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Original Research Article

Amygdalin suppresses the proliferation, migration and EMT of gastric cancer cells by inhibiting TGF-β/Smad signal pathway

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Abstract

Purpose: *To investigate the effects of amygdalin on the proliferation, motility and epithelial mesenchymal transformation (EMT) of gastric cancer cells and to elucidate the operating mechanisms of action.*
Methods: *Gastric cancer AGS cells were treated with amygdalin (2.5, 5 and 10 mg/L). MTT and colony formation assays were used to investigate the effect of amygdalin on gastric cancer cell proliferation, while wound healing and Transwell assays were also carried out to determine its effect on the motility of gastric cancer cells. Immunoblot assays were used to investigate the effects of amygdalin on epithelial mesenchymal transformation (EMT) process and TGF-β/Smad pathway in gastric cancer cells.*
Results: *Treatment with amygdalin suppressed the proliferation of gastric cancer AGS cells ($p < 0.05$). Amygdalin suppressed the migration and invasion of AGS cells in vitro ($p < 0.05$). Additionally, amygdalin suppressed epithelial mesenchymal transformation (EMT) in AGS cells, and suppressed TGF-β/Smad pathway ($p < 0.05$), thereby suppressing growth, motility, and EMT in AGS cells.*
Conclusion: *Amygdalin may be useful for the treatment of gastric cancer; however, further studies are required ascertain this.*

Keywords: *Gastric cancer, Amygdalin, Proliferation, Epithelial mesenchymal transformation (EMT), TGF-β/Smad pathway*

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INTRODUCTION

Malignant tumors are the second leading causes of death in humans, 60-70 % of which occur in the digestive system [1]. Gastric cancer is still a major cause of death from malignant diseases. Early onset of gastric cancer is recessive and usually has no specific clinical symptoms in the digestive tract, which makes early diagnosis difficult. Therefore, the mortality and morbidity of gastric cancers are high. Surgical resection is still the main treatment strategy for the management of gastric cancer [2]. During the postoperative period, other combined treatment measures,

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such as adjuvant chemotherapy, may be used [3]. However, currently available treatments do not produce satisfactory outcomes. Therefore, new and more effective natural anticancer drugs need to be explored. Amygdalin is one of the main bioactive components of bitter almond in China [4]. In the human body, Amygdalin is hydrolyzed into benzaldehyde, glucose and cyanide which are considered to be the active components of amygdalin [5]. It also has many pharmacological effects, including antioxidant, antibacterial, anti-inflammatory and immunomodulatory effects [6]. It has been reported that amygdalin regulates the adhesion and migration of prostate cancer cells *in vitro* by downregulating α6 integrin [7]. Another study showed that amygdalin inhibited the expressions of CDK1 and Cyclin B, thereby inhibiting the progression of renal cancer cell cycle and the growth of tumor cells [8]. However, few studies on the anti-tumor effects of amygdalin were revealed in gastric cancer.

Epithelial mesenchymal transformation (EMT) is a specific cellular process by which tumor cells lose their epithelial features and acquire mesenchymal markers. Previous studies indicated that amygdala alleviated streptozotocin-induced excessive oxidative stress, inflammation, and renal fibrosis in diabetic rats by inhibiting the TGF-β/ Smad pathway and mediating ECM degradation [9].

EXPERIMENTAL

Antibodies

The antibodies used in this study include the following: E-cadherin antibody (1:1000 dilution, ab181296, Abcam, Cambridge, UK), N-cadherin antibody (1:1000 dilution, ab245117, Abcam, Cambridge, UK), vimentin (VIM) antibody (1:500 dilution, ab92547, Abcam, Cambridge, UK), Snail antibody (1:1000 dilution, ab216347, Abcam, Cambridge, UK) and TGF-β1 antibody (1:1000 dilution, ab215715, Abcam, Cambridge, UK). Others are Smad2 antibody (1:1000 dilution, ab40855, Abcam, Cambridge, UK), Smad3 antibody (1:1000 dilution, ab40854, Abcam, Cambridge, UK), p-Smad2 antibody (1:500 dilution, ab280888, Abcam, Cambridge, UK), p-Smad3 antibody (1:500 dilution, ab52903, Abcam, Cambridge, UK), and GAPDH antibody (1:2000 dilution, ab8245, Abcam, Cambridge, UK).

Cell culture

Human gastric adenocarcinoma AGS cells were obtained from ATCC (USA) and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10 % fetal bovine serum (FBS), 100 U/mL penicillin (Sigma, USA) and 100 mg/mL streptomycin (MedChem, USA). The cells were cultured in 95 % air and 5 % CO_2 at 37 ℃. Human gastric adenocarcinoma AGS cells were treated with amygdalin 2.5, 5 and 10 mg/L, Sigma) for 24 h.

Immunoblot

Protein samples were isolated using 8 % SDS-PAGE, sequentially transferred onto the PVDF membranes for 2 h, blocking with 5 % fat-free milk in TBST buffer for 2 h at room temperature. Polyvinylidene fluoride (PVDF) membranes were subsequently cultured with primary antibodies for 1 h at room temperature. Subsequently the membranes were incubated with HRP-conjugated secondary antibodies for another 1 h. Specific proteins were visualized with enhanced chemiluminescence detection reagent (Pierce Biotechnology).

Cell viability assay

Cell viability assay was conducted with MTT kit (Bimake, USA). Cells were maintained in 96-well plates. 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide (MTT) solution was put into the each well for 72 h and the cells were incubated for 4 h. Then, the stained cells were isolated with 150 μL DMSO. The absorbance of each well was read at 490 nm.

Colony formation assays

A number of 1000 AGS cells, after treatment with amygdalin, were seeded into 6-well plates. After maintaining for 14 days, the cells were fixed with 4 % paraformaldehyde (PFA) for 25 min and stained with 0.1 % crystal violet buffer for 20 min. The colony numbers were then counted.

Wound healing assay

The cells were maintained for 48 h and a mechanical wound was made by a 10 uL-pipette, subsequently washed with PBS, and then the serum-free culture medium was added to induce healing. Photographs were taken at 0 and 24 h time point, and the width was measured.

Transwell assay

Cells were re-suspended in serum-free medium. The upper chamber contained 20 % matrigel and the cells were incubated at 37 ˚C for 30 min. Cells were then plated into the upper chambers to induce migration towards the lower chamber

containing complete medium. After 24 h, cells in the upper chambers were removed, and the remaining cells were fixed in 4 % paraformaldehyde (PFA) for 30 min and stained with 0.1 % crystal violet. Invasive cell number was counted.

Statistical analysis

Data are presented as mean ± standard deviation (SD). GraphPad Prism 6 software was used for data analysis. $P < 0.05$ indicates a statistically significant difference.

RESULTS

Amygdalin suppressed the proliferation of gastric cancer cells

Amygdalin treatment suppressed the proliferation of AGS cells in a concentration-dependent manner (Figure 1 A). Similarly, colony formation assays also indicated that Amygdalin treatment suppressed the colony formation of AGS cells, with the decreased colony numbers (Figure 1 B and C). Amygdalin treatment therefore suppressed the proliferation of gastric cancer cells.

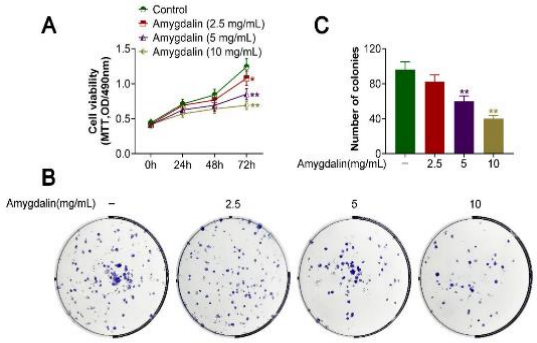

Figure 1: Amygdalin suppressed the proliferation of gastric cancer cells. (A). MTT assays showing the OD value at 490 nm wavelength of AGS cells after the treatment of amygdalin at the indicated concentration for 24, 48, and 72 h. (B and C). Colony formation assays showing the proliferation capacity of AGS cells treated with amygdalin at the indicated concentration, and the colony number was counted. Results are presented as mean ± SD; *$p < 0.05$, **$p < 0.01$

Amygdalin treatment suppressed the motility of gastric cancer cells

Performing wound healing assays, amygdalin treatment suppressed the migration of AGS cells, with increased relative wound width (Figure 2 A). Similarly, transwell assays showed that amygdalin treatment suppressed the invasion of AGS cells in a dose-dependent manner, with decreased invasive cell numbers (Figure 2 B). Therefore, Amygdalin treatment inhibited the motility of gastric cancer cells.

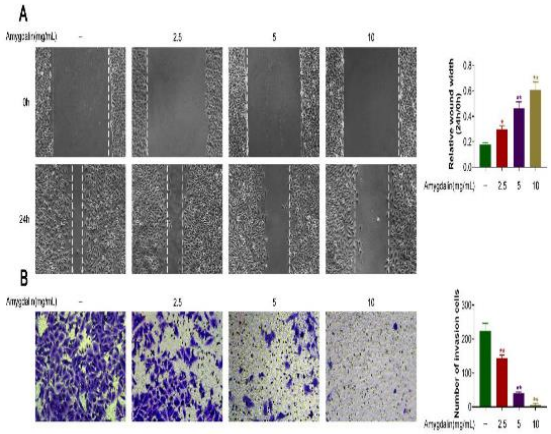

Figure 2: Amygdalin treatment restrained the motility of gastric cancer cells. (A). Wound healing assays showing the migration capacity of AGS cells after the treatment of amygdalin at the indicated concentrations, and the relative wound widths were measured. (B). Transwell assays showing the invasion capacity of AGS cells after the treatment of amygdalin at the indicated concentrations, and the invasive cell number was quantified. Results are presented as mean ± SD, *$p < 0.05$, **$p < 0.01$

Amygdalin affected the EMT process of gastric cancer cells

The expression of E-cadherin, a EMT marker, was significantly increased after the treatment of amygdalin in AGS cells (Figure 3). However, other 3 EMT markers, i.e., N-cadherin, vimentin and Snail, were significantly down-regulated after amygdalin treatment in AGS cells (Figure 3). These findings confirm that amygdalin suppressed the EMT process of gastric cancer cells.

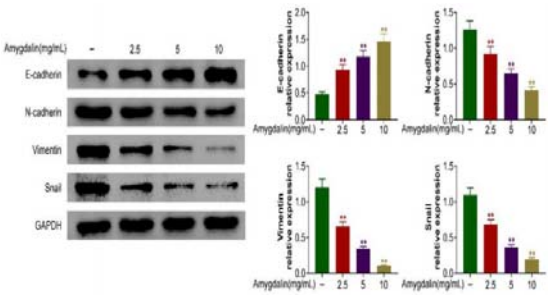

Figure 3: Amygdalin affected the EMT process of gastric cancer cells. Immunoblot assays showing the expression levels of E-cadherin, N-cadherin, Vimentin, and Snail in AGS cells upon treatment in amygdalin at the indicated concentrations. Results are presented as mean ± SD, **$p < 0.01$

Amygdalin suppressed the TGF-β/Smad signaling pathway in gastric cancer cells

Amygdalin treatment suppressed the expression of TGF-β1 in AGS cells (Figure 4). Smad2 and Smad3 are two key proteins of TGF-β/Smad pathway, and amygdalin treatment also suppressed the phosphorylation levels of Smad2 and Smad3 in AGS cells (Figure 4). Therefore, amygdalin suppressed this pathway in gastric cancer cells.

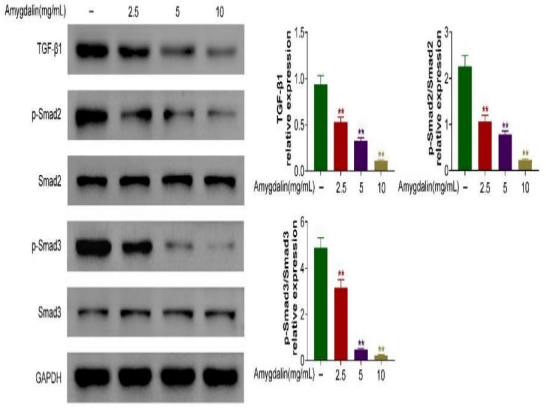

Figure 4: Amygdalin suppressed the TGF-β/Smad signaling pathway in gastric cancer cells. Immunoblot assays showing the expression levels of TGF-β1, Smad2, Smad3, and the phosphorylation levels of Smad2 and Smad3 in AGS cells upon the treatment of amygdalin at the indicated concentration. Results are presented as mean ± SD, $^{**}p < 0.01$

DISCUSSION

Gastric cancer is thought to be the most common tumor of the digestive tract in China. Therefore, there is an urgent need to develop effective therapeutic methods and drugs for gastric cancer[10]. Recently, gastric cancer was discovered to now affect younger people [11]. In the early stage of gastric cancer, there are no obvious symptoms. For advanced gastric cancer, traditional surgical resection and chemoradiotherapy have little effect, and so there is still the need to develop effective treatment drugs [12]. Amygdalin was found to possess huge potentials for the treatment of gastric cancer [13]. The data confirmed that amygdalin suppressed the proliferation, motility, as well as EMT in gastric cancer cells, and therefore restrained cancer progression.

Amygdalin is hydrolyzed in the human body to benzaldehyde, glucose and cyanide, which are thought to be the bioactive components of amygdalin [4]. The biological activities of amygdalin have been widely reported, and they include anti-oxidant, anti-bacterial, anti-inflammatory and immunomodulatory effects [14]. Amygdalin suppresses the progression of multiple types of cancers such as prostate cancer and renal cancer. It mediates the adhesion and migration of prostate cancer cells by suppressing α6 integrin.

Amygdalin also decreases the adhesion and migration of breast cancer cells, and inhibits the expression of CDK1 and Cyclin B, thereby restraining the progression of renal carcinoma by affecting the cell cycle [15]. Furthermore, amygdalin promotes T cell activity, in order to suppress the progression of hepatocellular carcinoma via the JAK2/STAT3 pathway, and the effects of amygdalin on TGF-β/Smad pathway were found in gastric cancer cells, suggesting the different mechanisms of Amygdalin on different tumors.

In this study, the effects of amygdalin on EMT of gastric cancer was revealed. Epithelial-mesenchymal transition (EMT) is a biological process that causes tumor cells to become more migratory and aggressive. The EMT process is controlled by transcription factors and several signaling pathways [16]. For example, SALL4 activated the TGF-β/ Smad pathway to induce EMT and promoted the metastasis of gastric cancer. Recent studies have also revealed that amygdalin induced excessive oxidative stress and inflammation in rats by inhibiting the TGF-β1/Smad pathway [17]. Amygdalin affected the process of EMT in gastric cancer cells.

Transforming growth factor (TGF)-β/ Smad pathway affected multiple downstream cellular processes such as proliferation, motility, and apoptosis, contributing to cancer development. Multiple proteins promoted the progression of gastric cancer via the TGF-β/Smad pathway. For example, fibroblastic galectin-1-fostered metastasis was mediated by TGF-β1-induced EMT in gastric cancer [18]. Another study indicated that TOPK promoted EMT and invasion of breast cancer cells via the TGF-β1/Smad pathway. In this study, amygdalin suppressed the proliferation, motility and EMT of gastric cancer cells via this pathway.

CONCLUSION

Amygdalin suppresses the proliferation, motility and EMT process of gastric cancer cells by inhibiting TGF-β/Smad pathway. Thus, it can potentially be developed for the management of gastric cancer.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this study.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Junwei Xu and Wenbo Jiang designed the study and supervised the data collection; Junwei Xu analyzed and interpreted the data; Ning Qin and Tao Chen prepared the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the manuscript.

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