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Original Article

Inhibitory effects of curcumin on epithelial-mesenchymal transition in human gastric cancer cells and the possible mechanism

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Abstract: *Background:* Curcumin is the main active ingredient in *Curcuma longa L.*, and has a wide range of medicinal values. Tumor cells can obtain the interstitial cell phenotype and invasiveness through epithelial-mesenchymal transition (EMT), thus invading the surrounding tissues and promoting the transfer process. This study aimed to investigate the inhibitory effects of curcumin on EMT of gastric cancer cells and explore the possible mechanism. *Methods:* Human gastric cancer SGC7901 cells were divided into control, model and treatment group. In model and treatment groups, the EMT model of SGC7901 cells was established by treating with 10 ng/ml transforming growth factor-β1 (TGF-β1). In addition, the treatment groups were added with curcumin, with final concentration of 10, 20 and 30 μmol/L, respectively. After treatment, the migration ability of cells was detected by in vitro scratch assay. The invasion ability of cells was detected by transwell chamber assay. The expressions of EMT-related marker proteins and Notch signaling pathway proteins were determined by Western blot analysis. *Results:* Themigration and invasion ability of SGC7901 cells in treatment groups were obviously decreased compared with model group. After 72 h of treatment, the relative expression level of E-cadherin protein in treatment group was significantly higher than model group ($P < 0.05$); N-cadherin and vimentin protein expression levels in treatment group were significantly lower than model group, respectively ($P < 0.05$); Notch1 and Hes-1 protein expression levels in treatment group were significantly lower than model group ($P < 0.05$). *Conclusions:* Curcumin can inhibit the EMT of SGC7901 cells induced by TGF-β1. The possible mechanism may be related to its down-regulation of protein expression in Notch signaling pathway.

Keywords: Curcumin, epithelial-mesenchymal transition, gastric cancer, Notch signaling pathway

Introduction

Curcuma longa L. is a perennial herb cultured in Asia, which contents many active components [1]. Curcumin is a phenolic pigment which is separated from the tuber of *Curcuma longa L.*. It is the main active ingredient in *Curcuma longa L.*, and has a wide range of medicinal values. Previous studies find that, curcumin has the anti-inflammatory [2], anticoagulant [3] and anti-atherosclerosis [4] activity. In addition, curcumin has the anticancer functions [5]. Gastric cancer is one of the most common malignant tumors, and its mortality is very high in the malignant tumors [6]. As gastric cancer is often in the middle and late stages when onset, the surgery and chemotherapy are the main treatment methods, but the curative effect is not satisfactory [7]. Metastasis is one of the most important characteristic of advanced gastric cancer. It is an important cause of death in patients with gastric cancer, and is also the biggest obstacle to the treatment of gastric cancer [8]. It is found that, tumor cells can obtain the interstitial cell phenotype and invasiveness through epithelial-mesenchymal transition (EMT), thus invading the surrounding tissues, thereby promoting the transfer process [9]. Notch signaling pathway is a signal transduction system which has conservatism in evolution. The Notch receptor interacts with the ligand, thus transducing the signal. Notch signaling pathway plays an important role in cell proliferation, differentiation and apoptosis [10]. Recent studies have shown that, the abnormal activation of Notch signaling pathway is com-

mon in a variety of malignant tumors, such as breast cancer [11], colorectal cancer [12], renal cell carcinoma [13], etc.. This study investigated the inhibitory effects of curcumin on EMT of human gastric cancer SGC7901 cells induced by transforming growth factor-β1 (TGF-β1) and explored the possible mechanism. The objective was to provide the theoretical and experimental basis for the clinical application of curcumin to treatment of gastric cancer.

Materials and methods

Cell culture

Human gastric cancer SGC7901 cells (Shanghai Cell Bank, Chinese Academy of Sciences, Shanghai, China) were cultured in RPMI1640 medium containing 1% penicillin, 1% glutamine and 10% fetal bovine serum (FBS) in incubator under condition of 5% CO_2 and 37°C. When the fusion reached 80%, the cells were digested with 0.02% EDTA, followed by passage.

Cell grouping and treatment

SGC7901 cells were seeded in 96-well plates, with concentration of 1×10^6 cells/well. After culture (5% CO_2, 37°C) for 24 h, the cells were divided into control, model and 3 treatment groups, 18 wells in each group. The model and treatment groups were added with TGF-β1, with final concentration of 10 ng/ml. In addition, the 3 treatment groups were added with curcumin, with final concentration of 10, 20 and 30 μmol/L, respectively. The control group did not receive further treatment. The cells in 5 groups were cultured (5% CO_2, 37°C) for 24, 48 and 72 h, 6 wells for each time point in each group.

Determination of cell migration ability

The migration ability of SGC7901 cells was determined by in vitro scratch assay. After treatment of 24, 48 and 72 h, the scratch was made on the plate using the head of 10 μl Eppendorf pipette, and the scratch wound model was established. The scratched cells were washed away using PBS, and the serum-free RPMI1640 medium was added, followed by culture (5% CO_2, 37°C) for 24 h. The plate was photoed under the microscope. Five points were randomly selected in each wound area, and the scratch space was measured, and analyzed using Image ProPlus 5.0 analysis software.

Determination of cell invasion ability

The invasion ability of SGC7901 cells was determined by Transwell chamber assay. After treatment of 24, 48 and 72 h, the FBS-free cell suspension with concentration of 1×10^6 cells/ml was prepared. 100 μl of cell suspension was added to the Transwell chamber. The bottom of Transwell chamber was immersed in RPMI1640 medium containing 20% FBS, followed by culture (5% CO_2, 37°C) for 24 h. The Transwell chamber was taken out. The filter membrane was fixed with methanol for 1 min, and the superfluous methanol was swabed with cotton. The Giemsa staining was performed. The filter membrane was observed under microscope. Five fields of vision in each filter membrane were selected to calculate the number of penetrating cells, which presented the migration ability of cells.

Determination of EMT-related marker protein expression

SGC7901 cells were treated with 30 μmol/L curcumin for 72 h. The expressions of EMT-related marker proteins including E-cadherin, N-cadherin and Vimentin in cells were determined by Western blot analysis. After treatment, the cells were collected, followed by washing with PBS for two times. The total protein of cells was extracted using RIPA lysis buffer. The concentration of protein was measured, followed by SDS-PAGE electrophoresis. After trarsmembrane and blocking, the primary antibody (anti-E-cadherin, anti-N-cadherin, anti-Vimentin) was added, followed by incubation for overnight at 4°C. After washing using TBST solution for 3 times, the enzyme labeled second antibody (IgG) was added, followed by incubation for 1 h at 4°C. The membrane was washed using TBST solution, followed by coloration. β-actin was used as the internal reference. The relative expression level of target protein was presented by the ratio of integral optical density of target protein to β-actin. The primary and second antibodies were provided by Fuzhou Maixin Biotechnology Development Co., Ltd. (Fuzhou, China).

Determination of Notch1 and Hes-1 protein expression

SGC7901 cells were treated with 30 μmol/L curcumin for 72 h. The expressions of Notch1

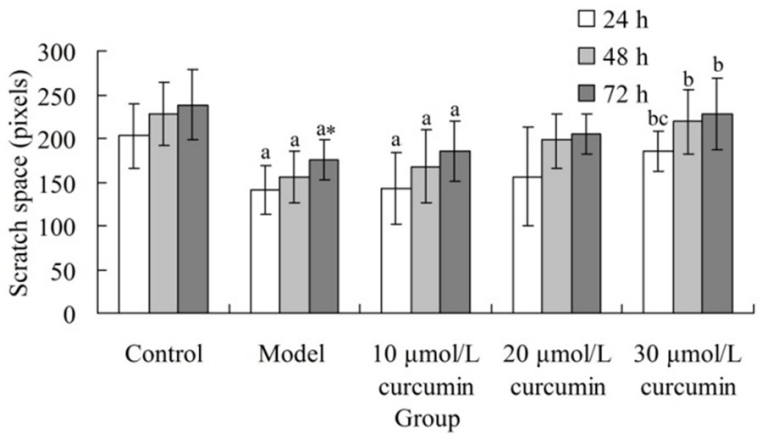

Figure 1. Comparison of SGC7901 cell migration ability among five groups. [a]P < 0.05 compared with control group; [b]P < 0.05 compared with model group; [c]P < 0.05 compared with 10 μmol/L curcumin group; [d]P < 0.05 compared with 20 μmol/L curcumin group; *P < 0.05 compared with 24 h time point.

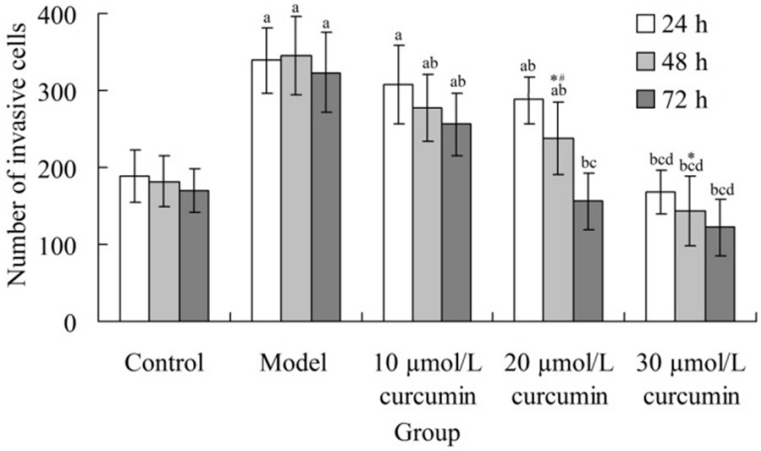

Figure 2. Comparison of SGC7901 cell invasion ability among five groups. [a]P < 0.05 compared with control group; [b]P < 0.05 compared with model group; [c]P < 0.05 compared with 10 μmol/L curcumin group; [d]P < 0.05 compared with 20 μmol/L curcumin group; *P < 0.05 compared with 24 h time point; #P < 0.05 compared with 48 h time point.

and Hes-1 protein in cells were determined by Western blot analysis. The operation process was the same with determination of EMT-related marker protein expression. The relative expression levels of Notch1 and Hes-1 protein were presented by the ratio of integral optical density to β-actin. The primary and second antibodies were provided by Fuzhou Maixin Biotechnology Development Co., Ltd. (Fuzhou, China).

Statistical analysis

All statistical analysis was carried out using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). Each measurement was repeated for three times. Data were presented as mean ± SD, and were compared using t test. P < 0.05 was considered as statistically significant.

Results

Curcumin decreasing the migration ability of SGC7901 cells induced by TGF-β1

The in vitro scratch assay showed that, after treatment, the scratch space of cell migration in model group was significantly lower than that in control group at 24, 48 and 72 h time point, respectively (P < 0.05). This indicated that, after treatment with TGF-β1, the migration ability of SGC-7901 cells was obviously increased. The scratch space in 30 μmol/ml curcumin group was significantly higher than that in model group at each time point, respectively (P < 0.05), with no significant difference from control group (P > 0.05). This indicated that, curcumin could obviously decrease the migration ability of SGC7901 cells induced by TGF-β1 (**Figure 1**).

Curcumin decreasing the invasion ability of SGC7901 cells induced by TGF-β1

Transwell chamber invasion assay showed that, after 24, 48 and 72 h of treatment, the number of invasive cells in model group was significantly higher than that in control group, respectively (P < 0.05). This indicated that, TGF-β1 could increase the invasion ability of SGC7901 cells. The number of invasive cells in 10 μmol/L curcumin group at 48 and 72 h, and 20 and 30 μmol/L curcumin groups at 24, 48 and 72 h was significantly lower than that in model group, respectively (P < 0.05). The number of invasive cells in 30 μmol/L curcumin groups at each time point had no significant difference with control group (P > 0.05). This indicated that,

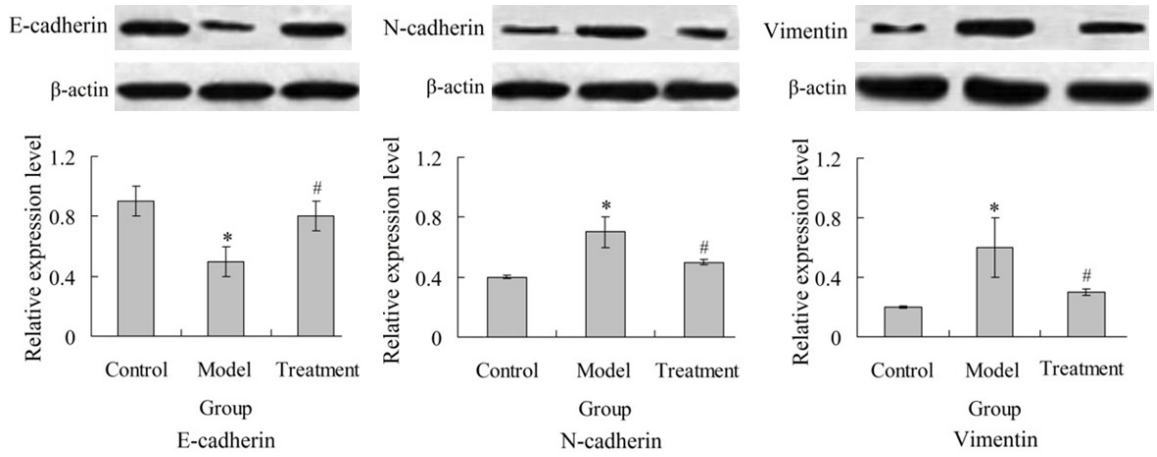

Figure 3. Comparison of E-cadherin, N-cadherin and vimentin protein expression in SGC7901 cells among three groups. *P < 0.05 compared with control group; #P < 0.05 compared with model group.

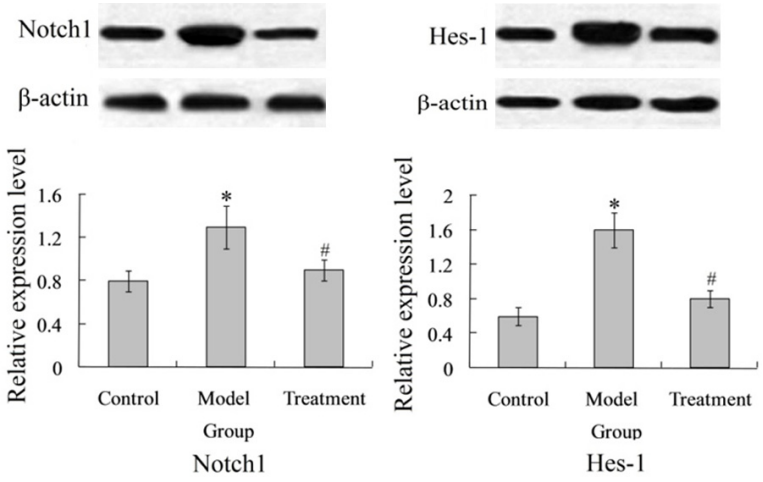

Figure 4. Comparison of Notch1 and Hes-1 protein expression in SGC7901 cells among three groups. *P < 0.05 compared with control group; #P < 0.05 compared with model group.

curcumin could obviously decrease the invasion ability of SGC7901 cells induced by TGF-β1 (**Figure 2**).

Curcumin regulating EMT-related marker protein expression in SGC7901 cells

After 72 h of treatment, the relative expression level of E-cadherin in model group was significantly lower than that in control group (P < 0.05), and that in treatment group was significantly higher than model group (P < 0.05). The N-cadherin and vimentin expression levels in model group were significantly higher than those in control group, respectively (P < 0.05), and those in treatment group were significantly lower than model group, respectively (P < 0.05). There was no significant difference of each index between control and treatment groups (P > 0.05) (**Figure 3**).

Curcumin regulating Notch1 and Hes-1 protein expressions in SGC7901 cells

As shown in **Figure 4**, after 72 h of treatment, the relative expression levels of Notch1 and Hes-1 protein in model group were significantly higher than that in control group, respectively (P < 0.05), and those in treatment group were significantly lower than model group, respectively (P < 0.05). There was no significant difference of Notch1 or Hes-1 protein level between control and treatment groups (P > 0.05).

Discussion

The anti-tumor effects of *Curcuma longa L.* and curcumin were firstly proposed by Indian scholar Kuttan et al in 1985 [14]. After that, other researchers have found that curcumin can reduce the weight of skin tumors in mice. A large number of pharmacological studies show that, curcumin has the spectral anticancer effect. It can inhibit the development of many kinds of tumor, such as gastric cancer, liver

cancer, nasopharyngeal carcinoma, lung cancer, colon cancer, leukemia, prostate cancer and melanoma, etc. [15]. Kuttan et al [14] have reported that, curcumin can inhibit the development of lymphoma in mouse ascites. Sindhwani et al [16] have found that, the rate of tumor formation of bladder cancer is significantly decreased after treatment with curcumin. Menon et al [15] have confirmed that, curcumin can inhibit the lung metastasis of bearing melanoma in mice. Azuine et al [17] have found that, the benzopyrene-induced tumor can be inhibited by curcumin. Ikezaki et al [18] have also confirmed the inhibitory effect of curcumin on colonic adenocarcinoma in mice.

EMT is an important step in the process of malignant tumor metastasis. Research shows that, the loss of the epithelial phenotype and obtaining of interstitial status are related to the poor differentiation of gastric cancer. EMT can reduce the adhesion ability of tumor cells and enhance the migration ability, playing an important role in the invasion and metastasis of gastric cancer [9]. It is shown that, TGF-β1 can induce the EMT of tumor cells, and promote the tumor metastasis during the cancer development [19]. This study has established the EMT model of human gastric cancer SGC7901 cells, and applied curcumin to intervene the modeled cells. Results found that, after 24, 48 and 72 h of treatment, the in vitro scratch assay and Transwell chamber assay showed that, the scratch space in model group was significantly lower than that in control group ($P < 0.05$), and the number of invasive cells in model group was significantly higher than that in control group ($P < 0.05$). This indicates that, the EMT model of gastric cancer SGC7901 cells has been successfully obtained. The scratch space in 30 μmol/L curcumin group at each time point was significantly higher than that in model group ($P < 0.05$), and the number of invasive cells in 10 μmol/L curcumin group at 48 and 72 h, and 20 and 30 μmol/L curcumin groups at 24, 48 and 72 h was significantly lower than that in model group ($P < 0.05$). This indicates that, curcumin can inhibit the EMT of SGC7901 cells.

Malignant biological behaviors of epithelial-derived tumor including invasion, migration and tolerance to chemotherapy are associated with the acquisition of EMT phenotype [20]. The decrease or deletion of E-cadherin expression and over-expression of N-cadherin and vimentin are thought to be a marker of tumor cell EMT [21, 22]. Results of this study showed that, the E-cadherin expression level in model group was significantly lower than that in control group ($P < 0.05$), and the N-cadherin and vimentin expression levels in model group were significantly higher than those in control group, respectively ($P < 0.05$). This indicated that, the EMT of gastric cancer SGC7901 cells has been successfully induced by TGF-β1. The E-cadherin expression level in treatment group was significantly higher than model group ($P < 0.05$), and the N-cadherin and vimentin expression levels in treatment group were significantly lower than those in model group, respectively ($P < 0.05$). This further confirms that, curcumin can inhibit the TGF-β1-induced EMT of gastric cancer SGC7901 cells.

Notch signaling pathway has more extensive and versatile influence on cell growth and development. Notch gene encodes the membrane protein receptors which are composed of Notch receptor and Notch ligand and intracellular effector molecules [23]. Notchl is one of the Notch receptors. Its main function is to bind to the ligand and initiate the Notch. Notchl is one of the important signal transduction pathways involved in the EMT process. The over activation of Notch1 molecule can make the normal cells to transform into malignant cells [24]. Hes-1 is the downstream transcription factor of Notch signaling pathway. The activated Hes-1 plays a major role in the development and progression of cancer [25]. In this study, we used curcumin to interfere the TGF-β1-induced EMT of SGC7901 cells. Results found that, the Notch1 and Hes-1 protein levels in model group were significantly higher than control group ($P < 0.05$), and those in treatment group were significantly lower than model group ($P < 0.05$). This indicated that, curcumin can down-regulate the expression of Notch1 and Hes-1 protein in SGC7901 cells, thus exerting the inhibition effect on EMT of gastric cancer cells.

In conclusion, Curcumin can inhibit the EMT of SGC7901 cells induced by TGF-β1. The possible mechanism may be related to its down-regulation of Notch1 and Hes-1 protein expression in cells. This study has provided an experimental basis for the clinical application of curcumin to treatment of gastric cancer.

Disclosure of conflict of interest

None.

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