Original Article

Total Astragalus saponins promote ferroptosis in gastric cancer cells by upregulating SIRT3

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Background: Gastric cancer (GC) is a malignant tumor of the digestive tract originating from the epithelial cells of the gastric mucosa, which is highly invasive and heterogeneous, posing a serious threat to human health. In recent years, ferroptosis, as a novel mode of programmed cell death, has shown potential anticancer effects in tumor therapy. Total Astragalus saponins (TAS), a natural product derived from Astragalus membranaceus, have been shown to possess various pharmacological activities, including anticancer effects. This study aimed to investigate the effects of TAS on GC cells, focusing on the mechanism of action of its regulation of the silent information regulator 3 (SIRT3) in inducing ferroptosis in GC cells.

Methods: We treated SGC-7901 cells with TAS at concentrations of 50, 100, and 200 μg/mL. After TAS treatment, the SGC-7901 cells were transfected with a vector designed to knock down SIRT3 expression. We assessed cell proliferation, viability, and apoptosis using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay), colony formation assay, and flow cytometry. SIRT3 expression was measured by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR). Fe^{2+}, malondialdehyde (MDA), lactate dehydrogenase (LDH), and superoxide dismutase assay kits were used to detect the level of reactive oxygen species (ROS) by fluorescent probe assay. Western blot was used to detect apoptosis-related proteins and SIRT3 protein expression.

Results: TAS dose-dependently inhibited SGC-7901 cell proliferation and viability ($P<0.05$) and induced apoptosis ($P<0.05$). TAS promoted the expression of SIRT3 and ACSL4 proteins ($P<0.05$), inhibited the expression of SLC7A11 and GPX4 proteins ($P<0.05$), and induced ferroptosis of SGC-7901 cells ($P<0.05$). Knockdown of the SIRT3 gene attenuated the effect of TAS treatment on ferroptosis ($P<0.05$).

Conclusions: TAS has therapeutic potential for GC and can effectively inhibit the proliferation and viability of SGC-7901 cells, and the mechanism may be that TAS upregulates SIRT3 to promote the ferroptosis of SGC-7901 cells.

Keywords: Gastric cancer (GC); total Astragalus saponins (TAS); silent information regulator 3 (SIRT3); ferroptosis

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Introduction

Gastric cancer (GC) is a common gastrointestinal malignant tumor occurring in the epithelium and glandular epithelium of the gastric mucosa, accounting for the 5th and 4th place in the incidence and mortality of malignant tumors globally (1). The development of GC is a complex multi-stage process (2), and according to a survey, early GC patients have a high 5-year survival rate (3). However, GC is difficult to detect due to the lack of typical clinical features in the early stage of development, and most patients are diagnosed only when the tumor progresses. This significantly increases the likelihood of tumor metastasis and the development of drug resistance, resulting in a 5-year survival rate of only 5–20% for patients with advanced GC (4), and about 170,000 people die of GC every year (5). With the development of social processes, accelerated aging of the population, unhealthy lifestyles, and other factors accumulating, the number of incidences is still on a rising trend. Currently, the main treatments for GC are surgery, chemotherapy, molecular targeted therapy, immunotherapy and chemotherapy, but due to the higher heterogeneity within the tumor tissue, and epigenetic changes, which make GC treatment complex with the risk of adverse reactions and recurrence. To date, GC remains a worldwide health problem (6). Given this situation, discovering novel drugs or therapeutic strategies to enhance the prognosis for GC patients is critical.

Recent studies have shown that ferroptosis is closely related to the physiopathology of tumors (7,8), so inducing ferroptosis in GC cells may be a potential strategy for GC treatment (9). Ferroptosis is a novel mode of programmed cell death (10). This distinct form of cell death is mediated by iron-dependent lipid peroxidation and is controlled by various cellular metabolic processes (11). Ferroptosis proteins that inhibit the occurrence of ferroptosis, including glutathione peroxidase 4 (GPX4) and solute carrier family 7 member 11 (SLC7A11), are highly expressed in cancer cells, which is an important cause of resistance to chemotherapeutic drugs and drug resistance in cancer cells (12). Silent information regulator 3 (SIRT3), as a NAD^+-dependent deacetylase, is an important member of the Sirtuin family (13). It is predominantly found in mitochondria and is thought to play a key regulatory role in physiological processes such as cellular energy metabolism, anti-oxidative stress and cell death (14). Recent studies have shown that SIRT3 affects tumor cell metabolism, metastasis and patient prognosis, and that SIRT3 has the potential to inhibit GC cells (15,16).

Traditional Chinese medicine (TCM) has shown a variety of advantages in cancer treatment, including multi-targeting and low toxicity and side effects (17). Astragalus membranaceus, as TCM, has been widely used in the field of TCM since ancient times and is commonly used clinically in the treatment of cardiovascular and cerebrovascular diseases, tumor diseases, diabetes, stroke, and other diseases (18-20). Astragalus membranaceus is one of the largest genera of flowering plants in the family Leguminosae, and there are currently about 2,000 to 3,000 species of Astragalus membranaceus in the world, with its main active ingredients including three major groups of saponins, polysaccharides, and flavonoids (21). One of the

Highlight box

Key findings

- We found that total astragalus saponins have therapeutic potential for gastric cancer and can effectively inhibit the proliferation and viability of SGC-7901 cells, the mechanism of which may be to promote the ferroptosis of SGC-7901 cells through the upregulation of silent information regulator 3 (SIRT3).

What is known and what is new?

- Ferroptosis, as a new type of programmed cell death, is of great significance for treating tumors. SIRT3 acts as either an oncogene or tumor suppressor by regulating cell death and growth. Astragalus saponins have pharmacological effects such as regulating immunity, resisting oxidative stress damage, anti-tumor and promoting body metabolism.
- Total astragalus saponins induced ferroptosis in gastric cancer cells by regulating SIRT3, thereby inhibiting the development of gastric cancer.

What is the implication, and what should change now?

- Total astragalus saponins have potential therapeutic value in gastric cancer, providing new therapeutic strategies and drug targets for gastric cancer treatment, and are expected to bring new breakthroughs in clinical treatment.

main active ingredients, astragaloside, is believed to have a variety of biological activities, including antioxidant, anti-inflammatory, anti-tumor and immune-enhancing effects. Studies have shown that Astragalus saponin can potentially inhibit GC cells (22,23).

In the present study, we investigated the mechanism of action of total Astragalus saponins (TAS) on human GC SGC-7901 cells, to provide a theoretical and experimental basis for the use of TAS in novel and highly efficient GC therapeutic strategies. We present this article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1421/rc).

Methods

Cell culture and treatment

Human Gastric Cancer Cells SGC-7901 provided by Wuhan Procell Life Sciences Co. SGC-7901 cells were cultured in a Dulbecco's modified eagle medium (DMEM) cell culture medium containing 10% fetal bovine serum in a constant temperature cell culture incubator environment at 3–7 ℃, with 5% CO_2. Replace the fresh medium once every 2 days, when the cells were fused to about 85%, trypsin digestion was performed, and the cells were passaged according to 1:3, and the logarithmic growth phase cells were taken for experiments after 3 passages. SGC-7901 cells in the logarithmic growth phase were inoculated in 96-well plates at a density of 1×10^4 cells/well, and to evaluate the effect of TAS on SGC-7901 cells, SGC-7901 cells were treated with TAS at concentrations of 0, 50, 100, and 200 μg/mL, respectively. To investigate the association between SIRT3 and TAS, SGC-7901 cells exposed to TAS were transfected with SIRT3 CRISPR/Cas9 KO plasmid (Santa Cruz, Shanghai, China, sc-4006-75) to knockdown the SIRT3 plasmid (TAS + KO-SIRT3 group) or the blank plasmid (TAS + vector group). Twenty-four hours later, cell-related indexes were detected.

MTT assay

SGC-7901 cells were seeded into 96-well plates at a density of 1.0×10^3 cells per well and exposed to various concentrations of TAS for 0, 12, 24, and 48 hours. Following the treatment, cell proliferation was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay) (Trevigen, MC, NBP2-54883, USA). Specifically, 5 mg/L of MTT solution was added to each well, and the cells were incubated for 4 hours. After incubation, the MTT-containing medium was carefully removed, and 100 μL of dimethyl sulfoxide (DMSO) was added to each well. After 15 min, the relative absorbance at 5–70 nm was measured using a Multiskan FC microplate reader (Thermo Fisher Scientific, Shanghai, China).

Colony formation assay

The treated cells were seeded into 6-well plates at a density of 4×10^2 cells per well and incubated at 3–7 ℃ with 5% CO_2 for 10 days to allow colony development. Upon completion of the cloning process, the cells were washed twice with phosphate-buffered saline (PBS) and subsequently fixed with 4% paraformaldehyde (Wuhan Servicebio Biotechnology Co., Ltd., G4202, G1101, Wuhan, China) for 20 min. After fixation, the cells were washed again with PBS and stained with 5% crystal violet (G1014, Wuhan Servicebio Biotechnology Co., Ltd.) for 15 min. Colony counting was performed using a microscope.

Flow cytometry

SGC-7901 cells were double-stained with Annexin V-FITC/propidium iodide (PI) Cell Apoptosis Detection Kit (G1511, Wuhan Servicebio Biotechnology Co., Ltd.) to observe apoptosis. Following treatment with 0.05% trypsin and 0.02% ethylene diamine tetraacetic acid (EDTA), SGC-7901 cells were washed three times with PBS and then resuspended in binding buffer at a concentration of 1×10^6 cells/mL. Then 100 μL of the solution was transferred to culture tubes, and 5 μL of FITC Annexin V and 5 μL of PI were added. after incubation in the dark for 15 min, 400 μL of binding buffer was added to each tube, gently shaken, and analyzed by flow cytometry (NovoCyte, CA, USA).

Detection of reactive oxygen species (ROS)

SGC-7901 cells in the logarithmic growth phase were inoculated into 96-well plates, added with 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) working solution (G1706, Wuhan Servicebio Biotechnology Co., Ltd.), and incubated in a 3–7 ℃, CO_2 incubator in the dark for 30 min. During this period, the mixing was gently shaken every 5 min to ensure that the probe and the cells were in full contact. After incubation, centrifuge the plates at 1,000 rpm for 5 min, remove the DCFH-DA working solution, wash the cells with PBS buffer 3 times, then

Table 1 qPCR primer sequences

RNA	Sequences (5' to 3')	Fragment length
SIRT3	F: 5'-CTGGATGGACAGGACAGATAAGA; R: 5'-GTTGTGGTCTGGTTCATGTTTGT	133 bp
β-actin	F: 5'-GTGACGTTGACATCCGTAAAGA; R: 5'-GTAACAGTCCGCCTAGAAGCAC	136 bp

qPCR, quantitative polymerase chain reaction.

resuspend the cells and assay them by flow cytometry.

Detection of Fe^{2+}, malondialdehyde (MDA), superoxide dismutase and lactate dehydrogenase (LDH) levels

The levels of Fe^{2+}, MDA, and LDH in the cells were detected using a commercial kit from Wuhan Sevier Biotechnology Co., Ltd. (Wuhan, China). The samples and kits were used according to the manufacturer's instructions.

Western blot

SGC-7901 cells were seeded into 6-well plates at a density of 1×10^6 cells per well and were washed twice with PBS following treatment. The cells were lysed using radio immunoprecipitation assay (RIPA) Lysis Buffer (BioSharp, BL504A, Shanghai, China) to extract total protein. Protein concentrations were measured with a Bicinchoninic Acid (BCA) assay kit (G2026, Wuhan Servicebio Biotechnology Co., Ltd.). Twenty μg of protein was mixed with 5× sampling buffer, boiled to denature, and then separated via SGC-7901 sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were transferred to a polyvinylidene fluoride (PVDF) membrane and blocked with 5% skim milk for 1 hour. Primary antibodies against SIRT3, SLC7A11, ACSL4, and GPX4 (Abcam, ab246522, ab307601, ab155282, ab262509, Cambridge, UK) were applied and incubated overnight at 4 ℃ on a shaker. The membrane was then washed three times with Tris-Buffered Saline with Tween-20 (TBST), incubated with a secondary antibody at room temperature for 1 hour, and subsequently washed three times with TBS. Enhanced chemiluminescence (ECL) chemiluminescent reagent was used to visualize the protein bands, which were imaged using a gel imaging system (Wuhan Servicebio Biotechnology Co., Ltd.). Band intensity was analyzed using ImageJ software 1.0, and protein levels were quantified relative to β-actin as an internal control.

Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was isolated from cells using TRIzol reagent (Invitrogen, 15596018CN, CA, USA) following the manufacturer's protocol. The RNA was subsequently reverse-transcribed into complementary DNA (cDNA) using M-MLV reverse transcriptase (RT) (Promega, Milano, Italy). Messenger RNA (mRNA) expression levels were quantified using a SYBR GREEN kit according to the manufacturer's instructions. The quantitative polymerase chain reaction (qPCR) reaction mixture included 1 μg of RNA, 10 μL of reaction buffer, 1 μL of RT primers, and 1 μL of deoxyribonucleoside triphosphates (dNTPs), with a final volume of 15 μL. The mixture was initially heated at 70 ℃ for 5 min and then rapidly cooled on ice. On ice, 4 μL of RT buffer, 1 μL of M-MLV RT, and 1 μL of RNase inhibitor were added. The reverse transcription reaction was conducted at 42 ℃ for 60 min, followed by 85 ℃ for 5 min. The qRT-PCR data were analyzed using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control, and relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences used are listed in *Table 1*.

Statistical analysis

All experiments were repeated at least three times, and SPSS 22.0 software was used for statistical analysis, and measurements were expressed as $\bar{x}\pm s$. We used ImageJ software for image analysis of protein bands and Graph Prism 8.0 software for plotting and analysis. A one-way analysis of variance (ANOVA) was employed to evaluate intergroup differences across all experimental data. Subsequent comparisons between pairs of groups were conducted using a non-independent samples *t*-test. Additionally, Tukey's *post hoc* test was utilized to assess differences among multiple groups following the one-way ANOVA. $P<0.05$ indicated that the differences were statistically significant.

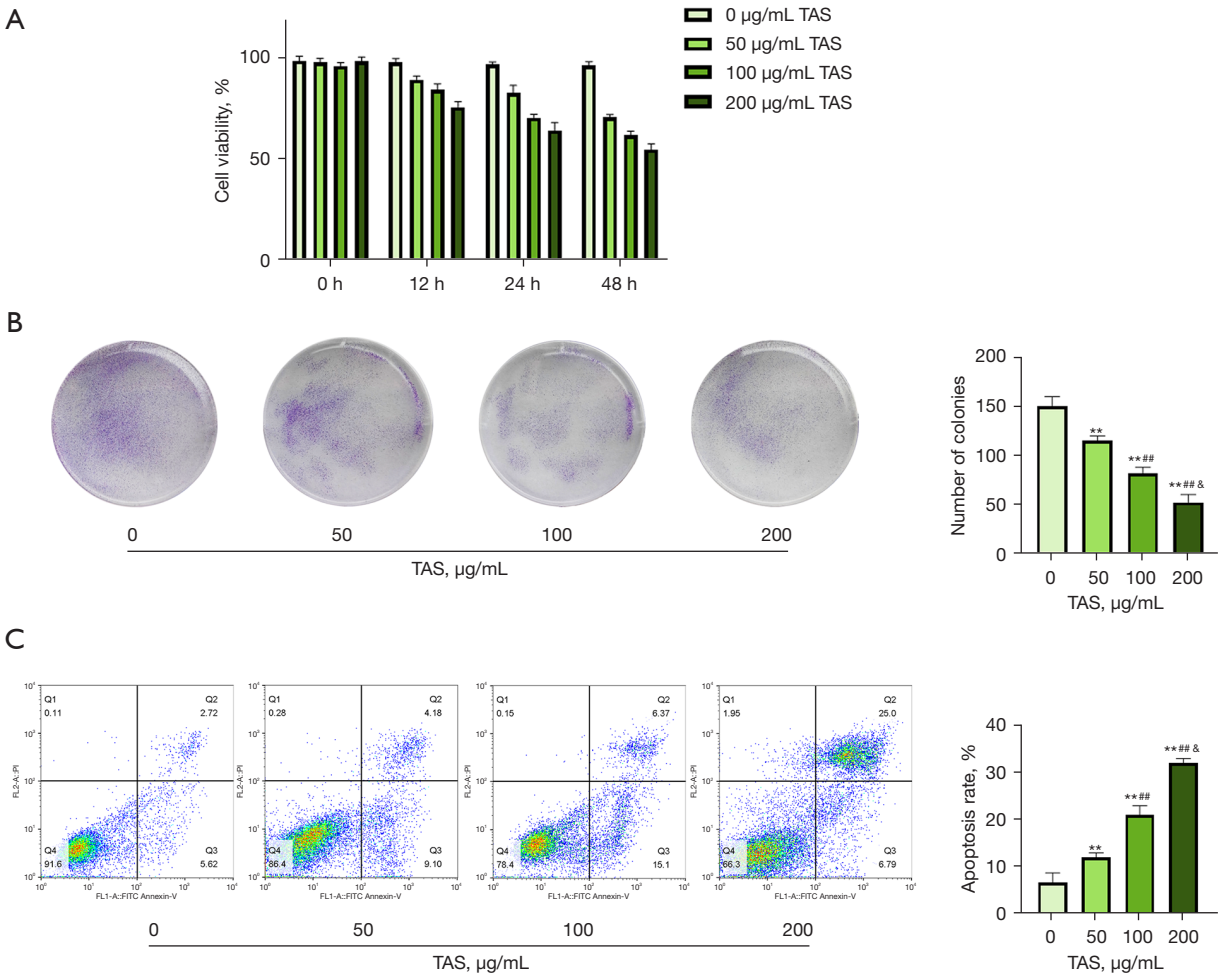

Figure 1 TAS was found to suppress the proliferation of SGC-7901 cells while enhancing their apoptosis. (A) The impact of different TAS concentrations on SGC-7901 cell proliferation was evaluated using the MTT assay. (B) Effects of different concentrations of TAS on the viability of SGC-7901 cells observed by crystal violet assay, magnification 1×. (C) The effect of varying TAS concentrations on the apoptosis levels of SGC-7901 cells was analyzed using flow cytometry. **, P<0.01, *vs.* TAS 0 μg/mL; ##, P<0.01, *vs.* TAS 50 μg/mL; &, P<0.05, *vs.* TAS 100 μg/mL (n=3). TAS, total astragalus saponin; SGC, stomach gastric carcinoma.

Results

TAS inhibits proliferation and promotes apoptosis of SGC-7901 cells

To explore the potential therapeutic effects of TAS on GC, we treated SGC-7901 GC cells with various TAS concentrations (50, 100, and 200 μg/mL) *in vitro*. The findings revealed a significant reduction in cell proliferation in a dose-dependent manner, with higher TAS concentrations leading to greater decreases in proliferation (P=0.007), as illustrated in *Figure 1A*. The inhibition rate of SGC-7901 cells was 48.7% when the concentration of TAS was 200 μg/mL, which was close to the half inhibitory concentration half-maximal inhibitory concentration (IC_{50}), and its effective action time was 48 h. Colony formation assays further demonstrated a notable decrease in cell viability with increasing TAS doses (*Figure 1B*). Additionally, flow cytometry analysis indicated that TAS treatment enhanced apoptosis levels in SGC-7901 cells in a concentration-dependent manner (*Figure 1C*). These results suggest that TAS effectively inhibits SGC-7901 cell proliferation and viability while promoting apoptosis.

TAS induces ferroptosis in SGC-7901 cells

In this paper, we explore the mechanism of action of TAS.

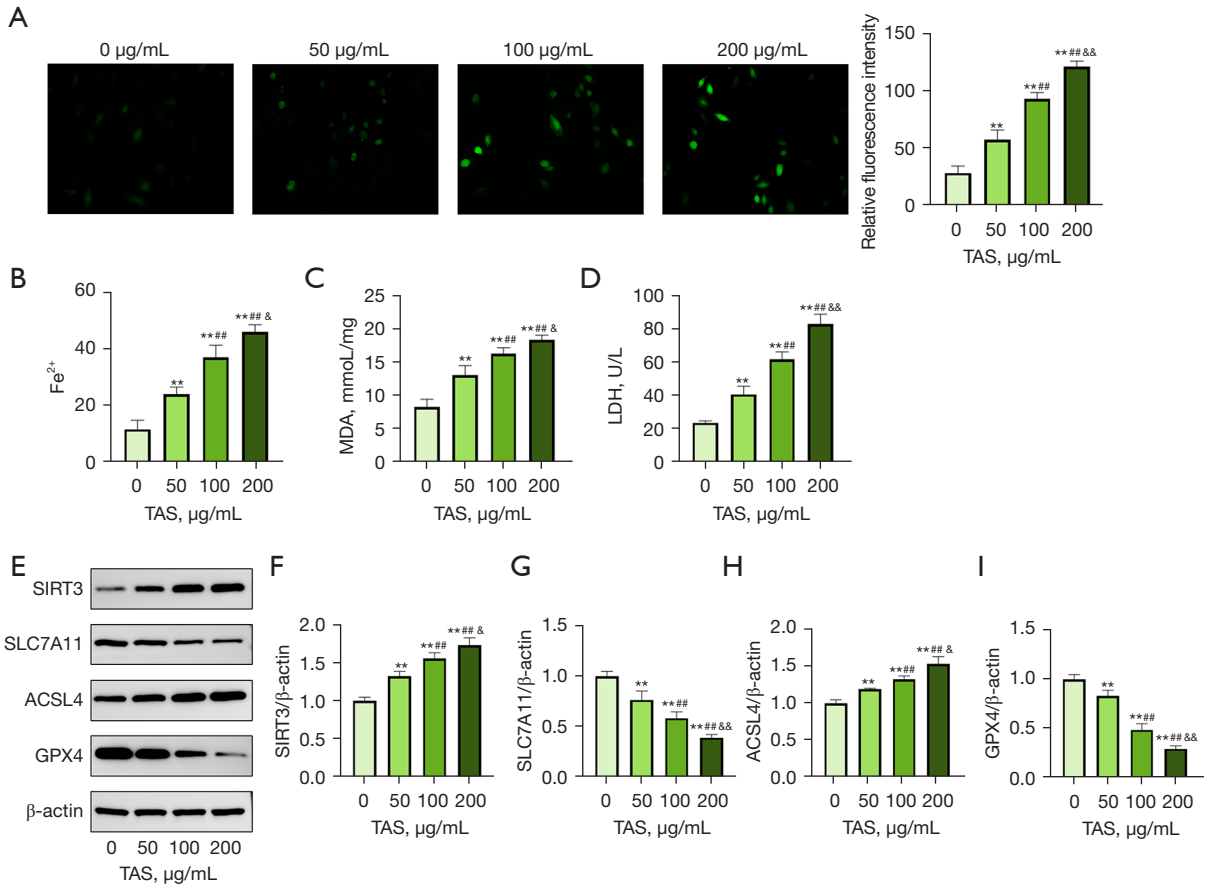

Figure 2 TAS induces ferroptosis in SGC-7901 cells. (A) The effects of different concentrations of TAS on ROS levels in SGC-7901 cells were detected and analysed by fluorescent probe method, magnification 200×. (B-D) The effect of varying TAS concentrations on (B) Fe^{2+}, (C) MDA, and (D) LDH levels in SGC-7901 cells. (E-I) Western blot analysis was conducted to examine the impact of different TAS concentrations on the expression of ferroptosis-related proteins (SIRT3, SLC7A11, ACSL4, and GPX4) in SGC-7901 cells. **, P<0.01, *vs.* TAS 0 μg/mL; ##, P<0.01, *vs.* TAS 50 μg/mL; &, P<0.05, &&, P<0.01, *vs.* TAS 100 μg/mL (n=3). TAS, total astragalus saponin; SGC, stomach gastric carcinoma; ROS, reactive oxygen species; MDA, malondialdehyde; LDH, lactate dehydrogenase; β-actin, internal reference.

Initially, we employed a probe to assess ROS levels in TAS-treated SGC-7901 cells. The data indicated that TAS markedly elevated ROS accumulation in a dose-dependent manner, with higher TAS concentrations correlating with increased ROS levels (*Figure 2A*). We then measured intracellular Fe^{2+} levels and observed a dose-dependent rise in Fe^{2+} accumulation following TAS treatment (P=0.003) (*Figure 2B*). Additionally, TAS treatment significantly enhanced MDA and LDH levels in cells (P=0.005, P=0.002) (*Figure 2C,2D*). To further elucidate the connection between TAS and ferroptosis, we analyzed the expression of ferroptosis-related proteins [SLC7A11, GPX4, and acyl-coenzyme A synthetase long-chain family 4 (ACSL4)] and SIRT3 by Western blot. Increasing TAS concentrations led to a significant reduction in SLC7A11 and GPX4 expression (P=0.003) and a gradual increase in ACSL4 expression (P<0.001) (*Figure 2E-2I*). These findings suggest that TAS may induce ferroptosis in SGC-7901 cells. In addition, the results showed that SIRT3 levels were significantly upregulated with the increase in TAS concentration (P=0.004). It is suggested that TAS can induce cellular ferroptosis by increasing the activity of SIRT3.

TAS upregulates the expression level of protein SIRT3

Here, we investigated the potential mechanism of TAS-regulated SIRT3 in SGC-7901 cells. qRT-PCR results showed that TAS caused a dose-dependent increase (P=0.002)

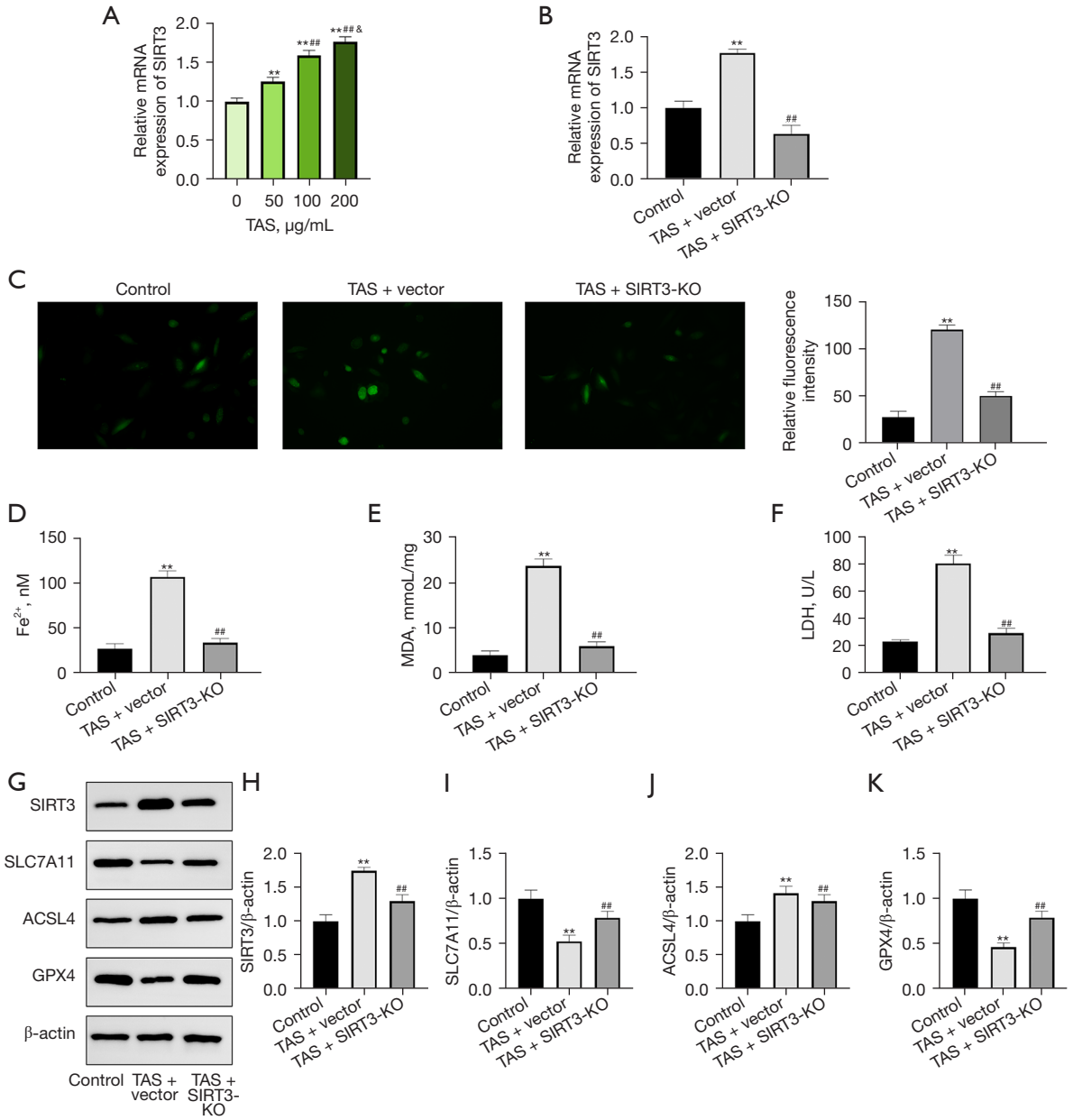

Figure 3 TAS increased the ferroptosis of SGC-7901 cells by upregulating SIRT3. (A) The impact of varying TAS concentrations on SIRT3 expression in SGC-7901 cells was assessed through qRT-PCR analysis, **, P<0.01, *vs.* TAS 0 μg/mL; ##, P<0.01, *vs.* TAS 50 μg/mL; &, P<0.05, *vs.* TAS 100 μg/mL (n=3); (B) the expression levels of SIRT3 in SGC-7901 cells following SIRT3 knockdown were quantified using qRT-PCR; (C) ROS levels were detected and SGC-7901 cells by fluorescent probe method, 200×; (D) detection of (D) Fe^{2+}, (E) MDA, and (F) LDH levels in cells of different treatment groups; (G-K) Western blot analysis was employed to evaluate the expression levels of ferroptosis-related proteins (SIRT3, SLC7A11, ACSL4, and GPX4) across different treatment groups. **, P<0.01, *vs.* Control; ##, P<0.01, *vs.* TAS + vector (n=3). TAS, total astragalus saponin; SGC, stomach gastric carcinoma; SIRT3, silent information regulator 3; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; ROS, reactive oxygen species; MDA, malondialdehyde; LDH, lactate dehydrogenase; β-actin, internal reference.

in SIRT3 expression in SGC-7901 cells (*Figure 3A*), suggesting that SIRT3 may be involved in the inhibitory effect of TAS on SGC-7901 cells.

To further investigate the potential role of SIRT3, we inhibited SIRT3 in SGC-7901 cells treated with TAS. The data indicated that transfection of the SIRT3 CRISPR/Cas9 KO vector resulted in down-regulation of SIRT3 expression in cells with a TAS concentration of 200 μg/mL, and this low expression reversed the activating effect of TAS on SIRT3 expression (P=0.005) (*Figure 3B*). These findings imply that the suppression of SGC-7901 cells by TAS may, at least partially, be mediated through the upregulation of SIRT3 expression.

TAS increases ferroptosis in SGC-7901 cells by upregulating SIRT3

Following SIRT3 inhibition, we examined whether TAS induced ferroptosis in SGC-7901 cells through SIRT3. We assessed ferroptosis-related markers in SGC-7901 cells treated with TAS and transfected with a SIRT3 inhibition plasmid. The data revealed that the downregulation of SIRT3 expression in TAS-treated cells (TAS + SIRT3-KO) notably decreased lipid ROS levels (*Figure 3C*), as well as the levels of Fe^{2+}, MDA, and LDH compared with the TAS + vector group (P=0.008, P=0.005, P=0.006) (*Figure 3D-3F*). Western blot analysis showed that the ferroptosis-associated indexes were significantly lower in the TAS-treated cells (*Figure 3G*) and that the levels of Fe^{2+}, MDA and LDH were also reduced compared with those in the TAS + vector group, the protein expression levels of SLC7A11 and GPX4 in the TAS + SIRT3-KO group were significantly higher (P=0.007, P=0.004), while the protein expression level of ACSL4 was substantially lower (P=0.009) compared to that of the TAS + SIRT3-KO group (*Figure 3H-3K*). This shows that reducing the expression level of SIRT3 can reverse the effect of TAS-induced ferroptosis in SGC-7901 cells.

Discussion

Stomach cancer is difficult to detect in the early stages and is usually diagnosed at a later stage, making its treatment unsatisfactory (24). Herbal medicines have shown promise against GC (25), and TAS, one of the active components of Astragalus, has the potential for cancer treatment (26). Several experiments have confirmed the anticancer effects of Astragalus extracts, and studies in relevant mouse animal models have verified the anticancer effects of Astragaloside IV, which has additionally been shown to improve drug resistance during antitumor chemotherapy (27,28). In the present study, we found that TAS inhibited the proliferation and viability of GC cells and promoted apoptosis in a dose-dependent manner. We further demonstrated that TAS may act on GC cells by regulating SIRT3 signaling activity. Importantly, this is the first *in vitro* demonstration of the inhibitory effect of TAS on SGC-7901 cell activity. SIRT3 is a key molecule in cancer therapy, and its dysregulated expression has been linked to a range of cancer-related processes across multiple types of cancer (29). The *in vivo* experiments in nude mice of Ma *et al.* (30) demonstrated that GC cell proliferation and invasion could be suppressed by upregulating SIRT3 expression. Overall, this research provides compelling evidence supporting the potential clinical use of TAS in the treatment of GC and underscores the need for further investigation.

As the clinical symptoms of GC in the early stage are not significant and lack typicality, most of the patients have already progressed to the middle and late stages when they are diagnosed, losing the best time for surgery and choosing non-surgical treatment. Radiotherapy is a common treatment method, which can prolong the survival time of patients (31). However, the chemotherapeutic drugs used in the clinic at this stage are not suitable for patients to tolerate because of their high side effects, including hematopoietic system failure and severe immunosuppression, so finding drugs with low toxicity and good effects has become a top priority. In this regard, TAS is considered a potential active substance for the treatment of GC (32). It has been reported that a variety of active components of Astragalus can reduce inflammatory damage of gastric mucosa, improve antioxidant capacity, regulate apoptosis and proliferation, inhibit glycolysis, improve blood circulation of gastric mucosa, and exert the protective effect of gastric mucosa through multiple pathways (33). Experiments by Kim *et al.* (34) showed that the aqueous extract of Astragalus induced apoptosis in prostate cancer cells. In addition, Wu *et al.* (35) performed molecular docking and protein blotting experiments and confirmed that gibberellin saponins induced apoptosis and enhanced the antitumor effect of T cells in HGC-2-7 and SGC-7901 GC cells. Therefore, a potential application may be the development of TAS-based therapies that can be used either alone or in combination with other treatments to enhance their effectiveness. In addition, the above findings could inform the development of diagnostic and prognostic tools to help identify patients who are most likely to benefit from TAS-based therapies and monitor their response to

treatment.

Ferroptosis, a novel form of iron-dependent programmed cell death, is a key tumor suppressor mechanism (36). Studies have shown that ferroptosis leads to the accumulation of ROS and lipid peroxidation, so ROS and MDA are often considered markers of ferroptosis (37,38). SLC7A11 is a subunit of the cystine/glutamate transporter system with multiple functions (39), including importing extracellular cysteines used for glutathione synthesis, scavenging ROS and enhancing cellular antioxidant activity, and also synergizing with the antioxidant effects of GPX4. GPX4 (40) has a catalytic role in reducing lipid hydroperoxides. ACSL4, a member of the ACSL family of long-chain acyl-CoA synthetases, is involved in fatty acid metabolism and catalyzes the generation of lipid peroxides (41). Therefore, SLC7A11, GPX4, and ACSL4 are often considered key regulatory proteins in the development of ferroptosis. Related reports have indicated that ferroptosis is associated with a variety of gastrointestinal cancers (42). In our investigation, TAS markedly elevated the levels of ROS, MDA, LDH, and Fe^{2+}, while reducing the protein expression of SLC7A11 and GPX4 and increasing the protein level of ACSL4 in SGC-7901 cells. These findings indicate that TAS may induce ferroptosis in SGC-7901 cells.

The silent information regulator (sirtuin, SIRT) family belongs to class III histone deacetylases (43), and members include SIRT1–7, which are widely expressed in the human body and are involved in the regulation of cellular stress response, metabolism, senescence, and apoptosis. Some studies have reported that SIRT3 can play an important role in p53-mediated ferroptosis by regulating cellular ROS levels (44,45). Therefore, the relevant mechanisms of SIRT3 in ferroptosis-related phenotypes remain to be further investigated. In our study, we found that TAS could significantly upregulate SIRT3, whereas the promotion of ferroptosis in SGC-7901 cells by TAS was significantly limited after knocking down SIRT3. Thus, it is clear that TAS acts by upregulating SIRT3 to induce ferroptosis in GC cells.

Additional research is required to clarify the effectiveness of TAS and the potential role of SIRT3 in treating GC, as well as to prepare for clinical trials. We conducted plasmid transfections in SGC-7901 cells to confirm the role of SIRT3 and TAS-induced ferroptosis within a complex biological framework. It is essential to explore the molecular mechanisms underlying TAS-induced ferroptosis and the involvement of SIRT3, including TAS's interactions with other proteins and signaling pathways related to ferroptosis. Identifying potential biomarkers that predict the efficacy of TAS treatment and the success of SIRT3-targeted therapies will also contribute to the development of personalized treatment strategies. Additionally, exploring the synergistic effects of SIRT3 in conjunction with other anticancer therapies could yield promising strategies for enhancing overall treatment efficacy and mitigating potential resistance to TAS-induced ferroptosis. Furthermore, developing patient stratification approaches based on SIRT3 expression levels, genetic profiles, and other pertinent factors will help ensure that clinical trials are tailored to the patient populations most likely to benefit from the treatment. In this study, we selected the SGC-7901 cell line as a model to investigate the effects of TAS on GC cells, primarily due to its widespread use and representative biological characteristics in GC research. However, given the high heterogeneity of GC, different cell lines may exhibit varying biological responses and drug sensitivities. Therefore, in future research, we plan to employ multiple GC cell lines for comparison to gain a more comprehensive understanding of the mechanisms of action and anticancer effects of TAS.

Conclusions

In summary, our results suggest that TAS has an antitumor effect. TAS induces ferroptosis in SGC-7901 cells by increasing the activity of ferroptosis-related proteins through upregulation of SIRT3 expression, which reduces cell proliferation and viability, and ultimately induces apoptosis. TAS holds significant promise for application in the treatment of GC. Nevertheless, the transition of these experimental findings into clinical practice for patients diagnosed with GC necessitates comprehensive further research. This includes *in vivo* studies, preclinical investigations, and rigorous clinical trials to evaluate the safety and efficacy of TAS therapeutic interventions. Should these endeavors prove successful, TAS therapies could emerge as a groundbreaking treatment option for GC. The potential impact of such advancements could be transformative, offering new hope for improved patient outcomes in a disease that has long posed challenges in effective management and treatment.

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Footnote

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