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THE EFFECTS OF GASTRIC CANCER INTERSTITIAL FLUID ON TUMORS BASED ON TRADITIONAL CHINESE MEDICINE 'PHLEGM' THEORY AND THE INVESTIGATION ON THE MECHANISM THROUGH MICRORNA-21 REGULATION

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This study aimed to investigate the effects of gastric cancer interstitial fluid (GCIF) on tumors and explore the possible mechanism of Xiaotan Sanjie decoction (XTSJ) on treatment of gastric cancer from the view of regulating microRNA-21 (miR-21) expression. The GCIF was extracted and identified by measuring the levels of interleukin-8 (IL-8), intercellular adhesion molecule 1 (ICAM-1) and miR-21. The effects of GCIF on the proliferation of SGC-7901 cells and tumor growing were assessed by cell counting kit-8 (CCK-8) assay and subcutaneously transplanted tumor-bearing nude mice model, respectively. Additionally, inhibition effect of XTSJ decoction on proliferation of SGC-7901 cells intervened by GCIF were assessed *in vitro* and anti-cancer effect of it was further assessed using orthotopic transplanted tumor-bearing nude mice model. The concentration of SGC-7901 gastric cancer cells were dependent on the concentration of the added GCIF. After 72 hours of continuous culture, the interstitial fluid had an obvious proliferative effect on the SGC-7901 tumor cells, which was the most significant in the high concentration group. XTSJ decoction could inhibit the growth-promoting effect ($P < 0.01$) of GCIF on gastric cancer cells. Intervention of the GCIF might promote the growth ($P < 0.05$) of the subcutaneously transplanted tumors in nude mice and decrease the net weight of the tumor-bearing nude mice ($P < 0.05$) after tumor removal. The GCIF was able to up-regulate the expression ($P < 0.001$) of miR-21 in the subcutaneously transplanted tumors. XTSJ decoction could downregulate the expression ($P < 0.05$) of miR-21 in SGC-7901 orthotopically transplanted tumors. XTSJ decoction can inhibit the multiplicative effect of GCIF on gastric cancer cells, growth of gastric tumor and promotion effect of GCIF on tumors, probably due to the down-regulating miR-21 expression in tumor tissues.

Key words: *gastric cancer, tumor microeuvironment, Phlegm evil, Xiaotang Sanjie decoction, gastric cancer interstitial fluid, microRNA-21, human gastric cell line*

INTRODUCTION

As one of the most common tumors, the incidence of gastric cancer ranks the fourth among the malignant tumors in China, and the mortality ranks the second (1, 2). Most patients are diagnosed at intermediate and advanced stages. At present, in view of the lack of effective radical treatment for the advanced gastric cancer, radiotherapy and chemotherapy may have an effect in prolonging the survival. However, many patients cannot tolerate these treatments because of weakness caused by the tumor. Therapeutic strategies to improve the tumor microenvironment (TME) of tumors are receiving increasing attention.

TME, the cellular milieu where tumor located, characterized by hypoxia, nutrient deficiency, low PH value, and high interstitial hydraulic pressure. It has reported that tumor may progress under the protection of the immunosuppressive environmental (3). Meanwhile, alterations in the patient's TME also have an influence on tumorigenesis, progression, metastasis (4, 5) and immune state of patients (6), indicating that the role of TME during tumor progression is beyond dispute (7).

Infiltrating around the tumor cells, the tumor interstitial fluid is an important microenvironment for tumor cell survival (8). Affected by various factors such as inflammation and immunosuppression, various types of cells such as tumor cells and infiltrating cells express large amounts of growth factors, cell chemokines or produce various metabolites, leading to the contents (e.g. regulatory molecules or metabolites) of tumor interstitial fluid different from that of normal interstitial fluid. It is clear that tumor interstitial fluid results from 'abnormal metabolism of the body', which was in accordance with the concept of generalized 'phlegm' (the pathological product of body metabolism) in traditional Chinese medicine (TCM). In other word, the abnormal interstitial fluid of patients with gastric cancer belongs to 'phlegm' category, which is also known as retention-of-phlegm in TCM.

Xiaotan Sanjie decoction (XTSJ) is an effective Chinese herbal formula developed from the theory of 'from the view of phlegm for cancer treatment'. Previous studies have shown that XTSJ decoction could perform anti-cancer efficacy *via* multiple targets and pathways. For example, Jun Shi *et al.* (9) reported that XTSJ decoction may inhibit adhesion, migration and

invasion of SGC-7901 gastric cancer cells partly through down regulating of IL-8. Additionally, XTSJ decoction could inhibiting angiogenesis in gastric cancer, which was associated with the vascular endothelial growth factor pathway through IL-8-linked regulation (10). Chun Jie Li *et al.* (11) revealed that by down-regulating the expressions of hTERT mRNA and protein, XTSJ formula exerts inhibitory effect on the growth and proliferation of gastric cancer cells. The possible mechanism of XTSJ decoction in gastric cancer treatment may be associated with affecting the phlegm evil, i.e., the interstitial fluid of the tumor, and intervening in upstream micro-RNA.

Additionally, multiple evidences have shown that microRNA-21 (miR-21) (12-15) of the mRNAs are closely correlated with gastric cancer, which suggests that this index is expected to as a valid diagnostic marker for gastric cancer. Therefore, this study aimed to explore the effects of GCIF on gastric cancer tumor *in vitro* and *in vivo* and whether XTSJ decoction exerted its efficacyagainst gastric cancer activity through miR-21 regulation.

MATERIALS AND METHODS

Materials

The SGC-7901 human gastric cancer cell line was purchased from the cell bank of the Shanghai Chinese Academy of Sciences. Female SD rats weighing 300 ± 20 g and male nude mice of seven-week-old were both provided by Shanghai SLAC Laboratory Animal Co., Ltd., (Production License: SCXK, Shanghai, 2007-0005, License No.: SYXK, Shanghai, 2007-0005), and fed under the specific pathogen free (SPF) condition.

Reagents and instruments

Mouse interleukin-8 (IL-8) kits (Shanghai Bio-Light Co., Ltd); mouse sICAM-1 ELISA kits (R&D Systems, Minneapolis, MN, USA); ThinPrep Cell Preservation Solution; 10% chloral hydrate (Sinopharm Co., Beijing, China); Dimethyl Sulfoxide (Amresco, Solon, OH, USA); 0.25% tryptase (Amresco, Solon, OH, USA); RMI1640 culture medium (HyClone, Logan, UT, USA); 10% fetal bovine serum (Gibco, Dublin, Ireland); miRNA Isolation Kit AM1560 (Ambion, Austin, TX, USA); RT Kit Taq Man®Micro RNA Reverse Transcription Kit (Applied Biosystems Inc., Wallthan, MA, USA); Real time PCR Kit, Hairpin-it miRNAs qPCR Quantitation Kit (miR21) (GenePharma Co., Shanghai, China); Real time PCR Kit, U6 snRNA Real-time PCR Normalization Kit (GenePharma Co., Shanghai, China).

Filter (Carrigtwohill, Co., Cork, Ireland) with a hole diameter of 0.22 μm; liquid-based cell automated processing system, ThinPrep 2000 (Cytyc Corp., Marlborough, USA); Biological camera microscope, Olympus BXH; Ultra clean table; Automatic enzyme spectrophotometer (Bio-Rad Labs. Inc., Des Plaines, IL, USA); CO_2 incubator (Heraeus, Hanau, Germany); and a fluorescence quantitative PCR instrument (Bio-Rad Labs. Inc., Des Plaines, IL, USA).

Preparation gastric cancer interstitial fluid of nude mice subcutaneously transplanted with SGC-7901 cells

1. Establishing the model of subcutaneously transplanted nude mice with SGC-7901 cells

Animal were assigned into two groups: control group and model group. And the detailed procedure of model establishing were as follows.

After disinfecting with 75% alcohol, the three generations of SGC-7901 solid tumors were removed from the axillary subcutaneous tissue of seven-week-old nude mice, followed by cutting them into blocks (approximately 1 × 1 × 1 mm in size) and stored in normal saline for further experiments. Then the animals were fasted for 12 h and anesthetized using chloral hydrate (0.03 mL/10 g). After disinfection of the right posterior axillary area, a distal puncture was carried out using an aseptic trocar in the posterior axillary line. The tumor blocks were pushed to the right axilla and then the mice were fed for four weeks under the SPF-grade environment until the tumor grows grown to approximately the size of a broad bean.

2. Preparation of gastric cancer interstitial fluid

The tumor tissues were completely peeled off, weighed, and the supernatant was prepared by homogenizing in normal saline before centrifuging it at 3000 r/s for 3 min. Then filtered the supernatant through a 0.22 μm filter membrane in an ultra-clean platform to obtain GCIF (19, 22).

Identification of gastric cancer interstitial fluid

1. Identification of obtained gastric cancer interstitial fluid by thin-cytologic test

The samples were centrifuged with the homogenate of the tumor tissue being collected by a broom sampler and washed into a small bottle containing ThinPrep cell preserving solution. The specimens in the preserving solution were processed by ThinPrep 2000 system. Briefly, mucus, blood and inflammatory cells from the specimen are separated from the epithelium, filtered through a high precision filter and transferred to an electrostatically treated slide to make a thin layer of cell smear of 2 cm in diameter, following by fixing with 95% alcohol, staining using the hematoxylin-eosin (H&E) staining and observing under a microscope.

2. Identification of obtained gastric cancer interstitial fluid by measuring the levels of IL-8 and sICAM-1

The levels of both IL-8 and sICAM-1 of obtained GCIF, tumor tissues of nude mice subcutaneously transplanted with human gastric cancer SGC-7901 cells and gastric tissue homogenate of nude mice in control group were detected by enzyme-linked immunosorbent assay (ELISA), according to the standard procedures.

3. Identification of obtained gastric cancer interstitial fluid by detecting miR-21 expression

The miRNA was isolated from the tumor tissue lysis solution by miRNA homogenate additive and acid-phenol chloroform reagents according to the instructions and reverse transcribed to cDNA. The conditions for Q-PCR were as follows: at 95ºC for three minutes, at 95ºC for 12 seconds for 40 cycles, and at 62ºC for 50 seconds. The CFX-Manager software was used to analyze gene expression data.

Effects of Xiaotan Sanjie decoction on the growth and proliferation of SGC-7901 human gastric cancer cell

1. Preparation of the Xiaotan Sanjie decoction-containing serum

XTSJ decoction is composed of Rhizoma Arisaematis, Pinellia ternata, scorpion, centipede, etc. The herbs were purchased from Shanghai Leiyunshang Pharmaceutical Co. Ltd. with the origin of the medicines being trackable (Main ingredients in XTSJ, please see *Table 1*). The concentrated

solution of XTSJ decoction was prepared by the Preparation Room of TCM of Changzheng Hospital (crude drug dose at 3.35 g/mL) and stored for use.

XTSJ decoction-containing serum was prepared according to the following procedure. Ten SD rats were randomly divided into two groups (n = 5/group): blank drug group (normal saline, 4 mL/d) and the XTSJ decoction group (XTSJ decoction, 4 mL/d). The concentration of XTSJ decoction Chinese medicine was calculated as 10 times the daily dose (90 mL) of the drug required by a standard person (60 kg). Rats in both groups were taken corresponding drugs twice daily by gavage for four consecutive days. Animals were fasting for food but free to water for 12 hours before the last medication. on the 5th day, blood was collected from the femoral artery of the rats under aseptic conditions one hour after the last gavage with corresponding drugs, following by centrifuging it at 3000 rpm for 10 min to collect the serum. Complements were inactivated in a water bath at 56ºC for 30 min, then filtered and sterilized using a 0.22 μm filter to obtain the blank drug serum and the XTSJ decoction-containing serum. The serum specimens were then stored at –70ºC for further experiments.

2. Effects of gastric cancer interstitial fluid on the proliferation of the SGC-7901 cells

The concentration of SGC-7901 cells in the logarithmic growth period was adjusted to 1 × 10^4/mL and seeded in 96-well plate (100 μL/well), following by adding 10 μL different concentrations (0.5, 0.05, or 0.005 g/mL) of tumor interstitial fluid or 0.9% normal saline (control group), respectively. After 24 h, 48 h or 72 h incubation (37ºC, 5% CO_2), 10 μL CCK-8 was added to each well and cultured in an incubator for another four hours. Then the absorbance (OD value) was measured at 450 nm by a microplate reader. At least 3 repeated wells were set up in each group, and the experiment was repeated 3 times.

3. Effects of Xiaotan Sanjie decoction-containing serum on the proliferation of the SGC-7901 cells intervened by gastric cancer interstitial fluid

100 μL SGC-7901 cells cell suspension (1 × 10^4/mL) was added to 96-well plate, following by adding 0.5 g/mL tumor interstitial fluid (10 μL) and corresponding intervention drugs (10 μL). Then the planked 96-well plate was placed in an incubator at 37ºC with 5% CO_2 for 72 h. OD value was recorded by addition 10 μL CCK-8 for another 4 h incubation. The cells in the present study were divided into three groups: GCIF group, GCIF + blank drug serum group, and GCIF + XTSJ decoction-containing serum group. Five repeated wells were set up and the experiment was repeated 3 times.

Effects of gastric cancer interstitial fluid on weight, pathological morphology and miR-21 expression of tumors of nude mice subcutaneously transplanted with SGC-7901 cells

1. Preparing tumor-bearing nude mice and grouping

The tumor-bearing nude mice subcutaneously transplanted with SGC-7901 cells were obtained according to the procure in 3.1 and divided into model group (n = 10), normal saline intervention group (n = 10), and SGC-7901 GCIF intervention group (n = 10).

2. Intervention of the SGC-7901 human gastric cancer interstitial fluid

The above tumor-bearing nude mice were subaxillary injected weekly with GCIF or saline adjacent to the tumor inoculation site for consecutive 4 weeks (0.8 mL/2 times/week). No intervention was conducted in model group.

3. Tumor weight, pathological observation and miR-21 expression

After 28 days, the mice were sacrificed under anesthesia. The tumors were removed and weighed, and the tumor tissues were preserved in a 4% paraformaldehyde solution for 48 h, followed by embedded in paraffin. Then sections were stained with H&E for further histopathological examination. Additionally, the rest of tumor tissues were stored in liquid nitrogen for detection of miR-21.

Effects of Xiaotan Sanjie decoction on weight, pathological morphology and miR-21 expression of tumors of nude mice orthotopic transplantation with SGC-7901 cells

1. Model establishment

The preparing procedure of three generations of SGC-7901 solid tumors blocks was according to 3.1. Then the animals were anesthetized using chloral hydrate (0.03 mL/10 g) before carefully exposing the peritoneum and the gastric wall. Seromuscular layer was carefully cut with an injection needle in the vascularized area of the greater curvature of the stomach, following by pushing the broken area inward with toothless forceps to form a concave box in the local gastric wall and implanting the prepared tumor tissue block into the concave box with dripping of one drop of medical OB biological glue. The wound was sutured closed after coagulation.

2. Animals grouping

A total of 40 nude mice were randomly divided into four groups (n = 10/group): XTSJ decoction group (3.35 g/mL),

Table 1. The ingredients of Xiaotan Sanjie (XTSJ) formula and corresponding percentages.

Chinese name	Latin name	Percentage
Ban-xia	Pinelliae rhizoma	12.7%
Nan-xing	Rhizoma arisaematis	12.7%
Fu-ling	Poria cocos	12.7%
Zhi-shi	Aurantii fructus immaturus	8.5%
Chen-pi	Citri reticulatae viride pericarpium	7.6%
Quan-xie	Scorpio	5.1%
Wu-gong	Scolopendra	7.6%
Ji-nei-jin	Galli gigerii endothelium corneum	12.7%
Bei-mu	Fritillariae cirrhosae bulbus	7.6%
Bai-jie-zi	Semen brassicae	7.6%
Gan-cao	Glycyrrhiza uralensis Fisch	5.1%

chemotherapy group (tegafur, 7.8 mg/mL), normal saline group (normal saline) and model group. All animals were given 0.4 mL of the specific medication per day by gavage, from Monday to Saturday, for consecutive four weeks.

Statistical methods

The SPSS 25 statistical software package was used, and the data were represented as mean ± standard deviation (x ± s). The measurement data were compared by an univariate ANOVA, and the Student-Newman-Keuls test was adopted for pairwise comparison between groups.

RESULTS

Observation of tumor cells of the extracted tumor interstitial fluid under the microscope

After grinding the tumor tissue, the tumor cells were found in the precipitates after centrifuging the suspension (*Figs. 1A* and *1B*). The extracted intercellular substance did not contain any tumor cells (*Figs. 1C* and *1D*).

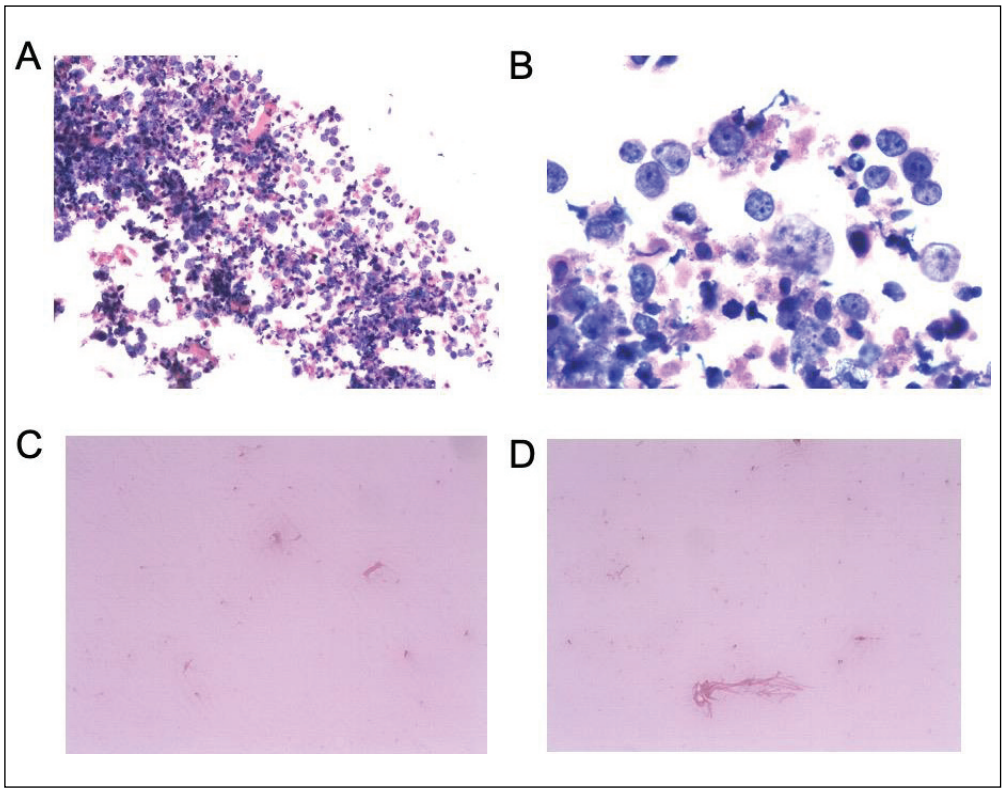

Fig. 1. (A) and (B): The suspension of the grounded SGC-7901 tumor tissue. The tumor cells (blue staining) were found in the suspension of the tumor tissue with the HE staining. The nuclear chromatin of the tumor cells were deep with obvious nucleoli ((A): H&E × 100, (B): H&E × 400). (C) and (D): There was no tumor cells in the extracted intercellular substance with the HE staining in the extracted gastric cancer interstitial fluid ((C): H&E × 100, (D): H&E × 400).

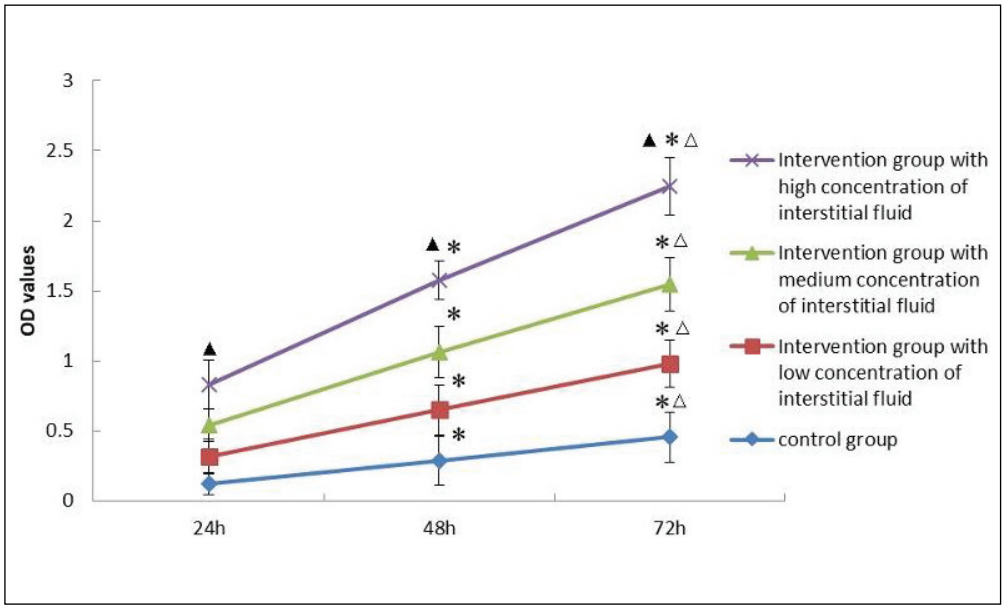

Fig. 2. The SGC-7901 tumor cell growth curve of different groups. According to the comparison based on time point, i.e., the horizontal comparison, the differences in OD among different time points in each intervention group were statistically significant (*P < 0.05, 24 h; ΔP < 0.05, 48 h). According to the comparison based on the concentration, i.e., the longitudinal comparison, there existed statistically significant differences among different groups at each time point. (ΔP < 0.05 when compared with the control group).

Table 2. Effect of tumor suspension (SGC-7901) and gastric cancer interstitial fluid (SGC-7901) on the concentration of IL-8, sICAM-1 and muR-21 expression. Note: * P < 0.05 vs. normal group.

Groups	No	**Concentration of IL-8** (pg/mL)	**Concentration of sICAM-1** (ng/mL)	**miR-21 expression**
Control group	14	1.143 ± 0.895	0.499 ± 0.285	0.062 ± 0.013
SGC-7901 Tumor suspension	14	2722.153 ± 107.244*	1.368 ± 0.041*	0.306 ± 0.069*
SGC-7901 Gastric cancer interstitial fluid	14	2686.930 ± 118.455*	1.266 ± 0.041*	0.264 ± 0.061*

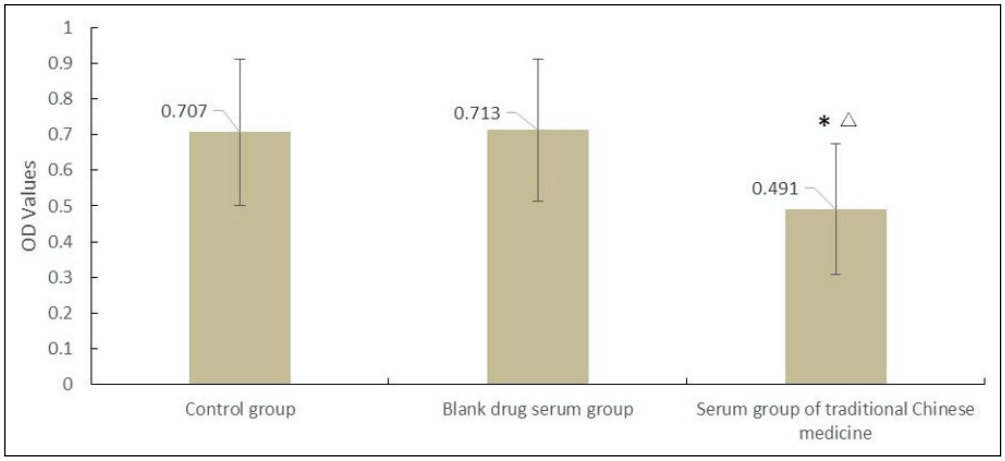

Fig. 3. The proliferation of SGC-7901 tumor cells in each group at 72 h (*P < 0.01, compared with the control group; ΔP < 0.01, compared with the blank drug serum group).

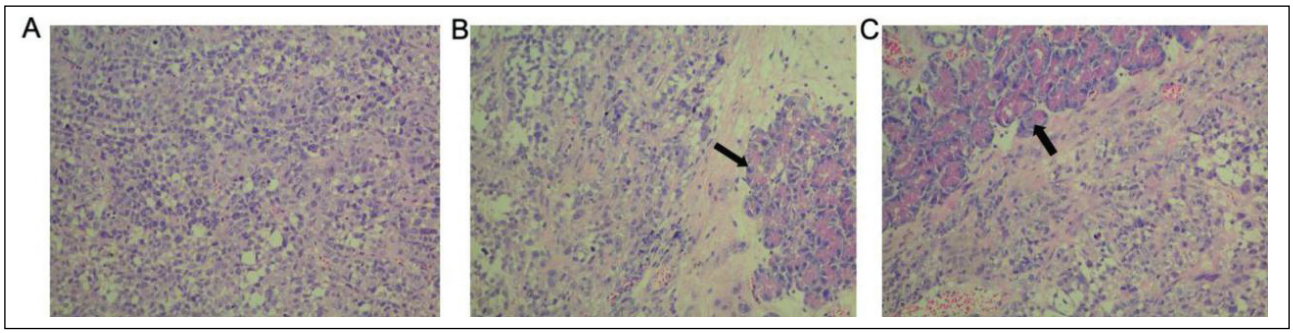

Fig. 4. Histopathology observation (H&E staining, × 200) of subcutaneously transplanted SGC-7901 gastric cancer in different groups. (A): SGC-7901 gastric cancer interstitial fluid intervention group. (B): normal saline intervention. (C): model group. The gastric gland tissue was visible at arrow.

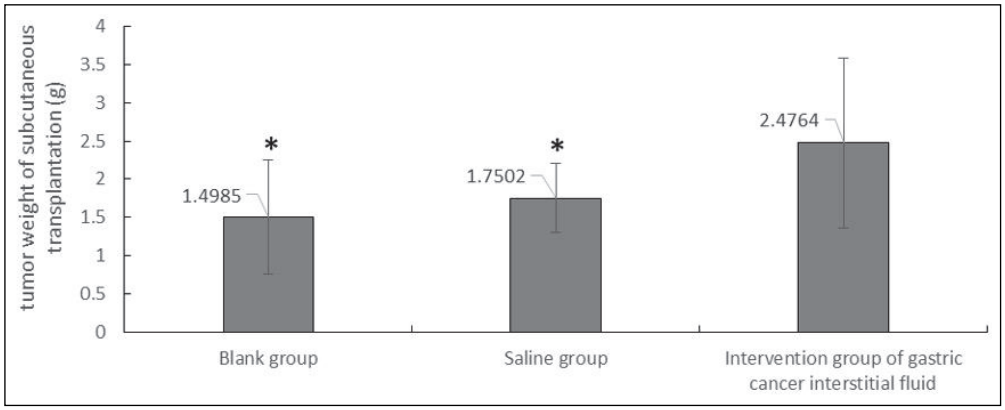

Fig. 5. Tumor weight of the SGC-7901 human gastric cancer nude mice in different groups. Compared with the gastric cancer interstitial fluid intervention group; ΔP < 0.05.

Comparison of levels of IL-8, sICAM-1 and miR-21 expression before and after filtration of subcutaneously transplanted tumor tissues

Shown as *Table 2*, no statistical difference in the levels of both IL-8 and sICAM-1 before and after filtration of subcutaneously transplanted tumor tissues could be observed, indicating that the extracted interstitial fluid without tumor cells could represent that of in tumor tissues.

Additionally, the miR-21 expression levels in the GCIF, the suspension of the SGC-7901 gastric cancer tissue, and the mice gastric tissue in the control group were measured. The results showed that the difference in the miR-21 expression was statistically significant among the three groups ($P = 0.0001$). Further pairwise comparison between the groups showed that the difference in miR-21 expression was statistically significant ($P < 0.05$) between the GCIF group and the control group. However, there was not statistically significant difference in the miR-21 expression when compared between the SGC-7901 gastric cancer suspension group and the GCIF group. This indicated that the expression of miR-21 in the GCIF group was higher than that in the control group, and was not different from that in the gastric cancer suspension (*Table 2*).

Gastric cancer interstitial fluid could promote proliferation of SGC-7901 cells

Fig. 2 shows that OD value was statistically significant between different groups at different time points ($P < 0.05$). The OD also increased with the increase in the concentration at the same time point. The concentration of the SGC-7901 tumor cells was dependent on the concentration of the added GCIF. After 72 days of continuous culture, there was an obvious proliferative effect on the SGC-7901 tumor cells in the interstitial fluid group, which was the most significant in the high concentration group. Therefore, 0.5 g/mL GCIF was chosen in the present study for continuous culture for 72 hours for subsequent experiments.

Xiaotan Sanjie decoction-containing serum could inhibit proliferation of SGC-7901 cells intervened by gastric cancer interstitial fluid

The result showed that intervention with XTSJ decoction-containing serum decreased the growth and proliferation rates of the gastric cancer cells. When compared with the control group and the blank drug serum group, the differences were statistically significant ($P < 0.01$). It was suggested that XTSJ decoction-containing serum could significantly inhibit the multiplicative effect of GCIF on the gastric cancer cells (*Fig. 3*).

Tumor weight, pathological observations and miR-21 expression of subcutaneous transplantation tumor in nude mice intervened by gastric cancer interstitial fluid

Effects of GCIF on pathological morphology of subcutaneous transplantation tumor in nude mice was shown in *Fig. 4.* The result showed that the tumor cells grew densely in the

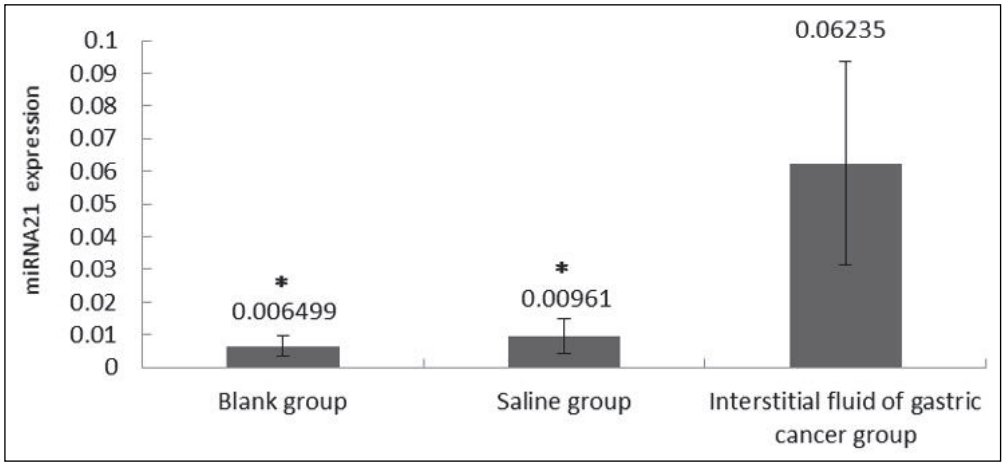

Fig. 6. Expression of microRNA-21 in the SGC-7901 subcutaneously transplanted tumor in different groups. Compared with the interstitial fluid group; $P < 0.05$.

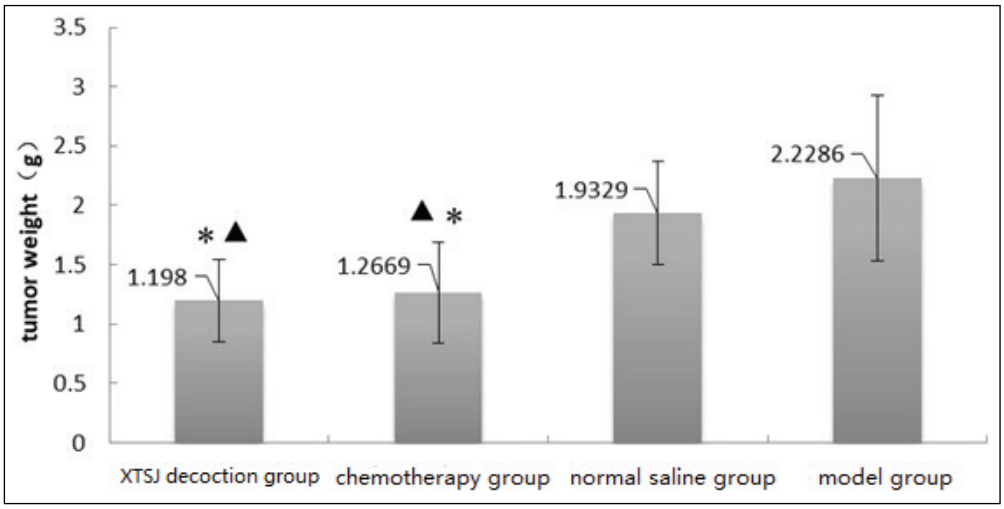

Fig. 7. Tumor weight in Xiaotan Sanjie decoction (XTSJ) group (3.35 g/mL), chemotherapy group (tegafur, 7.8 mg/mL), normal saline group (normal saline) and model group. (*$P < 0.05$ vs. the model group; Δ$P < 0.05$ vs. the saline group).

SGC-7901 GCIF intervention group but relatively sparsely in the normal saline and model groups.

Additionally, *Fig. 5* revealed the effect of GCIF on the weight of subcutaneously transplanted tumor in nude mice. When compared with the tumor weight in the other two groups (the normal saline intervention group and the model group), the overall difference was statistically significant with a $P < 0.05$ ($P = 0.0337$). The results showed that the difference in tumor weight was statistically significant between the GCIF intervention and the blank and the normal saline groups ($P < 0.05$). However, the difference in tumor weight was not statistically significant between the blank group and the normal saline group ($P > 0.05$).

The miR-21 expression in the subcutaneously transplanted tumor tissue with the intervention of the SGC-7901 GCIF has been performed in *Fig. 6*. It can be seen that miR-21 expression in GCIF group was significantly higher than in the normal saline intervention group and blank group ($P < 0.05$).

Comparison of tumor weight, pathological observations and miR-21 expression of orthotopically transplanted tumor in nude mice with the intervention by gastric cancer interstitial fluid

Fig. 7 showed that XTSJ decoction have positive effects on the weight of orthotopically transplanted tumor of nude mice. The difference in the tumor weight was statistically significant ($P < 0.001$; $P = 0.0001$) among the four groups, i.e., the model, normal saline, chemotherapy, and XTSJ decoction. For further pairwise comparison between the groups, the tumor weight in the XTSJ decoction group (1.198 ± 0.344) was lower than that in the chemotherapy group (1.267 ± 0.425), while the difference was not statistically significant. The tumor weight in both the XTSJ decoction group and the chemotherapy group was lower than that in the normal saline group and the model group, and the differences were statistically significant.

Histopathological observations of the orthotopically transplanted tumors of different group have been depicted in *Fig.8*.

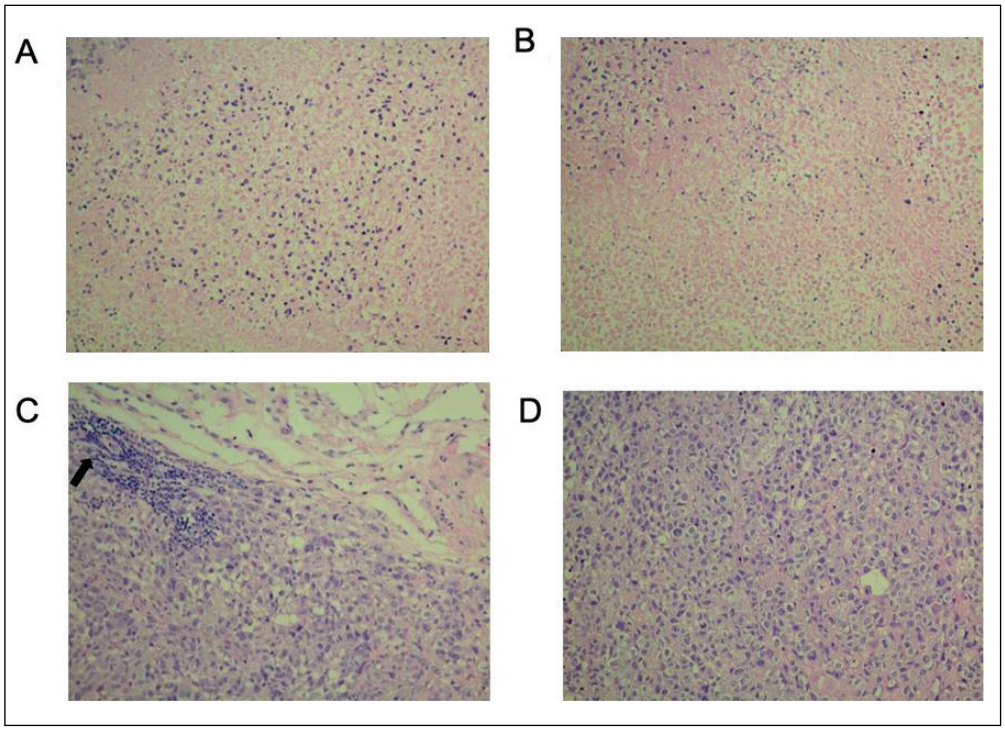

Fig. 8. Histopathology of the orthotopically transplanted tumors of different groups (H&E staining × 200). (A): Xiaotan Sanjie decoction (XTSJ) group; (B): chemotherapy group; (C): normal saline group; (D): model group.

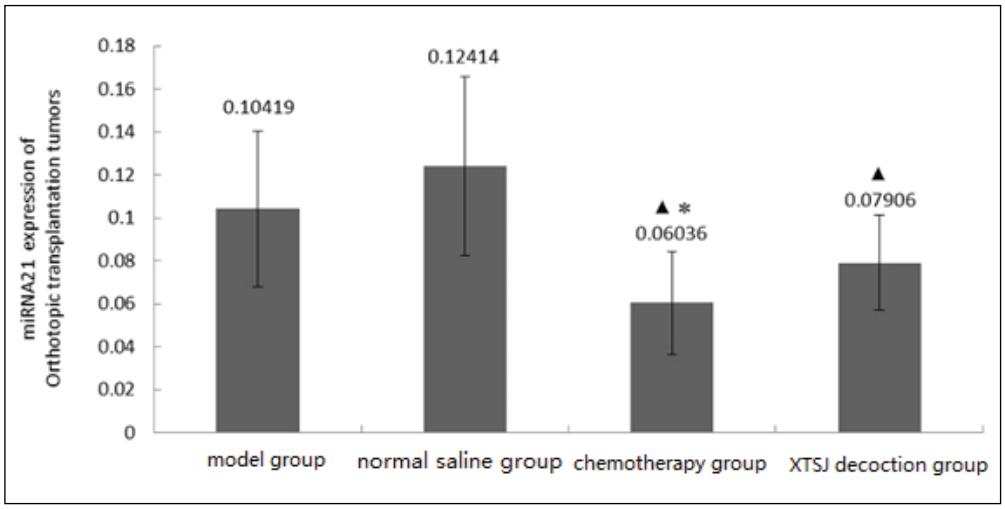

Fig. 9. microRNA-21 expression in the orthotopically transplanted tumor tissue of SGC-7901 human gastric cancer in Xiaotan Sanjie decoction (XTSJ) group, chemotherapy group, normal saline group and model groups ($\Delta P < 0.05$ vs. the blank group; $\Delta P < 0.05$ vs. the normal saline group).

The result indicated that tumor cells grew densely in the tumor interstitial fluid intervention group but relatively sparsely in normal saline group, model group and XTSJ decoction-treated group.

Additionally, the differences in miR-21 expression were statistically significant (P = 0.0005) among the blank group, normal saline group, chemotherapy group, and the XTSJ decoction group, which have been showed in *Fig. 9*. For further pairwise comparison between the groups, the difference in miR-21 expression was statistically significant between the chemotherapy group and both the blank group ($P < 0.05$), and the differences in the miR-21 expression were statistically significant between the chemotherapy group and the normal saline group and between the XTSJ decoction group and the normal saline group ($P < 0.05$ in both).

DISCUSSION

We provide a novel advancement in understanding of gastric cancer. The clinical treatment of gastric cancer by eliminating phlegm and removing stasis can prolong the survival and improve quality of life (16). A randomized double-blind study in advanced gastric cancer using QLQ-C30 International Universal Quality of Life Questionnaire and TCM Syndrome Scale showed that XTSJ decoction (Jinlongshe Granules) could significantly improve the quality of life in patients (17). The observations of the efficacy of XTSJ decoction in 104 patients with advanced gastric cancer who had lost the opportunity of chemotherapy showed that the effective rate of symptom improvement was 82.9%, and the improvement rate of the Karnofsky Performance Status was 84.4%. The median survival was 12.25 months (better than that of the one-year survival rate of 9 – 55.6% (18) in patients with advanced gastric cancer-in related foreign studies. Moreover, the three-year survival rate can reach 22.10%, indicating the significant therapeutic effect (19).

XTSJ decoction is mainly composed of Pinellia ternata, Rhizoma Arisaematis, scorpion, centipede, gecko, earthworm, baked Endothelium Corneum Gigeriae Galli, prepared Glycyrrhizae radix (licorice), and other drugs. The Pinellia ternata and Rhizoma Arisaematis dry dampness, resolve phlegm, remove stasis, and eliminate swelling. They complement each other to enhance the effectiveness at resolving the phlegm and removing the stasis. Both drugs act as the sovereign drug. The baked Endothelium Corneum Gigeriae Galli is a ministerial drug. It helps digestion, dissipates phlegm, resolves masses, and improves the efficacy of Rhizoma Arisaematis and Pinellia ternate. It also tones the spleen and assists in circulation. Therefore, it has the systemic effect of treating the symptoms and the causes. The scorpion, centipede, Teloon, and earthworm act together as assistant drugs. They are overpowering, quick acting, mobile, and penetrate rapidly. They are working internally in the viscera, externally in the meridians, and are resolving problems where sputum turbidity is coagulating. All drugs are used together and complement each other to eliminate phlegm poison, dissipate phlegm stasis, and either invigorate the spleen and stomach or dredge collaterals and relieve pain. It is applicable to cancer with symptoms of distention and fullness, poor appetite, nausea, vomiting, sputum, emaciation, fatigue, loose stools, *etc.* Baked licorice not only protects the spleen and stomach and reconciles all the herbal drugs, but it also alleviates the toxicity of all other drugs.

Consisting of all the nutrients needed for tissues cells metabolism and metabolic products of cells as well as unused substances, tumor interstitial fluid plays undisputed role in the progression of tumors. ICAM-1 and IL-8 are both overexpressed factors in gastric cancer tumor tissues, which have been demonstrated in clinical and pharmacological studies (10, 20, 21). Therefore, this study selected the two factors as indicators for identify gastric cancer interstitial fluid. Results showed that both tumor suspension and obtained gastric cancer interstitial fluid after filtration existed high levels of ICAM-1 and IL-8. The facts that no statistic difference between the two groups mentioned above suggested that gastric cancer interstitial fluid could represent that of in tumor tissues.

Additionally, intervention with GCIF can induce faster proliferation of SGC-7901 tumor cells *in vitro* and tumor growth *in vivo* have been demonstrated in present study, accompanied by worse animal condition such as lower net weight of the tumor-bearing nude mice. This result indicated that SGC-7901 GCIF might promote tumor growth and provide nutrients to it. Treatment with XTSJ decoction could reverse afore-mentioned phenomenon induced by GCIF, preliminary suggesting the positive effects of XTSJ decoction on anti-cancer and suppression of nutrient delivery to tumor.

The potential mechanism of XTSJ decoction on anti-cancer effect have been further investigated form the perspective of down-regulating miR-21 expression. As it is widely accepted, the miRNA controls genes in an organism, so if something goes wrong, it can cause abnormalities in the body, such as the more common phenomenon of uncontrolled cell division in cancer. As one of the most investigated tumor-related miRNAs, miR-21 overexpressed in gastric cancer tumor tissues plays a very important role in tumorigenesis. The detection of miR-21 in the peripheral blood can be used as a new tool to monitor the tumor cells in the circulation in patients with gastric cancer (22, 23). In addition, miR-21 also can be used as an effective target gene for the treatment of gastric cancer (24, 25). Various factors such as the *Helicobacter pylori* (Hp) infection can up-regulate the miR-21 expression, which leads to the occurrence of gastric cancer (26, 27). Tumor-bearing nude mice intervened with GCIF exhibited elevated miR-21 expression level, suggesting the promoting effect of gastric cancer tissue interstitial fluid on miR-21 expression. Of note, the decreased miR-21 expression in tumor-bearing nude mice treated with XTSJ decoction indicated that this Chinese herb compound exerted the anti-cancer effect partly *via* down-regulating miR-21 expression.

This study demonstrated that GCIF could promote the proliferation of SGC-7901 cells as well as gastric tumor growth. The XTSJ decoction could reversed afore-mentioned phenomenon induced by GCIF, possibly due to down-regulation miR-21 expression in tumor tissues.

Da-Zhi Sun, Min Ye and Da-Wei Ju contributed equally to this study.

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