Triptonide inhibits the pathological functions of gastric cancer-associated fibroblasts

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**A R T I C L E   I N F O**

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**A B S T R A C T**

Direct attacks on tumour cells with chemotherapeutic drugs have the drawbacks of accelerating tumour metastasis and inducing tumour stem cell phenotypes. Inhibition of tumour-associated fibroblasts, which provide nourishment and support to tumour cells, is a novel and promising anti-tumour strategy. However, effective drugs against tumour-associated fibroblasts are currently lacking. In the present study, we explored the possibility of inhibiting the pathological functions of tumour-associated fibroblasts with triptonide. Paired gastric normal fibroblasts (GNFs) and gastric cancer-associated fibroblasts (GCAFs) were obtained from resected tissues. GCAFs showed higher capacities to induce colony formation, migration, and invasion of gastric cancer cells than GNFs. Triptonide treatment strongly inhibited the colony formation-, migration-, and invasion-promoting capacities of GCAFs. The expression of microRNA-301a was higher and that of microRNA-149 was lower in GCAFs than in GNFs. Triptonide treatment significantly down-regulated microRNA-301a expression and up-regulated microRNA-149 expression in GCAFs. Re-establishment of microRNA expression balance increased the production and secretion of tissue inhibitor of metalloproteinase 2, a tumour suppressive factor, and suppressed the production and secretion of IL-6, an oncogenic factor, in GCAFs. Moreover, triptonide treatment abolished the ability of GCAFs to induce epithelial-mesenchymal transition in gastric cancer cells. These results indicate that triptonide inhibits the malignancy-promoting capacity of GCAFs by correcting abnormalities in microRNA expression. Thus, triptonide is a promisingly therapeutic agent for gastric cancer treatment, and traditional herbs may be a valuable source for developing new drugs that can regulate the tumour microenvironment.

**1. Introduction**

Malignant tumours are a leading cause of death worldwide [1]. Currently, the main therapeutic strategy for malignant tumours involves the removal and killing of tumour cells through resection, radiation therapy, or chemotherapy. However, these therapies themselves are associated with significant morbidity and mortality primarily due to their huge injuries to normal tissues [2,3]. Moreover, some chemotherapeutic drugs promote tumour metastasis and induce the conversion of common tumour cells to tumour stem cells [4]. Thus, new strategies are needed to combat malignant tumours.

Tumour cells cannot survive or expand in the body on their own and rely on stromal cells in the tumour microenvironment for nourishment and support. Tumour-associated fibroblasts, which differ from normal fibroblasts in gene expression profiles and functions, are the most prominent cell type in the tumour stroma [5]. Tumour-associated fibroblasts are not involved in the maintenance of tissue homeostasis or in wound repair but secrete proinflammatory cytokines, chemokines, and matrix metalloproteinases to create an inflammatory microenvironment suitable for activating and maintaining tumour cell proliferation, migration, invasion, and drug resistance [6]. Inhibition of the pathological functions of tumour-associated fibroblasts eliminate the “fertile soil” that sustains and promotes the malignancy of tumour cells, representing a robust therapeutic approach for various human tumours.

So far, two types of drugs have been developed for inhibiting the pathological functions of tumour-associated fibroblasts. The first drug type includes monoclonal antibodies and small-molecule inhibitors that block the enzymatic activity of fibroblast activation proteins expressed...
on the membranes of tumour-associated fibroblasts [7]. However, these drugs generated no tumour responses or showed only very weak efficacy in phase II clinical trials [8]. The second drug type includes cytokotoxic agents and T cells engineered with chimeric antigen receptors that kill tumour-associated fibroblasts [9]. However, these toxins and T cells are associated with serious side effects such as cachexia, and damage normal cells [10]. Therefore, developing effective new drugs against tumour-associated fibroblast is an urgent and challenging issue.

Traditional Chinese medicine has accumulated extensive knowledge on tumour treatments over thousands of years of clinical practice. Based on the unique concept of holism, traditional Chinese medicine explores the mechanism of tumour initiation and progression by investigating the interactions among the tumour, the human body, and the surrounding environment rather than focusing on tumour cells [11]. Used under the guidance of traditional Chinese medicine theories, some Chinese herbs exert anti-tumour effects not by killing or eliminating abnormal cells but by remodeling the gene expression patterns of the abnormal cells to suppress their pathological functions and restore them to normal states [12]. This makes the internal environment of the body unfavourable for tumour initiation and progression, prevents the tumour microenvironment from nourishing tumour cells and supporting their malignant behaviours, and deprives tumour cells of the conditions suitable for survival. Therefore, traditional Chinese herbs might be a promising source of tumour-associated fibroblast inhibitors.

Tripterygium wilfordii Hook F is a Chinese herb used for treating inflammatory diseases. It can inhibit the expression of proinflammatory cytokines and matrix metalloproteinases in synovial fibroblasts, exhibiting an ability to affect fibroblast functions [13]. Triptonide is a major active ingredient of Tripterygium wilfordii Hook F and has been proved able to regulate cytokine secretion by both in vitro and in vivo studies [14,15]. Considering this property of triptonide, we speculated that it can inhibit the pathological functions of tumour-associated fibroblasts. In the present study, for the first time, we treated gastric cancer-associated fibroblasts (GCAFs) with triptonide, observed its effects on the malignancy-promoting capacity of GCAFs, and explored the underlying mechanism.

2. Materials and methods

2.1. Cells and reagents

Human gastric carcinoma BGC-823 cells were purchased from the Cell Bank of Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). Triptonide (purity > 98%) was provided by Herbest Bio-Tech Co., Ltd (Baoji, Shaanxi, China) and dissolved in DMSO to the concentration of 100 mM as a stock solution. The stock solution was diluted with RPMI-1640 medium when used and the final DMSO concentration did not exceed 0.1% (v/v). A mouse monoclonal antibody against Pan-cytokeratin (Pan-CK) and rabbit polyclonal antibodies against PDGFR-β and alpha smooth muscle actin (α-SMA) were obtained from Abcam (Cambridge, UK). Rabbit polyclonal antibodies against E-cadherin, N-cadherin, and vimentin were obtained from Cell Signaling Technology (Beverly, MA, United States). Rabbit polyclonal antibodies against tissue inhibitor of metalloproteinase 2 (TIMP2), IL-6, and β-actin, and CY3-conjugated goat anti-mouse IgG, CY3-conjugated goat anti-rabbit IgG, and HRP-conjugated goat anti-rabbit IgG were obtained from Elabscience Biotechnology (Wuhan, Hubei, China). The 2′-O-methyl chemically modified oligonucleotides for microRNA-301a mimic (miR-301a mimic), miR-301a mimic negative control (301a-NC), microRNA-149 inhibitor (anti-miR-149), and anti-miR-149 negative control (149-NC), were produced by GenePharma (Suzhou, Jiangsu, China).

2.2. Isolation and culture of human gastric normal fibroblasts (GNFs) and GCAFs

Gastric tumour tissue and adjacent normal tissue (at least 2 cm from the outer tumour margin) were obtained from a patient with poorly differentiated gastric adenocarcinoma during gastric surgery and were immediately transported to our laboratory. The fresh tissue samples were minced into 0.5 to 1 mm³ fragments, seeded in 60-mm culture dishes containing RPMI-1640 supplemented with 20% fetal bovine serum (FBS), and cultured at 37 °C in a humidified atmosphere of 5% CO₂. The culture medium was changed twice a week for 2–3 weeks. Under these conditions, fibroblasts grew out from tissue fragments while other cells were mostly retained in the fragments. The cells were cultured until they reached confluence, after which cell monolayers were trypsinised and were passaged 1:2 (passage 1). Next, the fibroblasts were subcultured for another 3–4 passages to eliminate epithelial cells and were subsequently maintained in RPMI-1640 supplemented with 10% FBS, 2% penicillin and 2% streptomycin. GNFs and GCAFs between passages 6 and 8 were used for performing subsequent experiments. A signed consent was obtained from the patient. The study was conducted in accordance with the Declaration of Helsinki.

2.3. Immunofluorescence

GNFs and GCAFs grown on glass coverslips were washed with cold phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde, permeabilised with 0.1% Triton X-100, and then incubated with primary antibodies to Pan-CK (1:100), E-cadherin (1:100), vimentin (1:150), PDGFR-β (1:100), and α-SMA (1:100) overnight at 4 °C. The cells were subsequently incubated with fluorescence-conjugated secondary antibodies for 1 h at 37 °C. After 3 PBS washes, the cells were mounted with mounting medium containing DAPI for 5 min and were examined with a Nikon Eclipse TS2 fluorescence microscope equipped with a digital camera.

2.4. Preparation of conditioned media

To prepare conditioned media derived from GNFs and GCAFs, the cells were plated into 60-mm dishes (5 × 10⁵ cells/dish) and were cultured in RPMI-1640 supplemented with 10% FBS. After 12 h, the culture medium was replaced with 4 mL fresh RPMI-1640 medium, and the cells were cultured for an additional 48 h. Culture supernatants were collected, centrifuged at 1000 rpm, filtered using 0.1 μm membranes, and supplemented with 3% FBS.

To prepare conditioned media derived from triptonide-treated GCAFs, the cells were treated with 0.1% DMSO (vehicle control) or 10, 20, or 40 nM triptonide for 72 h. Next, the cells were collected and cultured in RPMI-1640 supplemented with 10% FBS in 60-mm dishes (5 × 10⁵ cells/dish) for 12 h. Then, the medium was replaced with 4 mL fresh RPMI-1640 medium, and the cells were cultured for an additional 48 h. Culture supernatants were collected, centrifuged at 1000 rpm, filtered using 0.1 μm membranes, and supplemented with 3% FBS.

2.5. Culture of gastric cancer cells with conditioned media

BGC-823 cells were maintained in RPMI-1640 medium supplemented with 10% FBS. At 80% confluence, the cells were collected, allocated into different groups, and culture with appropriate conditioned media for 48 h.

2.6. Colony formation assay

BGC-823 cells were placed in each well of 6-well plates and were maintained in RPMI-1640 medium supplemented with 10% FBS for 2 weeks. Colonies were fixed with methanol and stained with 0.1%
Fig. 1. Identification of GNFs and GCAFs. Fibroblasts were isolated from the tumour and adjacent normal tissues of a gastric adenocarcinoma patient. The expression of Pan-CK, E-cadherin, vimentin, PDGFR-β, and α-SMA in the cultured fibroblast cells was detected by immunofluorescence.
crystal violet in PBS for 15 min. Colony formation was determined by counting the number of stained colonies.

2.7. Wound healing assay

BGC-823 cells were seeded in 6-well plates at a density of $5 \times 10^5$ cells per well and were grown into monolayers. The monolayers were wounded by scratching with a plastic tip, washed twice with PBS to remove any debris, and photographed under a microscope. Next, the cells were cultured in RPMI-1640 at 37 °C in 5% CO$_2$ for 24 h and were observed and photographed using a microscope. The distance migrated by the cells was calculated from the photographs. Relative migration distance was calculated using the following formula: 1-(mean remaining breadth/mean wounded breadth) [16].

2.8. Transwell invasion assay

Invasion assay was performed using 24-well Transwell chambers (polycarbonate membrane, 8 μm pore size; Costar, Cambridge, MA, USA). The upper surfaces of the Transwell membranes were pre-coated with Matrigel (BD Biosciences, San Jose, CA, USA) which was allowed to solidify at 37 °C for 4 h. Thereafter, $1.5 \times 10^5$ BGC-823 cells suspended in 150 μL RPMI-1640 were seeded into each upper chamber, and 600 μL of RPMI-1640 supplemented with 20% PBS were added into each lower chamber. The plates were incubated at 37 °C for 24 h. Then, the media were removed from the Transwell chambers and the cells on the upper surfaces of the Transwell membranes were wiped off. Cells that had migrated to the lower surfaces of the Transwell membranes were fixed and stained with crystal violet, and the number of cells in five randomly selected fields at $\times 200$ magnification was counted.

2.9. Reverse transcription-quantitative PCR (RT-qPCR)

Total RNA was isolated using RNAiso Reagent (TaKaRa, Dalian, Liaoning, China) according to the manufacturer’s protocol. RNA concentrations were measured by a NanoDrop 2000 (Thermo Scientific, Wilmington, DE, USA). To evaluate the expression of microRNAs, reverse transcription and qPCR were performed with the Transcript Green miRNA Two-Step qRT-PCR SuperMix (Transgene, Beijing, China) using U6 as endogenous control. The qPCR cycling condition was as follows: 94 °C for 30 s, and 40 cycles of 94 °C for 5 s and 60 °C for 34 s. To evaluate the expression of E-cadherin, N-cadherin, and vimentin mRNA, reverse transcription was performed with a PrimeScript RT reagent kit with gDNA Eraser (TaKaRa) using 1 μg of total RNA from each sample, followed by qPCR with the SYBR Premix Ex Taq (TaKaRa) using GAPDH as endogenous control. The qPCR cycling condition was as follows: 95 °C for 30 s, and 40 cycles of 95 °C for 5 s and 60 °C for 31 s. Relative expression levels of each gene were calculated with the $\Delta\Delta Ct$ method. Primers are described in Supplementary Table S1.

2.10. Transfection of oligonucleotides

Oligonucleotides for 301a-NC, miR-301a mimic, 149-NC, and anti-miR-149 were transfected with siRNA-Mate* at a final concentration of 50 nM. After 48 h of transfection, cells were harvested for further experiment.

2.11. Western blot analysis

Western blot procedures were conducted as previously reported [17]. Antibodies against TIMP2 (1:500), IL-6 (1:3000), E-cadherin (1:1000), N-cadherin (1:1000), vimentin (1:1000), and β-actin (1:2000) were used as primary antibodies. HRP-conjugated goat anti-rabbit IgG diluted in 0.5% non-fat milk were used as second antibodies.

2.12. Enzyme-linked immunosorbent assay (ELISA)

The TIMP2 and IL-6 concentrations in the conditioned media were measured using an ELISA Kit (Baizhi, Beijing, China) according to the manufacturer’s instructions.

2.13. Statistical analysis

Results were summarized as mean ± SD. Student’s t-test and one-way analysis of variance were used to analyze the data and the significance level was set at $P < 0.05$.

3. Results

3.1. Identification of GNFs and GCAFs

First, cultured GNFs and GCAFs were assessed through the immunofluorescence staining of epithelial cell markers Pan-CK and E-cadherin and mesenchymal cell markers vimentin and PDGFR-β. GNFs and GCAFs yielded negative results for Pan-CK and E-cadherin and positive results for vimentin and PDGFR-β (Fig. 1). To further identify GCAFs, we examined the expression of α-SMA, a marker of activated fibroblasts typically expressed strongly in tumour-associated fibroblasts but weakly in quiescent fibroblasts [5]. We found that α-SMA expression was higher in GCAFs than in paracancerous GNFs (Fig. 1). These results indicate that paired GNF and GCAF cultures were successfully established.

3.2. GCAFs promoted colony formation, migration, and invasion of gastric cancer cells

To determine whether GCAFs affect the colony formation, migration, and invasion abilities of gastric cancer cells, BGC-823 cells were cultured with GNF- or GCAF-conditioned medium and were used to perform clone formation, wound healing, and transwell invasion assays. We observed that the BGC-823 cells cultured in the GCAF-conditioned medium showed significantly higher colony formation, migration, and invasion abilities than those cultured in the GNF-conditioned medium (Fig. 2), indicating that GCAFs promote the malignant behaviours of gastric cancer cells.

3.3. Triptonide inhibited the malignancy-promoting capacity of GCAFs

To examine whether triptonide affects the malignancy-promoting capacity of GCAFs, GCAFs were treated with vehicle control or incremental concentrations of triptonide and conditioned media were prepared to culture BGC-823 cells. The BGC-823 cells cultured in the conditioned media from triptonide-treated GCAFs exhibited lower colony formation, migration, and invasion abilities than those cultured in the conditioned medium from control-treated GCAFs (Fig. 3), indicating that triptonide inhibits the malignancy-promoting capacity of GCAFs.

3.4. Triptonide decreased miR-301a and increased miR-149 expression in GCAFs

Recent studies have shown that dysregulation of microRNA expression is largely associated with the malignancy-promoting capacity of tumour-associated fibroblasts [18,19]. We selected eight microRNAs that are related to the pathological functions of tumour-associated fibroblasts [18], and detected their expression in GNFs and GCAFs using RT-qPCR. The expression of miR-301a was higher and that of miR-149 was lower in GCAFs than in GNFs (Fig. 4). After triptonide treatment, miR-301a expression was significantly decreased and miR-149 expression significantly increased in GCAFs (Fig. 5).
3.5. MiR-301a and miR-149 mediated triptonide-induced inhibition of the malignancy-promoting capacity of GCAFs

To investigate whether miR-301a and miR-149 are involved in triptonide-induced changes in GCAF pathological functions, GCAFs were transfected with a miR-301a mimic or anti-miR-149, or were co-transfected with the miR-301a mimic and anti-miR-149. Then, the cells were treated with 40 nM triptonide and conditioned media were prepared to culture BGC-823 cells.

The colony formation assay revealed that transfection with the miR-301a mimic or anti-miR-149 attenuated triptonide-induced inhibition of the colony formation-promoting capacity of GCAFs (Fig. 6A). Moreover, co-transfection with the miR-301a mimic and anti-miR-149 blocked the inhibitory effect (Fig. 6A).

Similarly, the wound healing assay revealed that transfection with the miR-301a mimic or anti-miR-149 attenuated triptonide-induced inhibition of the migration-promoting capacity of GCAFs, and that co-transfection with the miR-301a mimic and anti-miR-149 blocked the inhibitory effect (Fig. 6B).

The transwell invasion assay also showed that transfection with the miR-301a mimic or anti-miR-149 attenuated triptonide-induced inhibition of the invasion-promoting capacity of GCAFs, and that co-transfection with the miR-301a mimic and anti-miR-149 blocked the inhibitory effect (Fig. 6C).

These results indicate that miR-301a and miR-149 mediate triptonide-induced inhibition of the colony formation-, migration-, and invasion-promoting capacities of GCAFs.
Fig. 3. Triptonide inhibited the colony formation-, migration-, and invasion-promoting capacities of GCAFs. Conditioned media were prepared from GCAFs treated by vehicle control, 10 nM triptonide, 20 nM triptonide, or 40 nM triptonide, and used to culture BGC-823 cells. After culture for 48 h, the colony formation, migration, and invasion capacities of the BGC-823 cells were measured. Data are plotted as mean ± SD of three separate experiments. *P < 0.05 and **P < 0.01 vs. the BGC-823 cells cultured with the conditioned medium from control-treated GCAFs.

Fig. 4. GCAFs expressed a higher level of miR-301a and a lower level of miR-149 than GNFs. **P < 0.01 vs. GNFs.

Fig. 5. Triptonide down-regulated miR-301a expression and up-regulated miR-149 expression in GCAFs. Data are plotted as mean ± SD of three separate experiments; **P < 0.01 vs. control-treated GCAFs.
3.6. Triptonide up-regulated TIMP2 expression and down-regulated IL-6 expression in GCAFs

TIMP2 and IL-6 are validated targets of miR-301a and miR-149, respectively [20,21]. We next investigated whether triptonide-induced changes in microRNA expression affected TIMP2 and IL-6 expression in GCAFs. Western blot analysis showed that triptonide treatment up-regulated TIMP2 expression and down-regulated IL-6 expression in GCAFs. Additionally, ELISA confirmed that triptonide treatment increased TIMP2 and decreased IL-6 protein expression.

![Graphs showing the effects of triptonide on TIMP2 and IL-6 expression](image)
secretion by GCAFs (Fig. 7B).

3.7. Triptonide prevented GCAFs from inducing epithelial–mesenchymal transition (EMT) in BGC-823 cells

Since TIMP2 and IL-6 both participate in the regulation of EMT [22,23], we examined whether treatment of GCAFs with triptonide affected EMT marker expression in gastric cancer cells. Western blot analysis showed that the BGC-823 cells cultured in the GCAF-conditioned medium expressed a lower level of E-cadherin and higher levels of N-cadherin and vimentin than those cultured in the GNF-conditioned medium, indicating that GCAFs can induce EMT in gastric cancer cells. The BGC-823 cells cultured in the conditioned medium from 40 nM triptonide-treated GCAFs expressed similar levels of E-cadherin, N-cadherin, and vimentin to those cultured in the GNF-conditioned medium, indicating that triptonide prevents GCAFs from inducing EMT in gastric cancer cells (Fig. 8).

4. Discussion

Triptonide is a key bioactive molecule identified in Tripterygium wilfordii Hook F, a traditional Chinese herb widely used for treating inflammatory diseases for centuries. In vivo experiments have shown that triptonide does not induce hepatotoxicity or nephrotoxicity and does not affect white blood cell, red blood cell, and platelet counts at high doses [24,25], indicating its excellent safety. However, previous studies on the pharmacological activities of triptonide have predominantly focused on its anti-inflammatory, anti-fertility, and neuro-protective functions [14,26,27]. To date, no study has assessed the effect of triptonide on the regulation of the tumor microenvironment. In the present study, we found that treatment of GCAFs with triptonide corrected the dysregulation of microRNA expression, increased the secretion of tumour suppressive factor TIMP2, and decreased the secretion of oncogenic factor IL-6. These results indicate that triptonide strongly inhibits the nourishment and support provided by GCAFs to tumour cells.

MicroRNAs, a class of small non-coding RNAs that typically reduce mRNA stability and suppress protein translation, are involved in tumour initiation and progression. MiR-301a is over-expressed in gastric cancer tissues, and its over-expression is positively associated with tumour size, invasion depth, and lymph node metastasis [28]. MiR-149 expression is abnormally down-regulated in gastric cancer tissues, and its down-regulation is correlated with lymph node metastasis and TNM stage [29]. However, expression and functions of these two microRNAs in GCAFs are unclear. In the present study, we found a higher miR-301a expression and a lower miR-149 expression in GCAFs than in GNFs. Triptonide treatment significantly down-regulated miR-301a expression and up-regulated miR-149 expression in GCAFs. Co-transfection with the miR-301a mimic and anti-miR-149 prevented triptonide-induced inhibition of the malignancy-promoting capacity of GCAFs. Thus, dysregulated miR-301a and miR-149 expression is crucial for the pathological functions of GCAFs; correcting microRNA expression abnormalities in GCAFs may be an effective anti-cancer mechanism of triptonide.
TIMP2 is a natural endogenous inhibitor of matrix metallopeptidases and is physiologically produced and secreted by fibroblasts and endothelial cells to maintain tissue homeostasis [32]. In the present study, we found that GCAFs secreted only small amounts of TIMP2, suggesting that TIMP2 concentration is low in gastric cancer microenvironment. Triptonide treatment significantly increased TIMP2 production and secretion by GCAFs. The TIMP2 released from GCAFs by triptonide stimulation could inhibit the activities of matrix metalloproteinases and thus disrupt the malignant behaviours of gastric cancer cells.

IL-6, a validated target of miR-149 [21], is a crucial oncogenic proinflammatory cytokine in the tumour microenvironment. Serum IL-6 levels are correlated with tumour progression and are an independent predictor of poor survival in patients with stages II and III gastric carcinoma [33]. IL-6 expression levels in gastric cancer tissues have been positively correlated with invasion depth and lymphatic metastasis [34]. In the gastric cancer microenvironment, IL-6 mainly derives from GCAFs and acts as a critical lynchpin between GCAFs and gastric cancer cells by strongly promoting the proliferation, migration, and invasion activities of gastric cancer cells [35]. In this study, triptonide significantly suppressed IL-6 production and secretion by GCAFs, suggesting that triptonide is able to decrease IL-6 concentration in the gastric cancer microenvironment and thus to weaken the oncogenic programs activated by IL-6.

EMT, characterized by decreased epithelial characteristics and increased mesenchymal characteristics, enhances the migratory potential of epithelial cells and is implicated in many physiological and pathological processes [36,37]. An EMT expression pattern in tumours, including decreased expression of the epithelial marker E-cadherin and increased expression of the mesenchymal markers N-cadherin and vimentin, is associated with the poor prognosis of cancer patients [38]. In gastric cancer, EMT is correlated with the malignancy of cancer cells and is a major contributor towards metastasis and chemotherapeutic resistance [39]. In the present study, GCAFs down-regulated E-cadherin expression and up-regulated N-cadherin and vimentin expression in

![Graphs showing the expression of E-cadherin, N-cadherin, and vimentin](image-url)

**Fig. 8.** Triptonide abolished GCAF-induced EMT in BGC-823 cells. BGC-823 cells were cultured with conditioned media from GNFs, GCAFs, control-treated GCAFs or 40 nM triptonide-treated GCAFs. Then, the mRNA (A) and protein (B) expression of EMT markers E-cadherin, N-cadherin, and vimentin in the BGC-823 cells was determined. Data are plotted as mean ± SD of three separate experiments. **P < 0.01.
BGC823 cells, indicating that induction of EMT in gastric cancer cells is an important pathological function of GCAFs. Treatment of GCAFs with triptone decreased the secretion of EMT promoter IL-6 and increased the secretion of EMT suppressor TIMP2, thus effectively inhibiting the ability of GCAFs to induce EMT in gastric cancer cells. Recent studies have shown that some commonly used chemotherapeutic drugs, such as gemcitabine, doxorubicin, and cytarabine, promote cancer cell migration and invasion by inducing EMT, and that this effect may be mediated by the tumour microenvironment [40, 41]. Thus, triptone may have advantages over some existing chemotherapeutic drugs in regulating the tumour microenvironment.

Previous researches have attempted to block the pathological functions of tumour-associated fibroblasts using monoclonal antibodies or small-molecule inhibitors to fibroblast activation proteins [7, 8]. However, these agents have shown poor efficacy in clinical trials [7, 8, 42]. One likely reason may be that inhibiting only one molecule alone is not sufficient to impact tumour-associated fibroblast functions. Differently, triptone showed a bi-directional and multiple-hit effect on GCAFs. It decreased the expression of the abnormally up-regulated miR-301a, and concurrently increased the expression of the abnormally down-regulated miR-149. Subsequently, the production and secretion of tumour suppressive factor TIMP2 were increased, and the production and secretion of oncogenic factor IL-6 were decreased. Consequently, gastric cancer cells lose the suitable environment provided by GCAFs for malignant progression. Therefore, triptone is potentially a powerful agent against GCAFs.

Malignant tumours are not simply local diseases. They reflect the systematic dysregulation of multiple organs, tissues, and cells. In addition to removing and killing tumour cells, correcting dysregulated internal environment of the body to eliminate the conditions suitable for tumour cell survival and progression is an important therapeutic approach for malignant tumours. Our results suggest that development of new drugs from traditional herbs that effectively inhibit the pathological functions of tumour-associated fibroblasts may contribute to tumour treatment.

5. Conclusions

This work reveals that triptone can inhibit the malignancy-promoting capacity of GCAFs by correcting their abnormalities in microRNA expression. Triptone is a promisingly therapeutic agent for gastric cancer, and traditional herbs may be a valuable source for developing new drugs that can regulate the tumour microenvironment.

Conflict of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at https://doi.org/10.1016/j.biopha.2017.10.046.


