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α-Asarone attenuates tumor-associated macrophages-induced gemcitabine resistance in pancreatic carcinoma via the transforming growth factor-beta 1/growth factor independent 1 axis

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Pancreatic cancer is characterized by aggressiveness and poor prognosis. The development of gemcitabine resistance, especially tumor-associated macrophage (TAM) -induced resistance in the tumor microenvironment, has greatly limited its therapeutic effectiveness. This study investigates the effects and underlying mechanisms of the plant-derived bioactive compound α-asarone in reversing gemcitabine resistance induced by TAMs in pancreatic cancer, offering potential therapeutic alternatives. Flow cytometry was used to assess the cell cycle and apoptosis in pancreatic cancer cells. Transforming growth factor-beta 1 (TGF-β1) secretion was measured by ELISA, and Cell Counting Kit-8 assays to evaluate the survival of PANC-1 cells treated with gemcitabine. Western blotting and quantitative real-time PCR were used to analyze growth factor independent 1 (Gfi-1) expression and its association with gemcitabine resistance. α-Asarone effectively reversed gemcitabine resistance in pancreatic cancer cells. Treatment with α-asarone reduced TGF-β1 levels in TAM condition medium, which in turn led to the upregulation of Gfi-1 expression. Gfi-1 was found to negatively regulate the expression of drug resistance factors, including connective tissue growth factor (CTGF) and high mobility group box 1 (HMGB1), thereby reversing gemcitabine resistance in pancreatic cancer cells. Those results indicate that α-asarone enhances Gfi-1 expression, downregulates CTGF and HMGB1, and restores gemcitabine sensitivity by reducing TGF-β1 secretion from TAMs. α-Asarone can effectively reverse gemcitabine resistance in pancreatic cancer by reducing TGF-β1 secretion from TAMs, upregulating Gfi-1, and downregulating resistance factors such as CTGF and HMGB1. This restoration of gemcitabine sensitivity may improve the therapeutic efficacy of gemcitabine in pancreatic cancer treatment. *Anti-Cancer Drugs* 36: 664–674 Copyright © 2025 The Author(s). Published by Wolters Kluwer Health, Inc.

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Introduction

Pancreatic cancer is one of the most aggressive malignancies, ranking as the fourth leading cause of cancer-related mortality, with a dismal 5-year survival rate of approximately 11% [1]. Despite advances in treatment, therapeutic options remain limited, with surgical resection rates falling below 20%. Chemotherapy, primarily with gemcitabine (GEM), remains the cornerstone of adjuvant therapy; however, the emergence of GEM resistance significantly hampers treatment efficacy, highlighting the urgent need for novel therapeutic strategies to overcome this challenge.

Tumor-associated macrophages (TAMs) are an important factor of the tumor microenvironment (TME), playing a key role in the occurrence, angiogenesis, metastasis, and immune escape of pancreatic cancer, and are an important factor in chemotherapy and immunotherapy resistance [2,3]. Moreover, TAMs contribute to both chemotherapy and immunotherapy resistance, making them a central target in cancer therapy. Transforming growth factor-beta 1 (TGF-β1), which is secreted primarily by tumor cells, stromal cells, and tumor-infiltrating macrophages, is closely associated with tumor metastasis and the progression of malignancy [4]. Research by Marta indicates that the expression of TGF-β1 is inversely correlated with the sensitivity to several chemotherapeutic agents, including GEM, cisplatin, and paclitaxel [5]. In the context of pancreatic cancer, TAMs promote resistance to GEM by

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upregulating TGF-β1 secretion, which contributes to therapeutic failure [6].

Growth factor independent 1 (Gfi-1), a transcriptional repressor of the zinc-finger protein family, plays an important role in regulating immune cell differentiation, myeloid cell maturation, and inflammatory responses [7]. Gfi-1 exerts its effects by binding to DNA and modulating the expression of genes involved in cellular growth, differentiation, and survival. Recent studies have implicated Gfi-1 in the development of drug resistance in multiple cancers. In pancreatic cancer, the expression of Gfi-1 is closely linked to resistance to GEM. Furthermore, treatments with compounds such as simvastatin have been shown to upregulate Gfi-1 expression in pancreatic cancer cells, suggesting that Gfi-1 may facilitate chemoresistance through modulation of the TME or by directly influencing tumor cell survival and proliferation [8].

Connective tissue growth factor (CTGF), a cysteine-rich protein, mediates interactions between tumor cells and the extracellular matrix. It has been identified as a key factor contributing to GEM resistance within the pancreatic cancer TME [9,10]. High mobility group box 1 (HMGB1), a nonhistone nuclear protein, plays a role in DNA replication, repair, and transcription. When released from cells, HMGB1 acts as a damage-associated molecular pattern, mediating inflammatory responses, immune activation, and drug resistance [11,12]. Both CTGF and HMGB1 are overexpressed in GEM-resistant pancreatic cancer cells, suggesting that they may contribute to the underlying mechanisms of chemoresistance [13,14].

α-Asarone, a bioactive component derived from *Acorus tatarinowii*, has demonstrated a range of pharmacological properties, including anti-inflammatory, antitumor, and lipid-lowering effects [15]. Previous studies have indicated that α-asarone exhibits anticancer activity in several malignancies, including esophageal, gastric, and colorectal cancers. It has also been shown to enhance chemotherapy efficacy in gastric cancer by modulating drug resistance mechanisms [16]. Simvastatin, a lipid-lowering agent, has been reported to reverse TAM-induced GEM resistance in pancreatic cancer [17]. Given that α-asarone exhibits lipid-lowering properties similar to simvastatin, we sought to investigate its potential role in modulating TAM-mediated GEM resistance in pancreatic cancer.

Materials and methods

Materials

PANC-1 cell and THP-1 cell-specialized medium were purchased from Wuhan Pricella Company, Wuhan, China. Penicillin/streptomycin, THP-1, and PANC-1 cell lines were purchased from ZQXZ Biotechnology, Wuhan, China. Phorbol-12-myristate-13-acetate (PMA), α-asarone, and GEM were purchased from MCE, Wuhan, China. Interleukin (IL)-4 and IL-13 were purchased from Peprotech, Wuhan, China. Cell Counting Kit-8 (CCK-8) kit, flow apoptosis assay kit, and cell cycle kit were purchased from Yeasen, Wuhan, China. ELISA test kit was purchased from Shanghai Jining Industry, Shanghai, China. The Luciferase reporter gene was purchased from Shanghai GenePharma. Shanghai, China. The BCA protein detection kit was purchased from Pierce, Shanghai, China. For the western blot experiments, the following antibodies were used: rabbit anti-Gfi-1 (14198-1-AP; Proteintech), rabbit anti-CTGF (25474-1-AP; Proteintech), rabbit anti-HMGB1 (10829-1-AP; Proteintech), mouse anti-GAPDH (60004-1-Ig; Proteintech), goat anti-mouse immunoglobulin G (IgG) (H+L) (SA00001-1; Proteintech), goat anti-rabbit IgG(H+L) (SA00001-2; Proteintech). Western Blot chemiluminescence reagent enhanced chemiluminescence (ECL) was purchased from Thermo Fisher Scientific, Shanghai, China. A predyed protein marker was purchased from Beyotime Biotechnology, Shanghai, China.

Cell culture

The human pancreatic cancer cell lines PANC-1 and human monocyte cell THP-1 were cultured in a specialized medium respectively, supplemented with 1% penicillin/streptomycin. The cells were maintained in a 37 °C incubator with 5% CO_2. The culture medium was replaced every 2–3 days depending on the cell growth density.

Tumor-associated macrophage induction in-vitro

THP-1 cells at the logarithmic growth stage were harvested, centrifuged to adjust the cell concentration to 1×10^6 cells/ml, inoculated into six-well plates, and cultured with 320 nM PMA, 20 ng/ml IL-4, and 20 ng/ml IL-13 for 48 h to establish a TAMs environment. We then constructed a coculture system of TAMs and PANC-1 cells. The upper compartment contained TAMs, and the lower compartment contained PANC-1 cells (RPMI-1640 medium supplemented with 10% fetal bovine serum). The resistance of PANC-1 cells was significantly enhanced after coculture for 1 week.

Cell Counting Kit-8 assay

PANC-1 cells and TAMs-PANC-1 cells were digested and collected, and the cells were inoculated into 96-well plates with 1×10^5 cells per well. GEM was added after the cells were fully attached to the plate. After 48 h, CCK-8 was added and incubated for 2–4 h. The absorbance was then measured at 450 nm. The absorbance was then measured at 450 nm. The relative cell viability was calculated based on the optical density (OD) values of treated cells compared with the control cells.

Cell transfection

PANC-1 cells were adjusted to 60–80%, CTGF small interfering RNA (siRNA) or HMGB1 siRNA were transfected, respectively. The transfected PANC-1 cells were collected 48 h later, and RNA was extracted to detect interference efficiency. The siRNA sequences were

listed in Supplemental Table S1, Supplemental digital content, *https://links.lww.com/ACD/A596*.

RNA reverse transcription and PCR quantification

Total RNA was extracted from cells using RNA extract reagent (Thermo Fisher Scientific) according to the manufacturer's protocol or reverse transcription, 1 μg of RNA was transcribed into cDNA using the PrimeScript RT Reagent Kit (Takara, Beijing, China). The reaction was performed at 37 °C for 15 min, followed by 85 °C for 5 s to inactivate the reverse transcriptase. Quantitative PCR (qPCR) was performed using the SYBR Green Master Mix (Takara). The PCR conditions were as follows: initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The primer sequences are listed in Supplemental Table S2, Supplemental digital content, *https://links.lww.com/ACD/A596*. GAPDH was used as an internal control to normalize gene expression. The relative expression of target genes was calculated using the $2^{-\Delta\Delta Ct}$ method. Each sample was run in triplicate, and data were analyzed using GraphPad Prism 10.

Western blot

PANC-1 cells were inoculated with 5×10^5/ml in each well of the six-well plate and cultured in a CO_2 incubator for 12 h. Then, 100 μl PANC-1 special medium and 100 μl TAM-conditioned medium (CM) were added to replicate wells in each group, respectively. After 24 h of coculture, the cells were lysed by adding 500 μl of protein lysis buffer containing phenylmethylsulfonyl fluoride to each well and were incubated on ice for 30 min. After lysis, the cell supernatant was collected by centrifugation at 12 000 rpm at 4 °C for 10 min. Protein concentration was determined by the BCA kit. The protein samples were separated by SDS-PAGE electrophoresis and then transferred to the PVDF membrane. The membrane was incubated with 5% non-fat milk in Tris-buffered saline with Tween 20 (TBST) for 1 h at room temperature. The membrane was then incubated overnight at 4 °C with a primary antibody (1 : 1000 dilution). The membrane was then washed three times with TBST, and the bound primary antibody was detected using a secondary antibody conjugated to horseradish peroxidase (HRP) (1 : 5000 dilution) for 1 h at room temperature. After further washing with TBST, the protein bands were visualized by the ECL detection system. The intensity of the bands was quantified by ImageJ, and the expression of proteins were normalized to internal control.

Transforming growth factor-beta 1 levels detection

TGF-β1 levels were measured using an ELISA kit following the manufacturer's instructions. Briefly, standards and samples (50 μl each) were added to wells, followed by 100 μl of HRP-labeled detection antibody. After incubation at 37 °C for 1 h, the wells were washed five times. Substrate solutions (50 μl each) were added, and the plate was incubated at 37 °C for 15 min in the dark. The reaction was stopped, and the OD at 450 nm was measured within 15 min. TGF-β1 concentrations were calculated using the standard curve.

Luciferase reporter gene detection

293T cells were seeded in 96-well plates at 70% confluence and cotransfected with luciferase reporter plasmids and microRNA. The cells were divided into eight groups: NC + pGL3-CTGF-wt, Gfi-1 + pGL3-CTGF-wt, NC + pGL3-CTGF-mut, Gfi-1 + pGL3-CTGF-mut, NC + pGL3-HMGB1-wt, Gfi-1 + pGL3-HMGB1-wt, NC + pGL3-HMGB1-mut, and Gfi-1 + pGL3-HMGB1-mut, with four replicates per group. After 6 h of transfection, fresh medium was added, and the cells were incubated for 48 h. Following incubation, the culture medium was discarded, and cells were lysed with 50 μl 1×PLB for 15 min at room temperature. Luciferase activity was measured by adding 100 μl luciferase assay reagent II to each well, followed by 100 μl Stop&Glo Reagent. Luminescence was detected using a microplate reader, with measurements taken 2 s after the addition of each reagent.

Xenograft mouse models

Female BALB/c nude mice (6 weeks old; Changzhou Cavins Biotechnology Co. Ltd., Changzhou, China) were randomly divided into two groups of five mice each. PANC-1 cell suspension (1×10^7 cells/100 μl) was inoculated subcutaneously. One group of mice received PANC-1 cells treated with TAM-CM and GEM, while the other received cells treated with TAM-CM, α-asarone, and GEM. Tumor size was measured every 3 days by calipers, and tumor volume was calculated using the formula: $V = 0.5 \times L \times W^2$, where L and W are the long and short diameters, respectively. Tumor growth and health status of the mice were monitored throughout the experiment. After the study, mice were euthanized, and tumor tissues were harvested. All animals were cared for according to the Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the Committee on the Ethics of Laboratory Animal Ethics Committee of Jiaxing University Medical College (Grant number: JUMC2021-180).

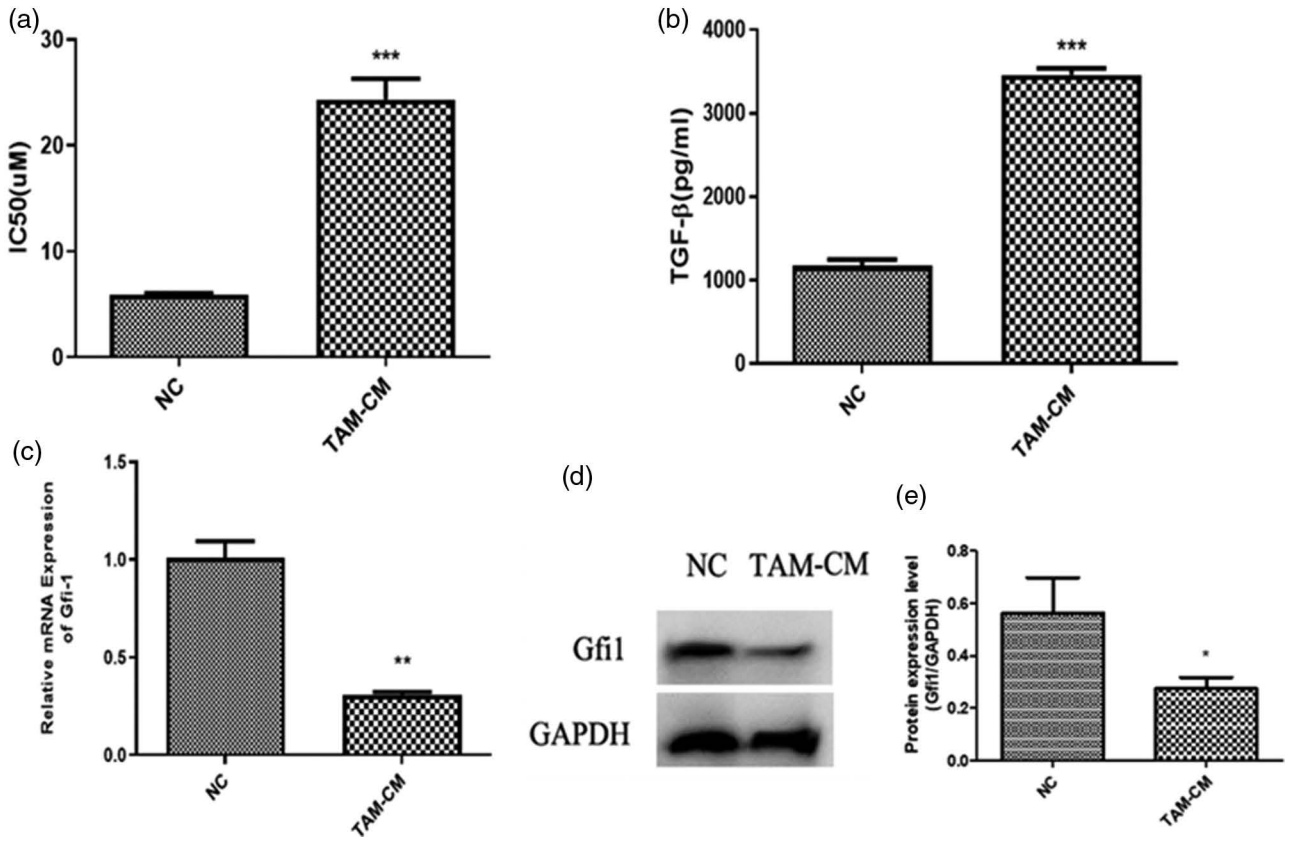
Cell cycle

Treated PANC-1 cells were collected and washed with 1 ml PBS. The cells were fixed with 2 ml precooled 70% ethanol at 4 °C for 30 min or overnight at −20 °C. After fixation, the cells were centrifuged, washed with PBS, and treated with RNase A (20 μg/ml) for 30 min at 37 °C. Following centrifugation, propidium iodide (50 μg/ml) was added, and the cells were incubated at room temperature for 30 min in the dark. The samples were analyzed by flow cytometry.

Apoptosis analysis

Treated PANC-1 cells were harvested and washed twice with precooled PBS. Cells ($1–5 \times 10^5$) were resuspended

Fig. 1



TAM-CM induces gemcitabine resistance in pancreatic cancer cells. (a) IC_{50} values of gemcitabine in the NC group and TAM-CM group ($n = 3$, $P < 0.001$). (b) ELISA analysis of TGF-β secretion levels in the NC group and TAM-CM group ($n = 3$, $P < 0.001$). (c) Real-time PCR analysis of Gfi-1 mRNA expression levels in the NC group and TAM-CM group ($P < 0.01$). (d) Western blot analysis of Gfi-1 protein expression levels. CM, conditioned medium; Gfi-1, growth factor independent 1; IC_{50}, half maximal inhibitory concentration; NC, negative control; TAM, tumor-associated macrophage; TGF-β, transforming growth factor-beta.

in 100 µl of binding buffer. A total of 5 µl of Annexin V-FITC and 10 µl of propidium iodide staining solution were added into cells, and the cells were then incubated at room temperature for 20 min in the dark. The cells were resuspended with 400 µl of binding buffer, and analyzed by flow cytometry within 1 h.

Statistics

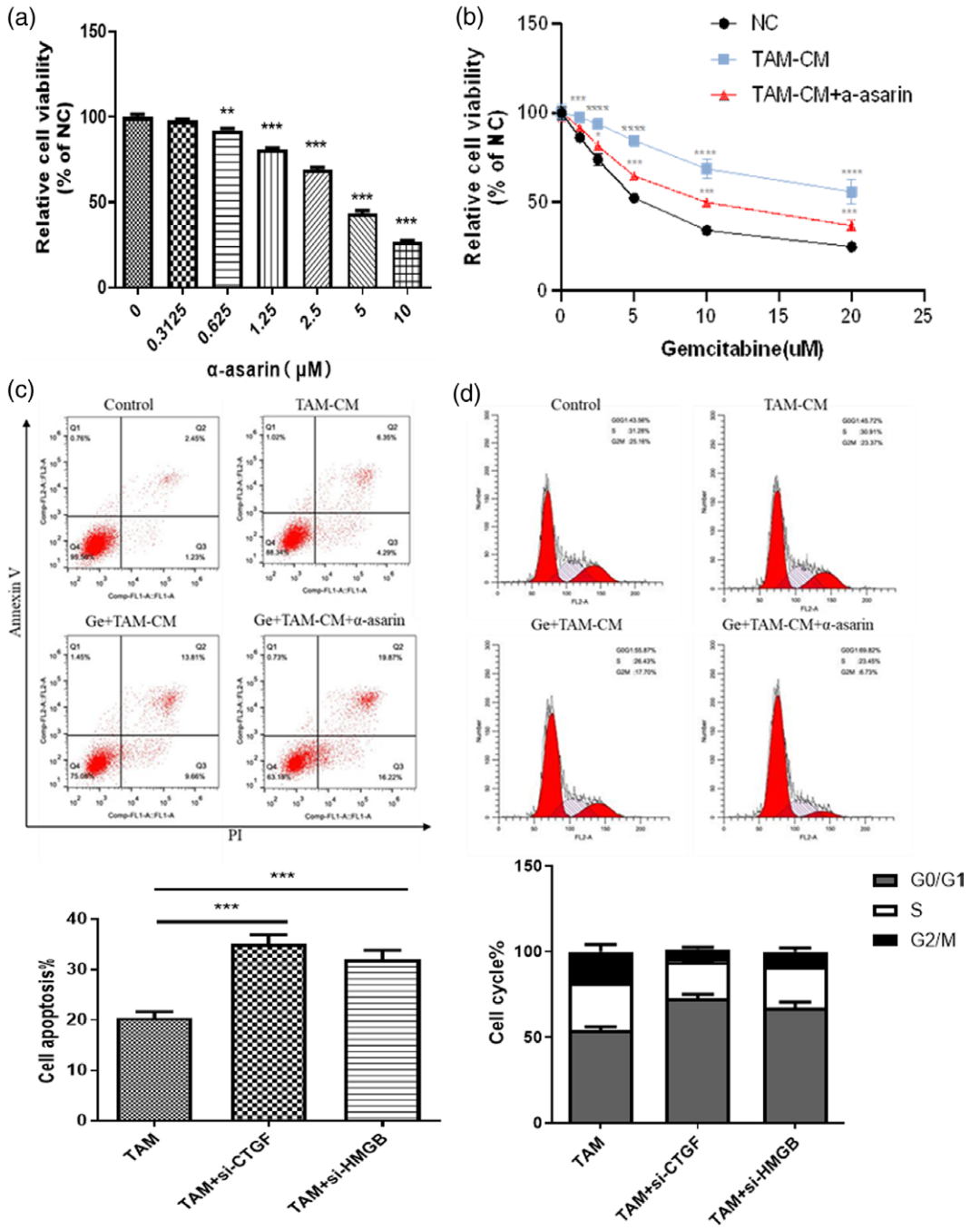
SPSS 20.0 software was used for statistical analysis. All experiments were repeated three times. Data were presented as means ± SD and compared using a *t* test. * or $^{\#}P < 0.05$, ** or $^{\#\#}P < 0.01$, ***$P < 0.001$ were considered statistically significant.

Results

Tumor-associated macrophage-conditioned medium induces gemcitabine resistance in pancreatic cancer PANC-1 cells

TAM-CM is a coculture system that mimics the TME of pancreatic cancer. The upper compartment is composed of TAMs, while the lower compartment contains PANC-1 cells. After 1 week of coculture, the drug resistance of PANC-1 cells was significantly increased. Upon induction, the half maximal inhibitory concentration of GEM in PANC-1 cells rose from 6.075 to 20.47 µM, compared with the control group, demonstrating a significant reduction in sensitivity ($P < 0.05$) (Fig. 1a). The secretion of TGF-β in the supernatant of PANC-1 cells was measured by ELISA. Compared with the blank control group, TGF-β secretion was markedly increased in the supernatant of PANC-1 cells after TAM-CM induction (Fig. 1b), indicating that TAM-CM induced an elevation in TGF-β levels in the PANC-1 cell supernatant. Furthermore, the mRNA and protein expression levels of the *Gfi-1* gene in the experimental group following TAM-CM induction were significantly lower than those in the control group (Fig. 1c and d). These experimental findings provide evidence that TAM-CM decreases the expression of the *Gfi-1* gene in PANC-1 cells.

Fig. 2

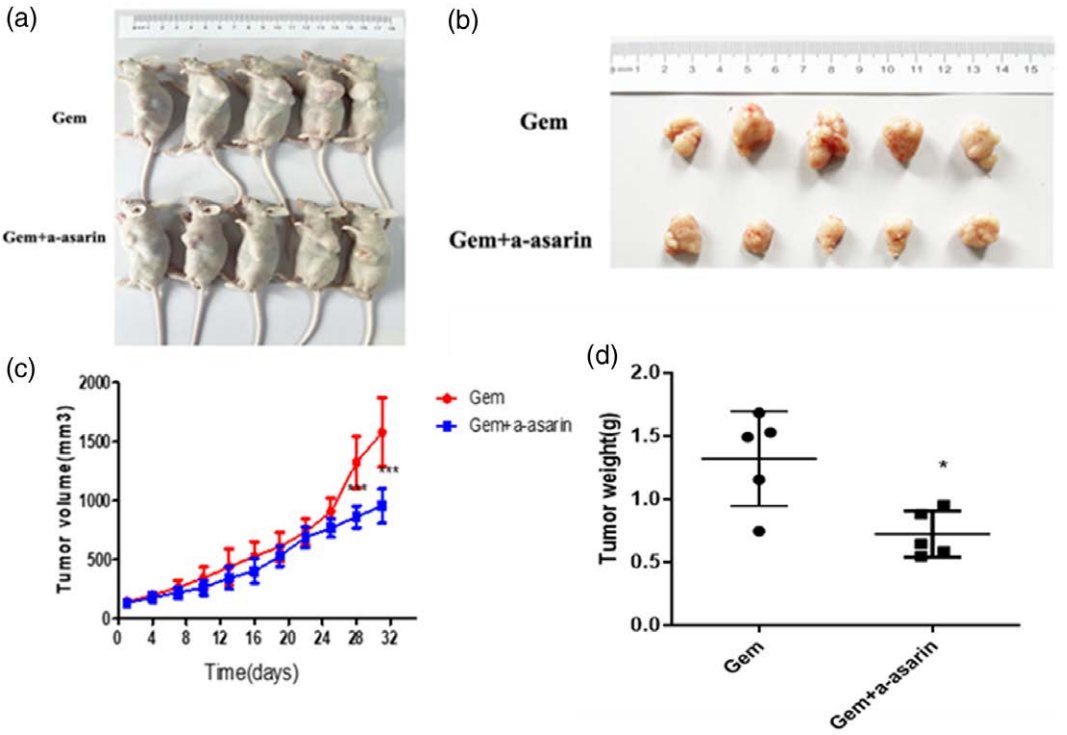


α-Asarone reverses TAM-CM-induced gemcitabine resistance in pancreatic cancer cells. (a) Relative cell viability of pancreatic cancer cells treated with different concentrations of gemcitabine, assessed by CCK-8 assay. (b) Effect of gemcitabine on the relative cell viability of pancreatic cancer cells in none (NC), TAM-CM, and TAM-CM + α-asarone treatment groups. (c) Apoptosis of pancreatic cancer cells analyzed by flow cytometry (Annexin V/PI). (d) Cell cycle distribution of pancreatic cancer cells analyzed by flow cytometry. CCK-8, Cell Counting Kit-8; CM, conditioned medium; CTGF, connective tissue growth factor; HMGB, high mobility group box; NC, negative control; PI, propidium iodide; TAM, tumor-associated macrophage.

α-Asarum restores gemcitabine sensitivity in tumor-associated macrophage-conditioned medium-induced PANC-1 cells

After 48 h of coculture with TAM-CM, pancreatic cancer PANC-1 cells were exposed to varying concentrations of GEM, and cell viability was assessed using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay over 72 h (Fig. 2a and b). PANC-1 cells cultured under TAM-CM conditions exhibited reduced sensitivity to GEM, while treatment with α-asarum

Fig. 3



α-Asarone reverses gemcitabine resistance in pancreatic cancer in-vivo. (a) Tumor-bearing mice. (b) Excised tumor tissues from the mice. (c) Tumor growth curve of tumor-bearing mice. (d) Tumor tissue weight ($N = 5$). GEM, gemcitabine.

(1.25 μM) restored the GEM sensitivity of these cells (Fig. 2b). Furthermore, TAM-CM induction led to a downregulation of Gfi-1 expression, which was reversed by α-asarum, resulting in the upregulation of Gfi-1 in the TAM-CM-treated PANC-1 cells. In addition, the proportion of apoptotic cells in the TAM-CM + GEM group increased significantly in the GEM treatment compared with the TAM-CM-only group; however, apoptosis was still considerably lower than that observed in the GEM-only group. This observation correlates with the development of GEM resistance induced by TAM-CM. In contrast, the group treated with α-asarum showed a 37% increase in apoptotic cells, suggesting that α-asarum can restore GEM sensitivity in resistant pancreatic cancer cells (Fig. 2c). Cell cycle analysis revealed that the TAM-CM + GEM group exhibited a G1 phase block, indicating that GEM was less effective in the TAM-CM-treated cells than in the TAM-CM + α-asarum-treated cells. These findings indirectly suggest that α-asarum enhances the sensitivity of PANC-1 cells to GEM (Fig. 2d).

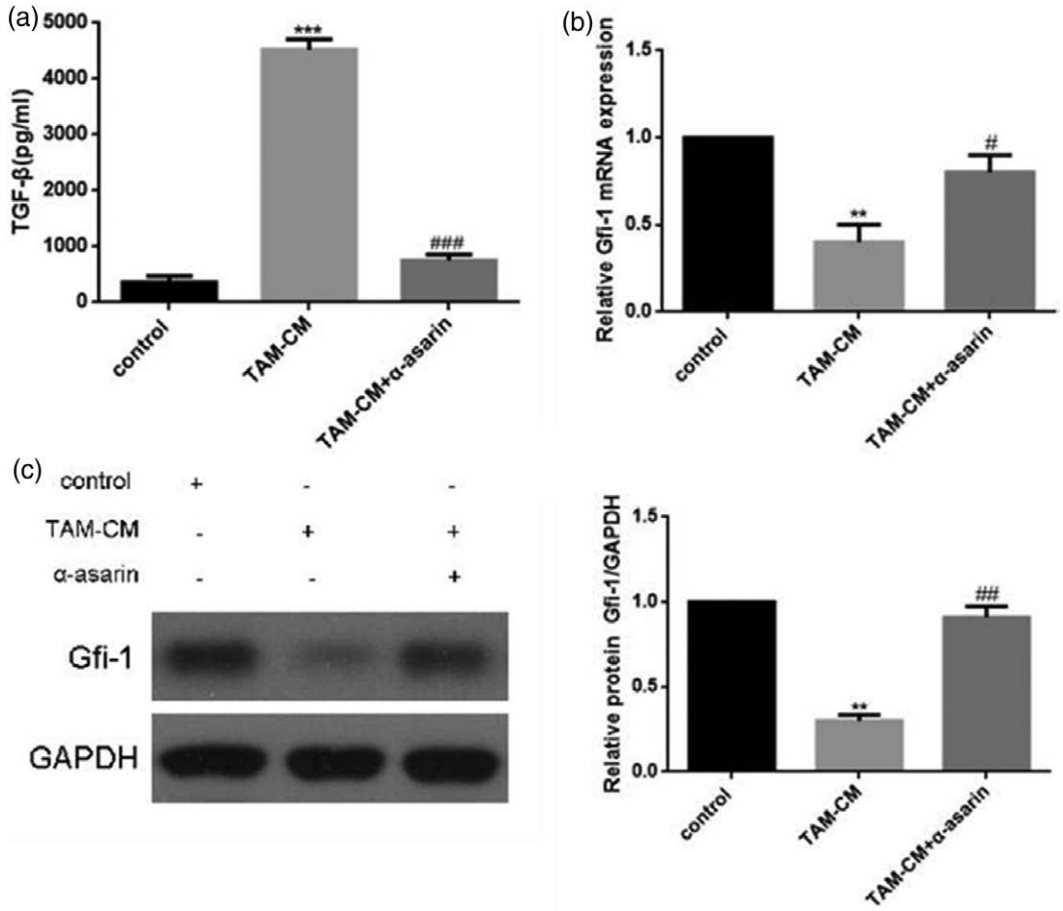
α-Asarone restores gemcitabine resistance in pancreatic cancer PANC-1 cells in-vivo

TAM-CM-induced GEM-resistant PANC-1 cells were inoculated in subcutaneous in nude mice. GEM or α-asarone plus GEM was injected into the tail vein of nude mice, and tumor formation experiments were observed. As shown in Fig. 3, the tumor growth rate in the α-asarone + GEM group was significantly slower, with a more pronounced reduction in tumor size after 24 days compared with the GEM-only group ($P < 0.05$). The average tumor weight in the α-asarone + GEM group was 1.3238 g, whereas the GEM-only group had an average tumor weight of 0.7252 g. The slower tumor growth in the α-asarone + GEM group suggests that α-asarone enhanced the sensitivity of nude mice to GEM, thereby inhibiting tumor progression.

α-Asarone restores growth factor independent 1 expression by inhibiting transforming growth factor-beta 1 secretion

TAM-CM induction in PANC-1 cells results in GEM resistance and elevated TGF-β1 levels. In this study, we investigate whether α-asarone, which has been shown to reverse GEM resistance, could also reduce TGF-β1 expression. The results demonstrate that TGF-β1 secretion was significantly elevated in the TAM-CM group compared with the control group, but treatment with α-asarone in the TAM-CM + α-asarone group significantly reduced TGF-β1 levels (Fig. 4a). Real-time PCR (RT-PCR) analysis showed that Gfi-1 mRNA expression was significantly lower in the TAM-CM group, while α-asarone treatment restored its expression (Fig. 4b).

Fig. 4



α-Asarone upregulates Gfi-1 expression by inhibiting TGF-β1 secretion. (a) ELISA analysis of TGF-β1 secretion levels. (b) Real-time PCR analysis of Gfi-1 mRNA expression levels. (c) Western blot analysis of Gfi-1 protein expression levels and semiquantitative analysis. CM, conditioned medium; Gfi-1, growth factor independent 1; TAM, tumor-associated macrophage; TGF-β, transforming growth factor-beta.

Similarly, western blot analysis revealed a reduction in Gfi-1 protein levels in the TAM-CM group, which was significantly reversed upon α-asarone treatment (Fig. 4c and d). These findings suggest that α-asarone effectively upregulates Gfi-1 expression by inhibiting TGF-β1 secretion in TAM-CM-treated pancreatic cancer cells, thus reversing GEM resistance.

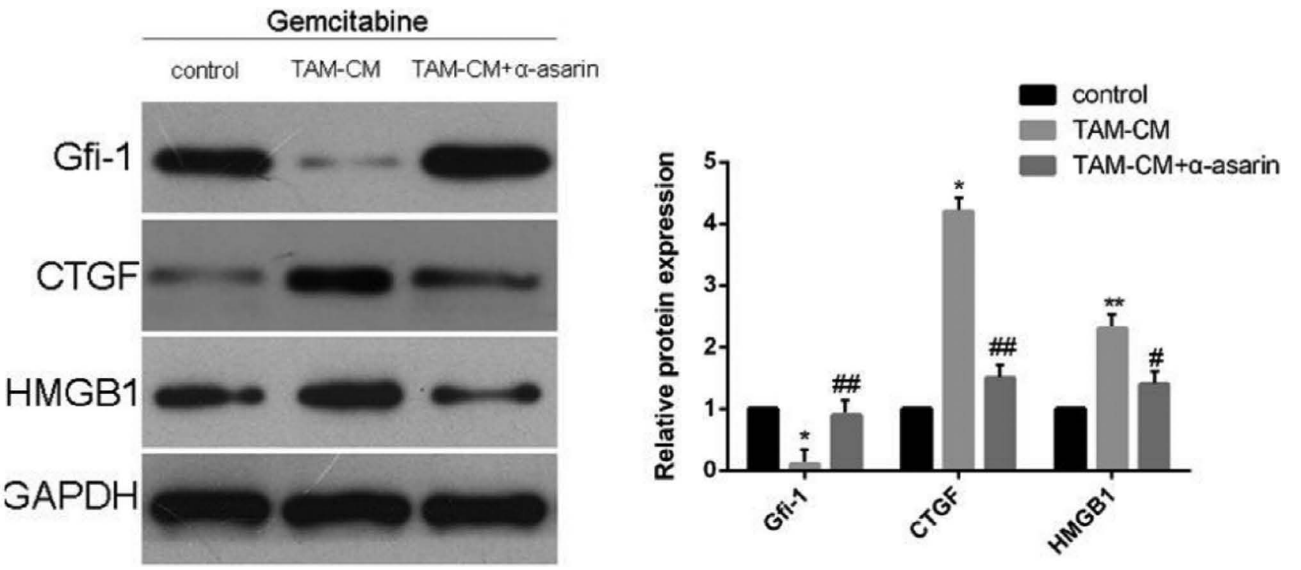
Growth factor independent 1 affects gemcitabine resistance in pancreatic cancer by regulating the expression of connective tissue growth factor and high mobility group box 1

CTGF and HMGB1 have been shown to be highly expressed in GEM-resistant pancreatic cancer cells and may play a role in the development of GEM resistance. Western blot analysis in Fig. 5 revealed that Gfi-1 protein expression was significantly decreased in the TAM-CM group compared with the control group ($^{*}P < 0.05$); however, treatment with α-asarone in the TAM-CM + α-asarone group significantly restored Gfi-1 expression ($^{\#}P < 0.05$). In addition, the expression levels of CTGF and HMGB1 were significantly elevated in the TAM-CM group ($^{**}P < 0.01$), suggesting their involvement in GEM resistance. α-Asarone treatment significantly reduced the expression of both CTGF and HMGB1.

Growth factor independent 1 affects gemcitabine resistance in pancreatic cancer by regulating the expression of connective tissue growth factor and high mobility group box 1

To explore the role of Gfi-1 in GEM resistance, luciferase assays were conducted with CTGF and HMGB1, showing significantly reduced expression in the original sequence (Fig. 6a–c). In addition, 48 h after knocking down CTGF and HMGB1 in PANC-1 cells using si-CTGF/si-HGMB-1, normal cultured PANC-1 cells and TAM-CM-cultured PANC-1 cells were treated with varying concentrations of GEM. RT-PCR and western blot analyses revealed that the downregulation of CTGF and HMGB1 restored the sensitivity of PANC-1 cells

Fig. 5



α-Asarone modulates the expression of Gfi-1, CTGF, and HMGB1 in gemcitabine-treated pancreatic cancer cells. Western blot analysis of Gfi-1, CTGF, and HMGB1 protein expression in control, TAM-CM, and TAM-CM + α-asarone-treated PANC-1 cells in the presence of gemcitabine. CM, conditioned medium; CTGF, connective tissue growth factor; Gfi-1, growth factor independent 1; HMGB1, high mobility group box 1; TAM, tumor-associated macrophage.

to GEM (Fig. 6d–j). These findings suggest that Gfi-1 regulates GEM resistance by modulating CTGF and HMGB1 expression in pancreatic cancer cells.

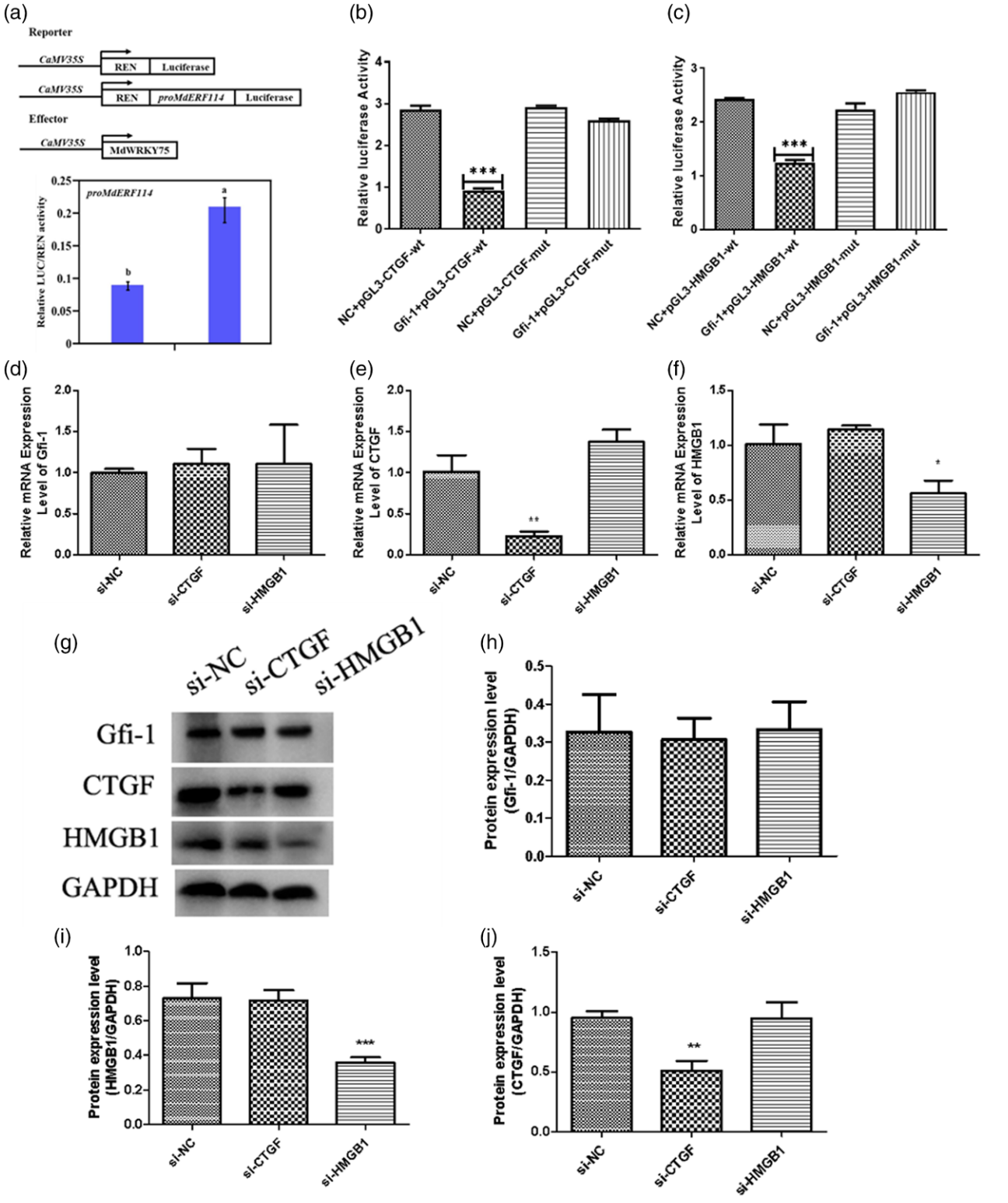
Discussion

Pancreatic cancer is an extremely invasive and highly malignant digestive system tumor. By 2030, it is likely to become the second leading cause of cancer-related deaths [18]. The onset of pancreatic cancer is insidious, and its progression is rapid, with extremely poor treatment efficacy and prognosis. Chemotherapy resistance represents a significant challenge in enhancing the survival rate of patients with pancreatic cancer. An increasing amount of clinical data has verified that GEM is only effective for a small fraction of patients with pancreatic cancer, with a low clinical benefit rate, and the majority of patients exhibit resistance to GEM [19]. GEM is a pyrimidine nucleotide analog belonging to the category of antimetabolic anticancer drugs. Once within the body, it is metabolized into an active form by deoxycytidylate kinase to inhibit DNA synthesis. Cytidine deaminase and other enzymes such as deoxycytidine deaminase can cause irreversible deamination of this nucleoside anticancer drug, resulting in its inactivation. Abnormal expression of these enzymes is associated with resistance to such drugs [20]. To improve the prognosis of patients with pancreatic cancer undergoing chemotherapy, in-depth comprehension of the mechanism of GEM resistance and methods to reverse it has emerged as one of the research focuses in pancreatic cancer treatment.

A distinctive feature of pancreatic cancer is the presence of dense connective tissue surrounding cancer cells, which can constitute up to 80% of the tumor mass. The abundant stromal components not only make it difficult for drugs to permeate into the tumor but also shape an immunosuppressive TME. TAMs are the principal immune cells in the TME and are among the earliest infiltrating cells in pancreatic intraepithelial neoplasia, with their numbers continuously increasing as the disease progresses to invasive cancer. TAMs are an independent prognostic factor in patients with human pancreatic cancer and are associated with a higher risk of disease progression, recurrence, metastasis, and shorter overall survival [21]. Simultaneously, preclinical pancreatic cancer mouse models have provided crucial evidence for the significance of TAMs in driving angiogenesis, stromal remodeling, immunosuppression, tumor cell invasion, and drug resistance [22]. Although the role of TAMs in GEM resistance in pancreatic cancer has received greater attention, the mechanism by which TAMs enhance GEM resistance in pancreatic cancer remains largely unclear.

Traditional Chinese medicine can act on multiple stages of tumorigenesis and development, characterized by multiple targets, multiple links, and multiple effects. It plays a vital role in inhibiting tumor cell growth, improving patient symptoms and signs, reducing adverse reactions to radiotherapy and chemotherapy, enhancing the sensitivity of chemotherapy drugs, and prolonging patient survival. Particularly, the antitumor and chemosensitizing activities of traditional Chinese medicine have become a

Fig. 6



Gfi-1 inhibits the transcriptional activity of CTGF and HMGB1 by directly binding to their promoters. (a) Schematic diagram of the reporter plasmid constructs used for luciferase assays. (b) Relative luciferase activity between Gfi-1 and CTGF. Significant differences are indicated (***$P < 0.001$). (c) Relative luciferase activity between Gfi-1 and HMGB1. Significant differences are indicated (***$P < 0.001$). (d–f) qPCR analysis of Gfi-1, CTGF, HMGB1 mRNA expression levels after knockdown of si-CTGF and siHMGB1. (g–j) Western blot analysis of Gfi-1, CTGF, HMGB1 protein expression levels after knockdown of si-CTGF and siHMGB1. CTGF, connective tissue growth factor; Gfi-1, growth factor independent 1; HMGB1, high mobility group box 1; qPCR, quantitative PCR.

research hotspot in recent years. *A. tatarinowii* is a traditional Chinese herb, that contains α-asarone as one of its active ingredients. Recent studies have revealed the significant antitumor effects of α-asarone. This study aimed to investigate whether α-asarone could enhance the sensitivity of GEM-resistant pancreatic cancer cells induced by TAMs and explore the underlying mechanisms.

In our study, the CCK-8 assay demonstrated that TAM-CM induces GEM resistance in pancreatic cancer cells, while α-asarone administration restored the sensitivity of these cells to GEM. Further experiments using qRT-PCR, western blot, and ELISA revealed that TGF-β expression was significantly higher in GEM-resistant pancreatic cancer cells induced by TAM-CM, while Gfi-1 expression was markedly reduced. This observation is consistent with a study by Xian *et al.*, which found that when GEM-resistant pancreatic cancer cells were treated with simvastatin and GEM, TGF-β secretion from TAMs decreased, Gfi-1 expression was upregulated, and apoptosis increased, thereby improving drug resistance. To further explore the downstream molecular mechanisms, we utilized the starBase v3.0 and Targetscan miRDB databases to identify two high-scoring target genes of Gfi-1: CTGF and HMGB1. miRanda analysis and dual-luciferase assays confirmed this interaction. We also found that knockdown of CTGF and HMGB1 resulted in reduced cell proliferation, G1 phase arrest, and restored sensitivity to GEM. These findings suggest that α-asarone regulates CTGF and HMGB1 expression via the TGF-β/Gfi-1 axis to alleviate GEM resistance in pancreatic cancer.

However, this study has certain limitations: (a) The impact of α-asarone on normal tissues, particularly renal function, was not evaluated. Further in-vivo organ and animal experiments are needed to determine the appropriate dosage, administration methods, and potential adverse reactions. (b) The study involved a limited number of cell and tissue samples, and additional validation studies with a larger sample size are warranted.

In conclusion, this study demonstrates that α-asarone mitigates GEM resistance in pancreatic cancer cells by reducing the secretion of TGF-β from TAMs and promoting Gfi-1 expression. The upregulation of Gfi-1 inhibits the transcription of CTGF and HMGB1, thus rendering PDAC cells more sensitive to GEM. α-Asarone, therefore, attenuates TAM-mediated GEM resistance in pancreatic cancer cells by blocking the TGF-β/Gfi-1 axis, offering a promising strategy for overcoming GEM resistance in pancreatic cancer treatment.

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All authors have read and approved the final version of the manuscript, and consent to the publication of this work.

Conflicts of interest

There are no conflicts of interest.

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