ORIGINAL RESEARCH

Effect and significance of Ginsenoside Rg3 targeting TREM2 to regulate the polarization of TAMs in gastric cancer cells

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Abstract

Objective

This study aims to investigate the effects of ginsenoside Rg3 on the proliferation, migration, and invasion of gastric cancer (GC) cells and its underlying molecular mechanism.

Methods

Normal gastric mucosal epithelial cells (GES-1) and GC cell lines were cultured. GC cells were treated with ginsenoside Rg3, and the expression of TREM2 (triggering receptor expressed on myeloid cells 2) was detected by qRT-PCR (quantitative reverse transcription PCR) or western blot. THP-1 cells were differentiated into M0 macrophages using PMA (phorbol 12-myristate 13-acetate), and then polarized towards an M2 phenotype (TAMs, tumor-associated macrophages) using IL-4 and IL-13. TAMs were co-cultured with ginsenoside Rg3-pretreated cells. The expression of M1 macrophage markers[TNF-α (tumor necrosis factor-α), IL-6 (interleukin-6), iNOS (nitric oxide synthase 2)] and M2 macrophage markers [(CD206, CD163, IL-10 (interleukin-10)] was detected by qRT-PCR. Immunofluorescence was used to assess changes in the M1 marker CD163 and the M2 marker CD68. Cell viability was measured using CCK-8 assay, cell proliferation by colony formation assay, and cell migration and invasion by Transwell assay. To validate the mechanism, TREM2 was overexpressed in the GC cell line BGC-823 combined with ginsenoside Rg3 treatment.

Results

TREM2 expression was elevated in GC. Ginsenoside Rg3 treatment inhibited TREM2 expression in GC cells. Furthermore, ginsenoside Rg3 promoted the polarization of TAMs towards the M1 phenotype, thereby reducing GC cell viability and inhibiting GC cell proliferation, migration, and invasion. Overexpression of TREM2 in GC reversed the promoting effect of ginsenoside Rg3 on M1 polarization of TAMs, while simultaneously increasing GC cell viability, proliferation, migration, and invasion capabilities.

Conclusion

Ginsenoside Rg3 inhibits TREM2 expression, promotes the polarization of TAMs towards the M1 phenotype, and consequently suppresses the proliferation, migration, and invasion of gastric cancer cells.

Keywords: Gastric cancer; Ginsenoside Rg3; TREM2; TAMs; Macrophage polarization

Introduction

According to global cancer statistics, the incidence rate of gastric cancer (GC) is 5.7%, and its mortality rate is 8.2%, making it the fifth most common cause of cancer-related deaths worldwide[1]. The early symptoms of GC are often inconspicuous, and most patients are diagnosed at an advanced stage, imposing a heavy burden on families and society[2]. Current treatments for GC primarily include surgery, radiotherapy, chemotherapy, interventional therapy, and biotherapy. Commonly used anti-tumor drugs such as 5-fluorouracil, cytarabine, and doxorubicin can alleviate symptoms, control tumor biological activity, and prolong survival time; however, they are expensive, highly toxic, prone to inducing drug resistance, and can damage immune function, bone marrow function, and digestive function[3,4]. Therefore, highly effective and low-toxicity anti-tumor drugs are urgently needed.

Ginseng is a natural herb widely distributed in China, Korea, Japan, and other Asian countries, and has been used for health promotion and treating various diseases[5]. Ginsenoside Rg3 is one of the most active monomers extracted from ginseng[6-8]. Studies have shown that ginsenoside Rg3 possesses various pharmacological effects, including antioxidant, anti-inflammatory, and anti-tumor activities[9-11]. It can reduce the cardiotoxicity and nephrotoxicity of chemotherapeutic drugs, alleviate multi-drug resistance in tumor cells, protect the central nervous and cardiovascular systems, and has anti-fatigue, hypoglycemic, and wound-healing properties[12]. Furthermore, ginsenoside Rg3 downregulates the expression of HIF-1α (hypoxia inducible factor-1) and VEGF (vascular endothelial growth factor) in human gastric cancer cells, thereby inhibiting tumor metastasis[13]. Another study found that ginsenoside Rg3 significantly increases the expression of pro-apoptotic proteins, activates caspase-3, -8, -9, and PARP cleavage, inducing apoptosis in cytotoxin-associated antigen A (CagA)-treated gastric cancer cells[14]. Additionally, ginsenoside Rg3 can upregulate miR-429, inhibiting the SOX2 and PI3K/Akt/mTOR signaling axis, thereby reducing cisplatin resistance in gastric cancer cells[15]. Despite these findings, the precise molecular mechanisms underlying the anti-cancer effects of ginsenoside Rg3, including its specific gene and pathway targets, require further elucidation.

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Research indicates that the occurrence and development of tumors are regulated by interactions with the surrounding tumor microenvironment (TME)[16]. Among the many factors influencing GC progression, tumor-associated macrophages (TAMs) play a crucial role[17]. Macrophages are generally classified into classically activated M1 type and alternatively activated M2 type[18]. TAMs tend to polarize towards the M2 phenotype, which is associated with poor prognosis in tumors[19]. TAMs are the most abundant immune cells in the TME and play a central role in resistance to immune checkpoint inhibitors (ICI)[20]. Multiple studies have shown that TAMs acquire a pro-tumor phenotype during tumorigenesis and promote tumor progression through angiogenesis, immunosuppression, and enhancing tumor cell invasion and migration[21]. It has been reported that M2 TAMs enhance the migration and invasion abilities of GC cells by increasing MMP2 secretion and promoting epithelial-mesenchymal transition (EMT)[22]. Moreover, a mixture of M1 and M2 TAMs promotes the malignant biological behavior of diffuse GC by secreting IL-1β, which increases Cancer-associated fibroblasts (CAFs)[23]. Therefore, investigating the polarization mechanisms of TAMs in GC is significant for exploring new therapeutic targets.

TREM2 (triggering receptor expressed on myeloid cells 2) is a transmembrane receptor of the immunoglobulin superfamily that can inhibit the phagocytic function of dendritic cells and macrophages, thereby influencing related immune signaling pathways[24]. In recent years, increasing evidence indicates that TREM2 also influences tumorigenesis and development. Downregulation of TREM2 significantly reduced the expression of CXCL10 (Chemokine ligand-10), CXCR3 (Chemokine receptor-3), MMP-2 (matrix metalloproteinases 2) andMMP-9, which play key roles in the TME. Furthermore, TREM2 regulates microglial phenotype transformation; downregulation of TREM2 promotes the transformation of microglia towards the M1 phenotype, induces the secretion of TNF-α, IL-1β, and IL-6, and reduces IL-10 and TGF-β[25]. TREM2 is associated with GC prognosis[26]. These results suggest that TREM2 plays a role in anti-tumor immunity, cell polarization, and tumor prognosis. Therefore, we hypothesize that ginsenoside Rg3 inhibits gastric cancer progression by targeting TREM2 to regulate TAM polarization. This study uses GC cell lines as a model system to investigate the effects of ginsenoside Rg3 on the biological activities of GC cells and its potential mechanism of action.

Materials and Methods

Cell culture

The normal gastric mucosal epithelial cell line GES-1, four gastric cancer (GC) cell lines (BGC-823, HGC-27, MGC-803, and SGC-7901), and the human monocytic leukemia cell line THP-1 were purchased from ScienCell Research Laboratories (Carlsbad, CA, USA). GES-1, GC cell lines, and THP-1 cells were cultured in RPMI-1640 medium (Roswell Park Memorial Institute) supplemented with 10% fetal bovine serum (Clark, Shanghai, China) and 100 U/mL penicillin-streptomycin (Life Technologies, Shanghai, China) at 37°C in a 5% CO2 atmosphere.

Cell grouping and treatments

THP-1 cells were treated with 100 ng/ml PMA (HY-18739, MedChem Express, Monmouth Junction, NJ, USA) for 48 h to induce differentiation into M0 macrophages. Subsequently, IL-4 (20 ng/ml) + IL-13 (20 ng/ml) were added to induce polarization of M0 macrophages into M2-type TAMs for 48 h. The experimental groups were as follows: Control group: GC cells treated with DMSO for 48 h; Rg3 group: GC cells treated with 50 μg/ml Rg3 for 48 h; TAMs + Control group: GC cells pretreated with DMSO for 48 h, then co-cultured with TAMs for 24 h; TAMs + Rg3 group: GC cells pretreated with 50 μg/ml Rg3 for 48 h, then co-cultured with TAMs for 24 h; TAMs + Rg3 + oe-NC group: BGC-823 cells transfected with empty vector plasmid, pretreated with 50 μg/ml Rg3 for 48 h, then co-cultured with TAMs for 24 h; TAMs + Rg3 + oe-TREM2 group: BGC-823 cells transfected with TREM2-overexpressing plasmid, pretreated with 50 μg/ml Rg3 for 48 h, then co-cultured with TAMs for 24 h. For co-culture of GC cells with TAMs, GC cells were seeded into Corning Costar Transwell inserts (0.4 μm pore size; Sigma-Aldrich, St. Louis, MO, USA), which contain membranes permeable to liquid but not to cells. The inserts were then placed into compatible wells plated with the differentiated M0 THP-1 cells.

Cell transfection

The TREM2-overexpressing plasmid and its empty vector control (pcDNA-TREM2, pcDNA-NC) were provided by Sangon Biotech (Shanghai, China). The above plasmids were transfected into BGC-823 cells using Lipofectamine 3000 transfection reagent (L3000015, Thermo Fisher, Waltham, MA, USA). Transfection efficiency was assessed 48 hours post-transfection before proceeding with subsequent experiments.

CCK-8 assay

Cells (1 × 104) were seeded into 96-well plates. After treatment, 10 μL of CCK-8 reagent (CK04, Dojindo Laboratories, Japan) was added to each well and incubated at 37°C for 4 hours. The absorbance of cells was measured at 450 nm using a microplate reader (BioRad, Hercules, CA, USA).

Colony formation assay

GC cells were collected, resuspended in PBS, and counted. Cells (1× 103) were seeded into 6-well plates with 2 ml of complete culture medium. After 14 days of culture at 37°C, visible cell colonies formed. The medium was discarded, cells were washed with PBS, fixed with 4% paraformaldehyde for 30 minutes at room temperature, and then stained with 0.1% crystal violet. Colonies were imaged and counted using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA).

Transwell assay

GC cells were centrifuged, resuspended in serum-free medium, and counted. Cell suspensions containing 1×106 cells were added to the upper chamber of Transwell inserts (BD Biosciences, USA). The lower chamber was filled with complete medium containing 10% FBS. For the invasion assay, the upper chamber was pre-coated with Matrigel. After 24 hours, cells that migrated/invaded were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. Cells were imaged and counted using ImageJ software (National Institutes of Health).

qRT-PCR

Total RNA was extracted from cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA was reverse transcribed into cDNA using the PrimeScript RT reagent

kit (RR037A, Takara, Tokyo, Japan). The relative mRNA expression levels were detected using the SYBR Green PCR Mix Kit (4309155, Applied Biosystems, Foster City, CA, USA) on a real-time PCR system (Applied Biosystems). GAPDH was used as the internal reference gene. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method[27]. Primer sequences are listed in Table 1.

Western blot

Total protein was extracted from cells using a protein extraction kit (BC3710, Solarbio, Beijing, China). Proteins were separated on 10% SDS-PAGE gels and transferred to PVDF membranes. The membranes were blocked with 5% skim milk and incubated overnight at 4°C with primary antibodies against TREM2 (1:1000, ab209814, Abcam, Cambridge, MA, USA) and GAPDH (1:2500, ab9485, Abcam). After washing, the membranes were incubated with a goat anti-rabbit IgG secondary antibody (1:5000, ab205718, Abcam). Target protein expression was detected using ECL Western Blotting Substrate (PE0010, Thermo Fisher Scientific, Waltham, MA, USA), with GAPDH serving as the loading control.

Immunofluorescence staining for CD163 and CD68 levels in TAMs

TAMs from each group were fixed with 4% paraformaldehyde and blocked with 5% BSA containing 0.3% Triton X-100. Then, the cells were incubated overnight at 4°C with primary antibodies against CD163 (1:50, ab316218, Abcam) and CD68 (1:100, ab213363, Abcam). The next day, cells were incubated with secondary antibodies for 1 hour, followed by DAPI staining. After quenching fluorescence and sealing with neutral gum, fluorescently stained cells were observed under a microscope.

Statistical analysis

Statistical analysis was performed using SPSS 21.0 software (IBM SPSS Statistics, Chicago, IL, USA) and graphing was done with GraphPad Prism 8.0 software (GraphPad Software Inc., San Diego, CA, USA). Measurement data are presented as mean ± standard deviation. Normality and homogeneity of variance were tested first. For data conforming to normal distribution and homogeneity of variance, comparisons between two groups were performed using the t-test. Comparisons among multiple groups were analyzed using one-way ANOVA or two-way ANOVA, followed by Tukey's multiple comparisons test for post-hoc analysis. P-values were two-sided, and $P < 0.05$ was considered statistically significant.

Results

TREM2 is highly expressed in GC cells

First, we analyzed the expression of TREM2 in gastric cancer tumor samples using the UALCAN database (http://ualcan.path.uab.edu/index.html). TREM2 expression was significantly upregulated in GC tumor samples compared to normal stomach tissue (Fig. 1A, $P < 0.05$). Next, we detected TREM2 expression levels in the normal gastric mucosal epithelial cell line GES-1 and four GC cell lines (BGC-823, HGC-27, MGC-803, and SGC-7901). TREM2 expression was significantly elevated in GC cells (Fig. 1B-C, $P < 0.05$). BGC-823 and MGC-803 cells, which showed relatively high expression levels, were selected for subsequent experiments.

(A) Analysis of TREM2 expression in normal gastric tissue and gastric cancer tumor samples from the UALCAN database. The normal gastric mucosal epithelial cell line GES-1 and gastric cancer cell lines (BGC-823, HGC-27, MGC-803, SGC-7901) were cultured. (B-C) Expression of TREM2 in cells was detected by qRT-PCR and Western blot. Experiments were performed independently three times. Data are presented as mean ± standard deviation. Figure 1A was analyzed by t-test; Figures 1B/C were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. * indicates $P < 0.05$.

Ginsenoside Rg3 inhibits TREM2 expression

To investigate the role of ginsenoside Rg3 in gastric cancer, BGC-823 and MGC-803 cells were treated with different concentrations (12.5, 25, 50, 100, and 200 μg/ml) of ginsenoside Rg3 for different durations (24 and 48 h). The effect of ginsenoside Rg3 on cell viability was detected by CCK-8 assay. The results showed that cell viability decreased at a gradient trend in a concentration- and time-dependent manner. Treatment with 50 μg/ml ginsenoside Rg3 for 48 h reduced cell viability by approximately 50% (Fig. 2A, $P < 0.05$); therefore, this concentration and duration were selected for subsequent experiments. Compared to the Control group, TREM2 expression was significantly decreased in the Rg3 group (Fig. 2B-C, $P < 0.05$).

BGC-823 and MGC-803 cells were treated with different concentrations (12.5, 25, 50, 100, and 200 μg/ml) of ginsenoside Rg3 for different times (24 and 48 h). The Control group consisted of DMSO-treated cells. (A) Cell viability was detected by CCK-8 assay. Based on the CCK-8 results, 50 μg/ml ginsenoside Rg3 and 48 h were selected for subsequent experiments. (B-C) Expression of TREM2 in cells was detected by qRT-PCR and Western blot. Experiments were performed independently three times. Data are presented as mean ± standard deviation. Figures 2A-C were analyzed by two-way ANOVA followed by Tukey's multiple comparisons test. * indicates $P < 0.05$.

Ginsenoside Rg3 promotes the polarization of TAMs towards the M1 phenotype

Studies have shown that TAMs tend to polarize towards the M2 phenotype, which is associated with poor prognosis in tumors[19]. We used PMA to induce THP-1 cell differentiation into macrophages and then induced polarization into TAMs using IL-4 and IL-13. TAMs were co-cultured with GC cells (BGC-823, MGC-803) pretreated with either DMSO or ginsenoside Rg3. Our qRT-PCR results showed that compared to the TAMs + Control group, the expression of CD206, CD163, and IL-10 (M2 macrophage markers) in TAMs was significantly decreased in the TAMs + Rg3 group (Fig. 3A, $P < 0.05$), while the expression of TNF-α, IL-6, and iNOS (M1 macrophage markers) was significantly increased (Fig. 3B, $P < 0.05$). Immunofluorescence results also confirmed that Rg3 promoted the polarization of TAMs towards the M1 phenotype (Fig. 3C, $P < 0.05$).

THP-1 cells were induced with 100 ng/ml PMA for 48 h to differentiate into M0 macrophages. Then, M0 macrophages were induced with 20 ng/ml IL-4 + 20 ng/ml IL-13 for 48 h to polarize into M2-type TAMs. TAMs were co-cultured for 24 h with GC cells (BGC-823, MGC-803) pretreated with DMSO or Rg3. (A) qRT-PCR detection of the expression of M2 macrophage markers (CD206, CD163, IL-10) in TAMs. (B) qRT-PCR detection of the expression of M1 macrophage markers (TNF-α, IL-6, iNOS) in TAMs. (C)

Table 1. qPCR primers

	Forward Primer (5'-3')	F Tm	Reverse Primer (5'-3')	R Tm
TREM2	CACAACTTGTGGCTGCTGTC	59.97	GGTAGAGACCCGCATCATGG	59.97
CD206	GCCTCGTTGTTTTGCGTCTT	59.9	GAGAACAGCACCCGGAATGA	60
CD163	CGGACTTCTCTCTGGAAGC	57.27	CTCATGTCCCTCACACTGG	57.15
IL-10	AAGACCCAGACATCAAGGCG	60.04	AGGCATTCTTCACCTGCTCC	60.03
iNOS	ATGGGAGAAGGGGATGAGCT	60.03	GTCCCAGGTCACATTGGAGG	60.04
IL-6	AGTGAGGAACAAGCCAGAGC	59.96	GGTCAGGGGTGGTTATTGCA	59.96
TNF-α	GTAGCCCACGTCGTAGCAAA	60.39	CCCTTCTCCAGCTGGGAGAC	61.62
GAPDH	GAGTCAACGGATTTGGTCGT	58.21	TGGGTGGAATCATATTGGAA	53.94

Figure 1. TREM2 expression is upregulated in gastric cancer

Figure 2. Ginsenoside Rg3 inhibits TREM2 expression

Figure 3. Ginsenoside Rg3 promotes the polarization of TAMs towards the M1 phenotype

Figure 4. Ginsenoside Rg3 inhibits GC cell proliferation, migration, and invasion by promoting TAM polarization

Figure 5. Ginsenoside Rg3 inhibits GC progression by targeting TREM2 to promote TAM polarization

Changes in the M2 macrophage marker CD163 and the M1 macrophage marker CD68 in TAMs were observed under a fluorescence microscope. Experiments were performed independently three times. Data are presented as mean ± standard deviation. Figures 3A-B were analyzed by two-way ANOVA followed by Tukey's multiple comparisons test. * indicates $P < 0.05$.

Ginsenoside Rg3 inhibits GC cell proliferation, migration, and invasion by promoting M1 polarization of TAMs

Compared to the TAMs + Control group, GC cell viability was significantly reduced in the TAMs + Rg3 group (Fig. 4A, $P < 0.05$), and the abilities of cell proliferation, migration, and invasion were significantly decreased (Fig. 4B-D, P <

0.05). This indicates that ginsenoside Rg3 can inhibit GC cell proliferation, migration, and invasion by promoting the polarization of TAMs towards the M1 phenotype.

(A) GC cell viability was detected by CCK-8 assay. (B) GC cell proliferation was detected by colony formation assay. (C-D) GC cell migration and invasion were detected by Transwell assay. Experiments were performed independently three times. Data are presented as mean ± standard deviation. Figures 4A-D were analyzed by two-way ANOVA followed by Tukey's multiple comparisons test. * indicates $P < 0.05$.

Ginsenoside Rg3 inhibits GC progression by targeting TREM2 to promote M1 polarization of TAMs

In summary, we hypothesized that ginsenoside Rg3 inhibits GC progression by suppressing TREM2 expression to promote M1 polarization of TAMs. To validate this mechanism, we overexpressed TREM2 in BGC-823 cells (Fig. 5A, $P < 0.05$) and treated the cells with 50 μg/ml ginsenoside Rg3 for 48 h. Then, the cells were co-cultured with TAMs for combined experiments. Our results showed that after TREM2 overexpression (Fig. 5B-C, $P < 0.05$), the expression of CD206, CD163, and IL-10 (M2 macrophage markers) in TAMs was significantly increased (Fig. 5D, $P < 0.05$), while the expression of TNF-α, IL-6, and iNOS (M1 macrophage markers) was significantly decreased (Fig. 5E, $P < 0.05$). Immunofluorescence results also confirmed that TREM2 overexpression inhibited the polarization of TAMs towards the M1 phenotype (Fig. 5F, $P < 0.05$). Furthermore, GC cell viability was significantly increased (Fig. 5G, $P < 0.05$), and the capacities for proliferation, migration, and invasion were significantly enhanced (Fig. 5H-J, $P < 0.05$). These results demonstrate that ginsenoside Rg3 inhibits GC progression by targeting TREM2 to promote the polarization of TAMs towards the M1 phenotype.

TREM2-overexpressing plasmid (oe-TREM2) , with empty vector plasmid (oe-NC) as control,was transfected into BGC-823 cells. TAMs were co-cultured with BGC-823 cells pretreated with ginsenoside Rg3. (A) Transfection efficiency was detected by qRT-PCR. (B-C) TREM2 expression in BGC-823 cells was detected by qRT-PCR and Western blot. (D) Expression of M2 macrophage markers (CD206, CD163, IL-10) in TAMs was detected by qRT-PCR. (E) Expression of M1 macrophage markers (TNF-α, IL-6, iNOS) in TAMs was detected by qRT-PCR. (F) Changes in the M2 macrophage marker CD163 and the M1 macrophage marker CD68 in TAMs were detected by immunofluorescence. (G) BGC-823 cell viability was detected by CCK-8 assay. (H) BGC-823 cell proliferation was detected by colony formation assay. (I-J) BGC-823 cell migration and invasion were detected by Transwell assay. Experiments were performed independently three times. Data are presented as mean ± standard deviation. Figures 5A, 5B, 5C, 5G, 5H, 5I, 5J were analyzed by t-test. Figures 5D and 5E were analyzed by two-way ANOVA followed by Tukey's multiple comparisons test. * indicates $P < 0.05$.

Discussion

Gastric cancer (GC) ranks as the fifth most fatal cancer globally, and its pathogenesis and treatment strategies remain hotspots in cancer research. Due to the heterogeneity and diversity of GC, treatment outcomes are often unsatisfactory[28]. Therefore, there is an urgent need to explore new therapeutic approaches, identify key molecules involved in gastric carcinogenesis, and gain deeper insights into the disease mechanisms of GC to provide novel and effective targets for its diagnosis and treatment.

Ginsenoside Rg3, one of the main active components of ginseng, possesses significant pharmacological effects including anti-tumor, anti-inflammatory, antioxidant, hypoglycemic, hepatoprotective, wound-healing, and immunomodulatory properties, and has been widely used in clinical adjuvant therapy[29]. The abilities to proliferate, migrate, and invade are major malignant biological behaviors of tumor cells. Numerous studies have shown that ginsenoside Rg3 exerts its anti-tumor effects primarily by inhibiting cancer cell proliferation, inducing apoptosis, and suppressing tumor angiogenesis. Ginsenoside Rg3 has demonstrated significant therapeutic effects against various cancers, including lung cancer, colorectal cancer, breast cancer, liver cancer, gastric cancer, and ovarian cancer[7,30-34]. In our study, treatment with 50 μg/ml ginsenoside Rg3 for 48 hours significantly reduced the viability, proliferation, migration, and invasion capabilities of GC cells, which is consistent with previous reports[35]. Studies have reported that macrophage induced by gastric cancer cells significantly promote metastasis by facilitating the EMT of GC cells. Based on their phagocytic capacity and cytokine production profiles, macrophages can be classified into immunogenic M1 macrophages and alternatively activated or reparative M2 macrophages. M2-type TAMs in the GC TME provide a favorable microenvironment for GC growth[36]. In our research, we investigated changes in TAM phenotypes. We used PMA to induce THP-1 cell differentiation into macrophages and IL-4/IL-13 to polarize these macrophages into M2-type TAMs. The polarization markers were detected via qRT-PCR and immunofluorescence. Our results indicated that ginsenoside Rg3 treatment promoted a shift in TAMs from the M2 towards the M1 phenotype. Research suggests that TREM2 is a crucial signaling hub mediating various pathological pathways involved in immunity. TREM2 expression plays a role in intracellular immunosuppression and can be induced in myeloid cells[37]. Trem2 knockout mouse models show greater resistance to the growth of various tumors, and improve checkpoint immunotherapy outcomes via TREM2's modulating function for tumor-infiltrating myeloid cells[38]. Furthermore, knocking down TREM2 expression inhibits the proliferation, migration, invasion, and progression of EMT in gastric cancer cells via the PI3K/AKT signaling pathway[39]. However, the functional role of TREM2 in GC and its relationship with TAM polarization remain largely unexplored. In our study, analysis via the UALCAN database revealed that TREM2 was highly expressed in tumor tissues from GC patients, and its expression was significantly elevated in GC cell lines, consistent with prior research[39]. Ginsenoside Rg3 treatment significantly suppressed TREM2 expression. Subsequently, combined experiments conducted in BGC-823 GC cells showed that compared to ginsenoside Rg3 treatment alone, overexpression of TREM2 reversed the effects by promoting a shift of TAMs towards the M2 phenotype, significantly enhancing GC cell viability, proliferation, migration, and invasion capabilities. This suggests that ginsenoside Rg3 modulates GC progression by targeting TREM2 to regulate TAM polarization.

In vivo validation is necessary to apply the results of our research in vitro to the physiological environment. We plan to further validate these findings by creating a gastric cancer

xenograft model or an in situ graft model. Based on the results of pharmacokinetic studies of existing preparations, we will administer clinically appropriate doses of ginsenoside Rg3 to mice to monitor tumor growth, metastasis trends, and overall survival of animals. Previous studies have shown that TREM2 can promote epithelial-mesenchymal transition (EMT) by activating the PI3K/AKT pathway in gastric cancer[39]. PI3K/AKT is a key pathway that regulates cell survival, metabolism, and protein synthesis[40]. When this pathway is continuously activated by TREM2, it further regulates several key downstream transcription factors, such as NF-κB and STAT3[41,42]. Therefore, we speculate that activation of the TREM2/PI3K/AKT axis can shape an immunosuppressive tumor microenvironment in vivo, and we will verify signaling through functional blockade experiments. These in vivo experiments integrate the internal signaling pathway of tumor cells with the regulation of the external immune microenvironment, providing a new perspective for understanding the multi-target role of Rg3 and laying a theoretical foundation for its combination with PI3K/AKT inhibitors or immunotherapy.

From a medical translational perspective, although our study demonstrated a potent anti-tumor mechanism of ginsenoside Rg3 (50 μg/mL) in vitro, its pharmacokinetic signature still needs to be considered in clinical translation. Existing clinical formulations and studies suggest that achieving effective systemic concentrations may rely on extended dosing times or advanced delivery strategies. Future research should focus on optimizing dose regimens and developing novel drug delivery systems (e.g., nanoparticles, liposomes) to fully realize their therapeutic potential for gastric cancer.

In conclusion, our findings demonstrate that ginsenoside Rg3 inhibits the progression of GC by targeting TREM2 to promote the polarization of TAMs towards the M1 phenotype, thereby suppressing GC cell proliferation, migration, and invasion. However, this study has certain limitations. We only validated the effects of ginsenoside Rg3 on GC cell proliferation, migration, and invasion in vitro; other potential effects, such as on tumor angiogenesis or EMT, were not explored. Furthermore, the specific mechanisms and signaling pathways through which TREM2 regulates TAM polarization require further elucidation. Future research should incorporate animal experiments and clinical data to confirm the therapeutic efficacy of ginsenoside Rg3 and delineate its detailed mechanisms in GC.

Declarations

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Conflicts of interest/ Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and material

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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