3K, p-Akt and Akt protein levels (A) and paeoniflorin inhibites cell proliferation (B). \**P <* 0.01 compared with control treatment group, b*P >* 0.05 *vs* control treatment group.

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**Figure 10 Overexpression of STAT3 inhibites the effect of paeoniflorin on cell proliferation.** Indicated representative western blotting analysis of p-STAT3 protein levels (A) and paeoniflorin inhibites cell proliferation (B). a*P <* 0.01 *vs* control treatment group, b*P >* 0.05 *vs* control treatment group.

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**Figure 11 Overexpression of microRNA-124 and the expression of PI3K/Akt.** Overexpression of microRNA (miR)-124 can accelerate the expression of miR-124 (A); inhibite PI3K, p-Akt, Akt and p-STAT3 protein expression (B). a*P <* 0.01 *vs* 0 μM paeoniflorin treatment group.

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**Figure 12 Anti-microRNA-124 and the effect of paeoniflorin.** Anti-microRNA (miR)-124 can reverse the effect of paeoniflorin on the expression of miR-124 in MGC-803 cells (A); After treatment with paeoniflorin (20 μM) for 72 h, anti-miR-124 significantly promoted the cell proliferation of MGC-803 cells (B); After treatment with paeoniflorin (20 μM) for 72 h, anti-miR-124 evidently inhibited cell apoptosis of MGC-803 cells (C); The anti-miR-124 significantly increased the expressions of PI3K, p-Akt, Akt and p-STAT3 protein in MGC-803 cells after paeoniflorin (20 μM) treatment at 72 h (D). a*P <* 0.01 *vs* 0 μM paeoniflorin treatment group and c*P <* 0.01 *vs* paeoniflorin-treated group transfected with negative control.