Huashi Jiedu decoction blocks cell cycle and inhibits EMT of gastric cells via LncRNA-p21

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Author contributions
Qiu SL and Sun LT conceived the study, and Qiu SL acquired the funding. Pang X, Zhang LY, Shi C, and He YT collected samples and conducted the experiments. Hong QR, He YT, and Shi C performed the genome assembly and analysis of the data. Pang X, Lin WY, and He YT wrote the manuscript. All authors have read and approved the final manuscript.

Competing interests
The authors declare no conflicts of interest.

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Abbreviations
TCM, traditional Chinese medicine; H2O, Huashi Jiedu decoction; WB, western blotting; EMT, epithelial-mesenchymal transition; NC, negative control; E-cad, E-cadherin; N-cad, N-cadherin.

Citation

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Abstract
Background: MicroRNAs and traditional Chinese medicine have emerged as pivotal regulators in the progression of gastric carcinoma. Huashi Jiedu decoction has been clinically used to enhance the quality of life in gastric carcinoma patients. Therefore, the present study aimed to elucidate the involvement of MicroRNAs and Huashi Jiedu decoction in gastric carcinoma metastasis and investigate their regulatory mechanisms in anti-tumor effects. Methods: Subsequently, the effect of Huashi Jiedu decoction on gastric carcinoma cell metastasis and proliferation was verified using cell counting kit-8, flow cytometry, wounding healing, and Transwell assays. LncRNA-p21 knockdown cells were successfully established in BGC-823 and MGC-803 cell lines. Mouse models inoculated with MGC-803 cells were generated, and the cellular morphology in tissues was assessed using hematoxylin staining. Additionally, western blot and immunohistochemistry analysis were performed to evaluate the expression of epithelial-mesenchymal transition-related proteins, with real-time PCR validation conducted in both in vitro and in vivo settings. Results: Network pharmacology revealed a close association between proteins involved in the epithelial-mesenchymal transition process and LncRNA-p21 in gastric carcinoma, consistent with the downregulated expression of E-cadherin, N-cadherin, and Twist following LncRNA-p21 knockdown. Cell counting kit-8 and flow cytometry assays confirmed that Huashi Jiedu decoction exhibited concentration-dependent cytotoxicity, effectively inhibiting the distant metastasis of gastric carcinoma cells in wound healing and invasion experiments. Treatment with Huashi Jiedu decoction resulted in increased expression of E-cadherin, N-cadherin, and LncRNA-p21 in both in vitro and in vivo models, as validated by western blot, real-time PCR, and immunohistochemistry. Conclusion: Collectively, our findings demonstrate that Huashi Jiedu decoction suppresses epithelial-mesenchymal transition and inhibits gastric carcinoma invasion and metastasis both in vivo and in vitro through the knockdown of LncRNA-p21, corroborating the initial findings from our network pharmacology analysis.

Keywords: gastric cancer; Huashi Jiedu decoction; long non-coding RNA-p21; epithelial-mesenchymal transformation; cell cycle; invasion and metastasis
**Highlights**

1. LncRNA is a non-coding single-stranded RNA that participates in the pathogenesis of malignant tumours, such as gastric cancer, through a variety of pathways. In this article, we investigated the possible mechanism of LncRNA-p21 against gastric cancer by blocking the cell cycle and reversing the EMT process, suggesting that LncRNA-p21 may be one of the molecular markers associated with gastric cancer.

2. Traditional Chinese medicine is a treasure tank for extracting anti-tumour active ingredients with unique advantages. In this paper, we extracted the active ingredients from *Patrinia villosa* (Thunb.) Juss, *Hedyotis diffusa*, *Radixia amethystoides* (Benth.) H.Hara and then regulated the mechanism of LncRNA-p21 against gastric cancer, which is expected to provide the readers with further ideas for research.

**Medical history of objective**

*Qi-Ge-San* is a classic formula for treating choking and difficulty swallowing, from Cheng Kuo Peng's "Medical Insights-Volume 3" (1732 C.E.). In ancient times, *Qi-Ge-San* variations were often used in the treatment of dysphagia. Modern pharmacological studies have shown that *Qi-Ge-San* variations had the effects of anti-tumour cell proliferation, improving memory, stimulating immune cell proliferation, and resisting distant invasion of tumours.

**Introduction**

Gastric carcinoma (GC) is the fifth most common cancer worldwide and the third most correlative cause of cancer death with a 3-year overall survival rate of 10.2 percent for advanced gastric cancer [1, 2]. The National Comprehensive Cancer Network guidelines for gastric cancer have completed a system of diagnosis and treatment, the median OS for distant metastases is hard to break through at 11 months, and the improvement in survival quality remains an intractability issue in clinical practice [3–5]. A meta-analysis illustrated that traditional Chinese medicine (TCM) can be effective in improving patients' prognosis and poor quality of life, as well as ameliorating the major factors responsible for the elevated incidence of adverse events [6]. However, the molecular mechanisms, pathway targets, and pharmacology of TCM-specific therapeutics are still not fully understood [7–9]. In the dialectical treatment, Huashi Jiedu decoction (HJD) is a commonly used empirical prescription in the gastric cancer with the treatment principle of "Removing Dampness and Detoxifying", contains multiple anti-tumor active ingredients such as rutin, geniposidic acid, and asperulosidic acid [10].

Distant metastasis of tumors is an epithelial-mesenchymal transition (EMT) process when epithelial cells are transformed into mesenchymal cells in a microscopic phenotype, which is significantly related to the catalytic induction of TGF-β1 [11, 12]. In addition, our network pharmacological also found that LncRNA-p21 and TGF-β1 have a strong bond, which has been confirmed to be correlated with carcinoma evolving by inhibiting proliferation and promoting apoptosis. LncRNA-p21 expression is reduced in a large number of tumors, such as prostate carcinoma, osteosarcoma, and hepatocellular carcinoma [13–16] More importantly, LncRNA-p21 is one of the conceivable prognostic markers of tumors in long-noncoding RNA [17]. Experiments have shown that LncRNA-p21 can regulate apoptosis and proliferation in gastric cancer cells by up-regulating ARHGEF9 expression and sponging out miR-514b-3p, and additionally, inhibit gastric cancer invasion and metastasis through p53 [18, 19].

The specific processes and mechanisms of how LncRNA-p21 suppresses gastric cancer metastasis remain murky, therefore, we aimed to investigate whether LncRNA-p21 is a key intermediate target for HJD-induced EMT in gastric cancer.

**Materials and methods**

**Cell culture**

MGC-803 and BGC-823 cell lines were purchased from a typical cancer preservation commission cell bank, Chinese Academy of Sciences (Shanghai, China), STR and mycoplasma were negative. The gene sequence of the shRNA is GgCUCTACATCUACTGCGWA (NM 078467), gaCACAGGAGGAAAGACCATGT (NM 078467), and gaACCATGUGGUCACTGTCAC (NM 078467). The experimental design aimed to inhibit the tumor-related biological behavior of gastric cancer cells using HJD. Therefore, our control group consisted of gastric cancer cells, while gastric mucosal cells and other cell types were excluded from the study. Cells were cultured in RPMI 1640 medium (Kino Co., Ltd, Hangzhou, China) which contained 10% fetal bovine serum (FBS; Gibco, Grand Island, USA) and in a cell culture incubator with 1% penicillin / streptomycin (Kino Co., Ltd, Hangzhou, China) under 5% CO₂ at 37°C. Figure 1 below shows our flow chart of experimental design.

**HJD preparation**

The HJD was prepared at the School of Pharmaceutical Sciences, Zhejiang Chinese Medical University (Hangzhou, China). Origin materials are *Patrinia villosa* (Thunb.) Juss. (MESH: *Patrinia*, *Hedyotis diffusa* (MESH: *Hedyotis*), and *Radixia amethystoides* (Benth.) H.Hara (MESH: *amethystoidin A*) (each 500 g), which were mixed and immersed in 1,000 mL of distilled water for half an hour. In Chinese medicine, the pathogenesis of gastric cancer is mostly due to the internal stagnation of evil heat and the blockage of phlegm and dampness. Therefore, in this paper, the pharmacological study of the anti-cancer activity of the formulae of *Patrinia villosa* (Thunb.) Juss, *Hedyotis diffusa*, and *Radixia amethystoides* (Benth.) H.Hara, which has the effect of clearing heat and detoxifying dampness, was conducted. Filtrates were then concentrated to 300 mL so that their crude drug content was 2 g/mL of HJD in the initial liqeur. Through HPLC-UV and HPLC-MS analysis confirmed the quality control as rutin (compound CID: 5280805), geniposidic acid (compound CID: 443354), chlorogenic acid (compound CID: 1794427), asperulosidic acid (compound CID: 11968867), and rabdosin (compound CID: 471121) (Supplementary Figure S1).

**TGF-β1-induced EMT cellular model**

MGC-803 and BGC-823 cells in the logarithmic growth phase were collected and subsequently seeded into culture flasks at a density of 2 × 10⁵ cells per flask. Following cell seeding, a final concentration of 5 ng/mL of TGF-β1 (recombinant human TGF-β1 mammalian, Preprotech, NewYork, NY, USA) was added to each flask and incubated for 48 hours. The expression levels of E-cadherin (E-cad) and N-cadherin (N-cad) were assessed by western blotting (WB) to confirm the successful induction of EMT by TGF-β1.

**Assay of cell counting Kit-8**

Cell proliferation was assessed using the CCK-8 assay (Genomcell, Jiangsu, China). MGC-803 cells were seeded at a density of 6 × 10⁴ cells/well, while BGC-823 cells were seeded at a density of 8 × 10⁴ cells/well. After the cells reached approximately 80% confluence (approximately 48 hours), the culture medium was replaced with different concentrations of HJD. Following 48 hours of further incubation, the CCK-8 reagent was added to the culture medium and incubated for an additional 1 hour. Absorbance was measured at 450 nm using a microplate reader (Biorad, NewYork, NY, USA). The proliferation inhibition rates (%) were calculated using the formula: (the average OD value of each replicate in the control group – the average OD value of each treatment group)/the average OD value of the blank control group × 100%.

**Wound healing assay**

Approximately 1 × 10⁵ MGC-803 cells and 3 × 10⁵ BGC-823 cells were seeded in 6-well plates. After cell attachment, 5 ng/mL of...
TGF-β1 was added to all wells for a duration of 48 hours to induce EMT. Subsequently, 1.5 mg/mL of HJD was added to each well. At 0 hours, 24 hours, and 48 hours after wound induction, three random fields of view were selected from each group and captured using an optical microscope (ix71, Olympus, Tokyo, Japan) at a magnification of 200 ×.

Trans well assay
The cell density was adjusted to 2 × 10^5 cells/mL. The transwell experiments were conducted in accordance with the instructions and procedures provided with the transwell kit. Subsequently, photographs were captured and analyzed using a light microscope (400 ×) (ix71, Olympus, Tokyo, Japan). The remaining steps were carried out following the same protocol as the transwell migration experiments.

Real-time fluorescent quantitative polymerase chain reaction (RT-qPCR) assay
Total RNA was extracted from each group using TRIzol reagent (Shanghai Pufei Biotech Co., Ltd., Shanghai, China), and cDNA was synthesized using the M-MLV Reverse Transcriptase kit (Promega, Madison, WI, USA). For two-step RT-qPCR, a 12 μL reaction solution was used for each reaction. This mixture contained 0.6 μL of template cDNA, 6 μL SYBR premix Ex Taq (Takara Bio, Shiga, Japan), 0.3 μL of the primer mix (5 μM), and 5.1 μL RNase-Free H2O. Primers were synthesized by Shanghai Sangon Biological Engineering and Technology Service (Shanghai, China). The relative expression level of each gene was calculated according to the 2^−ΔΔCt method [20]. The amplification primer sequences for each gene were detailed in the Supplementary Table S1.

Western blot assays

The experiments were performed by following the procedures and instructions of the guideline. Data were calculated and normalized to β-actin. Antibodies are listed below: β-actin (Cat#20536-1-AP, 1:1000, Sigma, Shanghai, China), N-Cad (Cat#76011, 1:500, Sigma, Shanghai, China), E-Cad (Cat#76319, 1:1000, Sigma, Shanghai, China), Vimentin (Cat#5741, 1:1000, Sigma, Shanghai, China), Peroxidase Conjugated Goat anti-Mouse IgG (H + L) (Cat#DW0990, 1:1000, Sigma, Shanghai, China).

Flow Cytometry assay
Cells at the logarithmic stage were incubated overnight in an incubator with an expected cell density of 50%–60% on day 2. The next day, the experimental group was treated with 50 μmol in LARTs 3 mL medium. The experimental group was added with 50 μmol in LARTs 3 mL medium, and the control group was set with 3 replicates in each group. After 48 h, cells were collected, fixed with ethanol, and then stained with propidium iodide. The data analysis of DNA was performed by Mod Fit LT software, and the percentage of each cell cycle (G1, S, G2 + M) was obtained.

Cell transfection assay
Construction, identification, and supply of human LncRNA-p21-shRNA lentiviral vectors were provided by Genechem Technology (Shanghai, China). Before MGC-803 and BGC-823 cells got infected at multiplicity of infection in 10 and 20 for 12 h, the correct sequence had been identified and the lentivirus had packed. LncRNA-p21 knockdown was successfully constructed through western blot confirmed.

Nude mouse modelling
This study was conducted by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the
Laboratory Animal Ethics Committee of Zhejiang Chinese Medical University (ethics approval number IACUC-20210125-13). The mice were housed in a specific pathogen-free barrier facility at the Animal Experimental Center, Zhejiang Chinese Medical University, under standard conditions of constant temperature and humidity (Approval number: 88471). MGC-803 cells from the Si-LncRNA-p21 group, NC-LncRNA-p21 group, and corresponding negative control (NC) group were collected. Each nude mouse was subcutaneously inoculated with 0.1 mL of cell suspension containing a cell concentration of $5 \times 10^7$/mL in the left limb. The development of subcutaneous tumors was assessed after two weeks. For the following two weeks, mice in the HJD group received daily administration of HJD (1.2 g/mL, 0.4 mL/20 g), while mice in the blank control group received daily administration of saline. On day 28, each mouse was weighed and the measurement was recorded. Subsequently, the spleen, liver, and tumor of each mouse were collected for further analysis.

**Immunohistochemistry**

For the estimation of protein concentration, all processes were conducted manually. Diaminobenzidine (Cat#ZLI-9065, Shanghai, China) visualized immunoreaction. Tissues were counterstained with hematoxylin (Cat#ZLI-9609, Shanghai, China), dehydrated and blocked with paraffin after Diaminobenzidine staining. The inverted microscope at 200× magnification was used to analyze and photograph results which were then analyzed with the help of Image-Pro Plus Version 6 software. Antibodies’ concentrations used were as mentioned in 2.8.

**Statistical analysis**

All measurement data are expressed as Mean ± standard deviation (X ± s) and analyzed using SPSS statistics version 22.0 (IBM, USA). All experiments were repeated in triplicate. Data are analyzed by one-way analysis of variance when they conform to normal distribution. Data are analyzed by the nonparametric test when they do not follow a normal distribution. It is the probability value of $P < 0.05$ that is considered statistically significant.

**Results**

**HJD blocks the cell cycle and cell inhibition of gastric cancer cells before transfection in vitro**

FCM was performed in both cells in three groups (control, EMT, and HJD). As shown in Figure 2A, 2B, the cell cycle is blocked in the

![Figure 2 Cell cycle and IC50 of gastric cancer cells, LncRNA-p21 participated in TGF-β1-stimulated EMT in gastric cancer (magnification: X 200). Flow cytometry was performed in MGC-803 and BGC-823 cells in three groups (NC, SiNC, LncRNA-p21) (A, B) Proliferation and inhibitory effect of HJD were performed (C–F). Knockdown of LncRNA-p21 and Western Blot assay was performed to confirm (G, H), groups were (a)MGC-803 LncRNA-p21; (b)MGC-803 SiNC; (c)MGC-803 NC; (d)BGC-823 LncRNA-p21; (e)BGC-823 SiNC, and (f) BGC-823 NC. Expression of CDK4 in gastric cancer cell lines with or without HJD intervention was analyzed. All experiments were performed three times. Mean ± SEM ($^\ast \ P < 0.05$) is used to present the data. The G1 phase to the S phase by modulating proteins such as Cyclin D1 and CDK4. Thus, these findings indicate that HJD can block cell cycle-related proteins and reverse the EMT status of gastric cancer cells, possibly in conjunction with LncRNA-p21. NC, negative control; HJD, Huashi Jiedu decoction; EMT, epithelial-mesenchymal transition; gastric cancer.**
G1/S-phase when compared to the control and EMT groups, suggesting that HJD interferes with the normal cell cycle of the cell, see in Figure 2I.

The present study aimed to investigate the inhibitory effect of HJD on the proliferation of gastric cancer cells and explore its potential role in cell cycle regulation and metastasis. Gastric cancer cells were treated with increasing concentrations of HJD (0, 0.25, 0.5, 1, 2, 4, 6, 8, 10 mg/mL) for 48 hours (P < 0.05; Figure 2A, 2B and 2I). The results showed a concentration-dependent suppression of cell proliferation, as evidenced by the positively correlated inhibitory effect of HJD on gastric cancer cell growth. The IC50 values for HJD ranged from 2.472 to 3.478 mg/mL in MGC-803 cells and from 2.944 to 3.929 mg/mL in BGC-823 cells (Figure 2C-2F). Based on these findings, subsequent experiments were conducted using a concentration of 3 mg/mL for MGC-803 cells and 3.5 mg/mL for BGC-823 cells. Furthermore, the expression of LncRNA-p21 and Si-NC in both cell lines was successfully established and confirmed using Western blot analysis. Additionally, the cytotoxicity and cell cycle distribution of HJD was assessed in three groups (NC, si-NC, and LncRNA-p21), as illustrated in Figure 2G and Figure 2I. Notably, the knockdown of LncRNA-p21 resulted in a significant decrease in CDK4 expression (P < 0.05), accompanied by an increase in Cyclin D1 expression (P < 0.05, Figure 2H, 2J). Building upon these results, further investigation is warranted to explore the potential of LncRNA-p21 in inhibiting gastric cancer cell invasion and metastasis.

**HJD reduces the migration potential of gastric cancer cells before cell transfection in vitro**

Cells from the control, EMT, and HJD groups were subjected to wound healing assays. The EMT model is built as described in the methods. The HJD group then administered 3–3.5 mg/mL HJD intervention for an additional 48 h. Compared to the control group, the migration distance of the HJD group was significantly greater (P < 0.05; Figure 3A, 3B). Similarly, trans well assays produced comparable results (P < 0.05; Figure 3C), and WB also verify the same pattern. In the previous cytotoxicity experiment, cell plateau appeared 48 h after slab laying,

**Figure 3** Huashi Jiedu decoction could inhibit TGF-β1-stimulated EMT process, cell invasion, and metastasis in gastric cancer (magnification: ×200, bar = 200 μm). Cells from three groups (control, EMT, and HJD) were subjected to wound healing assays (A, B). Trans well migration and invasion tests were conducted on the aforementioned groups (C). The expression of E-cad, N-cad, Vimentin, ZEB1 was determined using Western blot analysis. The groups consisted of (a) MGC-803 NC; (b) MGC-803 NC-EMT 24 h; (c) MGC-803 NC-EMT 48 h; (d) BGC-823 NC; (e) BGC-823 NC-EMT 24 h; and (f) BGC-823 NC-EMT 48 h. Each experiment was conducted in triplicate. Data are presented as the mean ± SEM (*P < 0.05). EMT, epithelial-mesenchymal transition; HJD, Huashi Jiedu decoction. EMT, epithelial-mesenchymal transition; HJD, Huashi Jiedu.

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but in the actual operation, cell migration distance became to show a difference 24 h after slay laying, therefore we extracted cells 24 h and 48 h to analyze the relationship between cell migration and EMT-related proteins. It was found that the difference in intervention proteins was also more significant at 24 h (P < 0.05; Figure 3D–3F). E-cad was significantly up-regulated in the HJD group relative to the control group (P = 0.05), while N-cad, Vimentin, Slug, and ZEB1 were all down-regulated (P < 0.05). However, there were no significant differences in the Twist (P > 0.05). The results suggest that HJD may not only inhibit the EMT process and the capacity of gastric cancer to invade and metastasize distant regions but also affect protein transcription from the G1 phase to the S phase by modulating proteins such as Cyclin D1 and CDK4. Thus, these findings indicate that HJD can block cell cycle-related proteins and reverse the EMT status of gastric cancer cells, possibly in conjunction with LncRNA-p21.

HJD-induced migration potential of gastric cancer cells after EMT or transfection in vitro

Wound healing and transwell results were repeated in these groups as NC, NC EMT, si-NC, si-NC EMT, LncRNA-p21, and LncRNA-p21 EMT, with HJD treated in each group. As shown in Figure 4A, 4B, the HJD group’s migration distance was substantially less than that of the control group. In Figure 4E–4G, the protein gene expression for E-cadherin was markedly up-regulated in si-NC-HJD and LncRNA-p21 HJD cells compared to NC-control cells, when N-cad expression was noteworthy down-regulated (P < 0.05), indicating reversal of EMT status after HJD treatment in 3–3.5 mg/mL or knockdown of LncRNA-p21. However, the difference between the si-NC and si-LncRNA-p21-HJD groups was less than that between the control and NC-HJD groups (P < 0.05), indicating that LncRNA-p21 suppression diminished the ability of HJD to reverse EMT in gastric cancer cells. In addition, the deletion of LncRNA-p21 can significantly reduce the HJD-induced reversal of EMT. Therefore, it can be concluded that the activation of LncRNA-p21 is responsible for the HJD-induced reversal of EMT and cell cycle block.

HJD suppresses LncRNA-p21 to inhibit tumor growth in vivo

Figure 5A illustrates the recorded weights of rats over a span of 14 days. There was a variance in the weight of the liver between NC, NC HJD, si-NC, si-NC HJD, LncRNA-p21, and LncRNA-p21 HJD on day 14. MGC-803 (1 × 10^6 cells) was chosen to establish the model by subcutaneous injection when the BGC-823 cell didn’t reach success in nude-mice modelling. Ascites increased the incidence of liver metastasis in some NC-control mice (Figure 5A, 5B). The EMT-related protein (E-cad, N-cad, Cyclin D1, and CDK4 (P < 0.05, Figure 5C–5D) was confirmed by WB in mice tumor tissues. After HJD intervention or LncRNA-p21 silencing, increased E-cad expression and decreased N-cad expression can be detected (P < 0.05). The variance in CDK4 and Cyclin D1 expression between the si-NC, si-NC HJD, LncRNA-p21, and LncRNA-p21 HJD groups (P < 0.05). Immunohistochemical staining was used to observe the expression of E-cad, N-cad, and Vimentin in tumor tissue (P < 0.05, Figure 5E), the expression variations of these proteins in tissue are comparable to those observed in vivo. These findings suggest the fact that the EMT of gastric cancer can be substantially reversed by HJD cell cycle intervention or LncRNA-p21 reduction.

Network pharmacology explores relevant targets among gastric cancer, HJD, EMT, and LncRNA-p21

The active components and proteins of Patrinia villosa (Thunb.) Juss., Hedyotis diffusa, and Rabdosia amethystoides (Benth.) H.Hara were retrieved from the TCMSP, ETCM, and HERB databases. A total of 359 proteins were identified using the Drug bank, PubChem database, and Swiss tar prediction. Additionally, 3,720 gastric cancer-related proteins were obtained from the diagenic and OME cancer tumor databases. By intersecting these proteins with the 196 drugs and CG protein interaction targets, we obtained a set of intersecting targets. The interactions between these targets were explored using the STRING database, and the resulting protein interaction data was visualized using Cytoscape software. Through network topology analysis, 96 key targets, including members of the STAT family and Interleukin family, were identified. The obtained data were further visualized using e-software, resulting in the generation of Venn diagrams (Supplementary Figure S2, S3). In the network pharmacology analysis, we discovered a close association between the STAT protein family and the EMT process in gastric cancer. This finding helps to explain how LncRNA-p21 regulates EMT by blocking the cell cycle. The detailed relationship between these factors is discussed further below.

Discussion

The first diagnosis of gastric cancer patients is often with distant metastases, especially lymph node metastases and implant metastases, which stands for the poor five-year survival rates [21]. Tumor metastasis is associated with EMT, a developmental process tightly related to TGF-β1, which has been reported to generate pre-metastatic microenvironment and neovascularization, accelerate cell division and proliferation, inhibit transcription methylation level, leading to distant invasion and metastasis [22–24]. Numerous studies have simultaneously confirmed that TGF-β1-induced up-regulation of LncRNA can promote the invasion and migration of gastric cancer cells through the activation of cell cycle enzymes and the promotion of cell proliferation [25, 26].

In this study, we observed that TGF-β1 can induce upregulation of LncRNA-p21, which in turn regulates the expression of Cyclin D1 and CDK, promoting cell DNA transcription, leading to cell entry into the G1/S cell cycle and accelerated replication. This process stimulates cell proliferation, and angiogenesis, and ultimately enhances the distant infiltration and metastatic capacity of gastric cancer (GC), establishing an EMT model for gastric cancer. However, our experiments using Western Blot analysis demonstrated that the expression of E-cadherin and other proteins did not show significant changes during this process, indicating that LncRNA-p21 knockdown alone does not reduce the TGF-β1-induced EMT process. Therefore, our next research focus will be on investigating whether the aberrantly upregulated LncRNA-p21 can be modulated to reverse the TGF-β1-induced EMT process.

It has been established that LncRNA-p21, functioning as a positive modulator of DNA gene transcription, exhibits enrichment and expression in gastric carcinoma, prostate tumors, ovarian cancer, head and neck carcinoma, and renal carcinoma [13, 18, 27, 28, 29]. Notably, its presence enhances the Warburg effect and sensitizes chemotherapy drugs, such as cisplatin [30]. These findings align with previous investigations into network pharmacological targets and enriched pathways related to the cell cycle, cell proliferation, and apoptosis.

In our preliminary clinical application, HJD could improve the quality of life of gastric cancer patients with distant metastasis and results remain to be published. In our preliminary clinical application, HJD could improve the quality of life of gastric cancer patients with distant metastasis and results remain to be published. Authors argue that the pathogenesis of gastric cancer in TCM is that cancer toxin and dampness become tangible evil, the balance of yin and yang (TCM thought Yin and Yang are the summarization of attributes of two opposite aspects of interrelated things or phenomena in nature.) in the human body depends on the proper transmission and distribution of essence and qi in the acquired body, and the regulation of qi and blood (TCM thought qi and blood were basic substances to constitute the body and maintain lifeactivities in human.). When qi, blood and Fluid transmission is blocked, which stays in the body and becomes accumulation, blocking the qi flow then the spleen and stomach fail to pass and descend, and finally, the fluids are not distributed, turning into dampness (TCM thought dampness is a disease caused by exogenous pathogenic factor, which mostly related to seasonal climate, living environment, and changes in the body's immunity), so as to brew dampness and poison into a disease. As a commonly employed herbal combination, HJD comprises Patrinia villosa (Thunb.)
Figure 4 Huashi Jiedu decoction could inhibit TGF-β1-stimulated EMT process, cell invasion, and metastasis in gastric cancer, particularly when LncRNA-p21 was knockdown (magnification: ×200, bar = 200 μm). Wound healing assays were performed in six groups in every cell line (A, B). Groups were (a) MGC-803 NC, (b) MGC-803 SiNC, (c) MGC-803 LncRNA-p21, (d) BGC-823 NC, (e) BGC-823 SiNC, (f) BGC-823 LncRNA-p21. Transwell migration and invasion assays were performed in groups listed above (C, D, F). The expression of E-cad, N-cad, CDK4, and Cyclin D1 was determined using Western blot analysis. Each experiment was conducted in triplicate. Data are presented as the mean ± SEM (\(* P < 0.05\)). NC, negative control.
LncRNA-p21 is a tumor promoter in gastric metastatic cancer in nude mice (magnification: ×200, bar = 200 μm). LncRNA-p21 is a tumor promoter in gastric metastatic cancer nude mice (A) while LncRNA-p21 modelling mice have lost more weight and got more liver metastasis (B). The expression of E-cad, N-cad, CDK4 was revealed using western blot (C, D) and immunohistochemical staining (E). Each experiment was conducted in triplicate. Data are presented as the mean ± SEM (⁎⁎⁎ P < 0.05).

Juss, Hedyotis diffusa, and Rabdosia amethystoides (Benth.) H. Hara, each exhibiting specific anti-tumor activities [31, 32]. The principal active ingredients encompass rutin, teniposide, chlorogenic acid, and aspergillus acid. Rutin not only modifies the energy metabolism of gastric cancer and promotes glycolysis through regulation of the PKM2/HIF-1α pathway, but it also activates targets within the p38/Caspase and BCL-2 family proteins, thereby enhancing gastric cancer sensitivity to oxaliplatin and 5-Fu [33–40]. Geniposidic, asperulosidic, and chlorogenic acids are recognized inhibitors of gastric cancer cell proliferation, with geniposide potentially possessing anti-inflammatory and antibacterial properties against H. pylori [41, 42]. Additionally, Chlorogenic acids exert an anti-inflammatory influence on the tumor microenvironment by scavenging oxygen-free radicals and promoting apoptosis [43, 44].

Upon careful consideration, the experimental findings and analysis convincingly validate our hypothesis, demonstrating that HJD effectively counteracts the promoting influence of up-regulated LncRNA-p21 on the TGF-β1-induced EMT process in gastric cancer cells, ultimately inhibiting distant metastasis in gastric cancer. Nevertheless, it is important to acknowledge certain limitations inherent in our experiments. For instance, we currently lack knowledge regarding the primary monomer present in the TCM compound under investigation. Furthermore, it is worth noting that our attempts to establish a successful model by knocking-down LncRNA-p21 in BGC-823 cells encountered some setbacks.

Conclusion

In summary, we have elucidated HJD regulatory mechanisms that prevent the progression of gastric cancer. LncRNA-p21 may block the cell cycle, reverse EMT, and inhibit the growth and dissemination of gastric cancer in HJD.
References


