European Review for Medical and Pharmacological Sciences 2017; 21: 1013-1019

TGF-β1 promotes human gastric carcinoma SGC7901 cells invasion by inducing autophagy

J. SHEN[1], D.-S. ZHAO[2], M.-Z. LI[1]

[1]Department of General Surgery, Beijing Chao-Yang Hospital, Capital Medical Univerisity, Beijing, China
[2]Department of Cardiovascular Medicine, Aerospace 731 Hospital, Beijing, China

Abstract. – OBJECTIVE: To investigate the role of TGF-β1 in autophagy and invasion ability in human gastric carcinoma cell line SGC7901.

MATERIALS AND METHODS: Cultured SGC7901 cells were treated with different concentrations of TGF-β1 for 24 h. The protein expression levels of autophagy relative marker LC3 and Beclin1 were detected by Western blot. The effect of TGF-β1 on invasion ability of SGC7901 cells was detected with transwell method.

RESULTS: The results demonstrated that TGF-β1 was able to induced autophagy of SGC7901 cells in a dose-dependent manner. Autophagy inhibitor 3-MA could inhibit TGF-β1 upregulated autophagy. Furthermore, TGF-β1 significantly enhances the invasion ability of SGC7901 cells. However, autophagy inhibitor 3-MA could effectively reverse this process.

CONCLUSIONS: TGF-β1 enhances SGC7901 cells migration by inducing autophagy.

Key Words:
Human gastric carcinoma, Autophagy, Transforming growth factor, Invasion.

Introduction

As the 4th malignant tumor in terms of the incidence rate in the world, gastric cancer ranks the 2nd position in the incidence rate and mortality rate according to the data of cancer in China in 2015[1]. The occurrence and development of gastric cancer are a gradual progression, which involves various factors and procedures, and gastric cancer, with a high incidence rate and mortality rate, is severely threatening the health of human beings. With the progress in the research of the treatment methods of the tumor, the treatment for patients with cancer in an early stage shows certain efficacy. Nevertheless, the 5-year survival rate of patients with gastric cancer remains lower than 30% at present[2]. Also, misdiagnosis of gastric cancer in early stage frequently occurs due to its insidious onset, which, plus the susceptibility to distant metastasis in the late stage, is significant to the research on the mechanisms relating to occurrence, development, invasion and metastasis of gastric cancer for improving the prognosis of patients.

Autophagy refers to a self-digestive process of cell induced by the stress state, such as hungry, hypoxia or deficiency in energy metabolism, in which the impaired organelle and protein are degraded into the absorbable biological macromolecules, like amino acid, to nourish the cells under stress state to maintain the survival of cells[3]. However, once the cell autophagy is excessively activated and persists on a high level for a long time, the cell will be injured, or even the autophagic cell death will be induced[4]. Studies have revealed that autophagy plays a role in every stage of tumors, such as formation, development, progression, invasion and metastasis[5].

As a kind of polypeptide secreted by various cells, transforming growth factor (TGF) is characterized by multiple biological effects, in which regulatory effect on the proliferation and differentiation of cells is relatively important. In addition, it also participates in many important biological processes, such as angiogenesis, fibrosis in damage repair and the occurrence of tumors[6]. TGF-β can be divided into 4 subtypes, i.e. TGF-β1, TGF-β2, TGF-β3 and TGF-β1β2, in which the expression of TGF-β1 is the highest. In the early stage of the tumor, TGF-β can inhibit the occurrence of a tumor, but after the formation of tumor lesion, TGF-β will promote the growth and distant invasion and metastasis of tumor[7]. Besides, some investigations reported that TGF-β1 participates in the regulation of the autophagic activity in the body[8].

Corresponding Author: Minzhe Li, MD; e-mail: limingzhe0213@163.com

However, studies on the effect of TGF-β1 on the gastric cancer SGC7901 cells remain very few. In this study, we aimed to investigate the effect of TGF-β1 on the regulation of autophagic activity, proliferation, invasion and migration capability of gastric cancer SGC7901 cells to provide an experimental basis for a research into the mechanism how TGF-β1 regulates gastric cancer.

Materials and Methods

Material

Gastric cancer SGC7901 cells (Institute of Cell Biology, SIBS, CAS, Shanghai, China); TGF-β1 (R&D, USA); 3-Methyladenine (3-MA, an autophagy inhibitor, Sigma-Aldrich, St. Louis, MO, USA); rabbit-anti-human LC3B and Beclin1 polyclonal antibody (Cell Signaling Technology, Danvers, MA, USA); antibody of β-Actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA); Protein marker (Thermo Fisher Scientific, Waltham, MA, USA); Electro-Chemi-Luminescence (ECL) kit (Millipore, Beijing, China); fetal bovine serum (FBS, Gibco, Grand Island, NY, USA); Roswell Park Memorial Institute (RPMI)-1640 medium (Hyclone, USA); Radio-immunoprecipitation Assay (RIPA) and bicinchoninic acid (BCA) kit (Beyotime Biotech Institute, Beijing, China); 24-well transwell chamber (size of well: 8 μm, Corning Costar, NY, USA); Matrigel for invasion experiment (BD, Shanghai, China).

Cell Culture

Gastric cancer SGC7901 cells were cultured in the RPMI-1640 medium containing 10% deactivated FBS, 100 U/mL penicillin and 100 μg/mL gentamicin in a cell incubator (37°C, 5% CO_2 and saturated humidity). Cells were digested and passaged using 0.25% trypsin. After 70 to 80% of cultured cells had been merged, the medium was replaced by serum-free medium for starving the cells overnight followed by the treatment of TGF-β1 for 24 h. Cells were divided into groups according to the requirement of the experiment.

Western Blot Assay

After the cells were treated by TGF-β1 in varying concentrations (0 ng/mL, 5 ng/mL and 10 ng/mL) for 24 h, the cells adhering to the wall of culture flask were rinsed using pre-heated phosphate buffered saline (PBS) and transferred into the EP tubes for centrifuge at 12000 rpm for 5 min and the supernatant was discarded. Then, RIPA was added into the collected cells for lysis, and the supernatant was collected and preserved at -20°C. Samples were loaded for electrophoresis at 80V to aggregate the protein which was later isolated at 100V followed by the membrane transfer. After the membrane was blocked using 5% skimmed milk for 1 h, a rabbit-anti-human polyclonal antibody of Beclin1 was added onto the membrane for incubation at 4°C overnight, and then the membrane was washed on the decolorizing shaking table using tris buffered saline and tween 20 (TBST) for 3 times (5 min/time). Subsequently, HRP-labeled goat-anti-rabbit IgG was added for incubation at room temperature for 1h and then the membrane was washed using TBST for 3 times (5 min/time). ECL was added onto the membrane for 1 minute of reaction followed by exposure to the X-ray, fixation, and development.

Cell Migration and Invasion Experiments

Cell migration and invasion experiments: a) Cell migration experiment: After 24 h of starving culture of gastric cancer SGC7901 cells in the logarithm phase in the serum-free RPMI 1640 medium for starving, they were digested using 0.25% EDTA-trypsin. Then, a single cell suspension was prepared using the cells in the serum-free RPMI-1640 medium, during which the cell density was adjusted to 3×10^5/mL. The cell viability was higher than 95% according to the trypan blue staining test. 200 μL serum-free cell suspension, together with the drugs according to the experiment requirement, was added to the upper surface of every invasion chamber and at the same time, and 3 duplication wells were set for each dosage group. RPMI 1640 medium containing 10% FBS was added onto the lower surface of the invasion chamber, 600 μL/well, and the cells were cultured in the incubator for 24 h. The chamber was taken out and culture medium was removed after being washed for 2 times. The cells that failed to pass through the upper surface of the chamber were wiped using a wet cotton swab and the chamber was fixated using methanol for 20 min, were dried at the room temperature and further dyed using crystal violet for 20 min. Then, They were placed under the inverted microscope for counting the cells that succeeded to pass the membrane, in which cells in the central part and four corners were counted at the high magnification (400x) and the average of cell count was

calculated. b) Cell invasion experiment: Matrigel was pre-melted at 4°C and 40 μL diluted matrigel was coated on the surface of each polycarbonate millipore (matrigel: serum-free medium = 1:3) and placed into the incubator for coagulation for later use; the density of cells that were prepared for inoculation was adjusted to 2×10^5/mL. Other procedures were the same as those in migration experiment.

Statistical Analysis

All data were presented as ($\chi \pm s$). For statistical analysis, ANOVA was carried out using SPSS19.0 (SPSS Inc., Chicago, IL, USA). Tukey's HSD (honestly significant difference) test is used in conjunction with an ANOVA to find means that are significantly different from each other. $p < 0.05$ suggested that the difference was statistically significant.

Results

Autophagy of Gastric Cancer SGC7901 Cells Induced by TGF-β1

After 24h of treatment using TGF-β1 in varying concentrations (0 ng/mL, 5 ng/mL and 10 ng/mL), the Western-blot assay revealed that the mRNA expression of LC3 and Beclin1 (labeling protein of autophagy) in the gastric cancer SGC7901 cells, after being stimulated by TGF-β1, was elevated with an increase in the concentration of TGF-β1 (Figure 1).

Blocking Effect of 3-MA on Cell Autophagy Induced by TGF-β1

After 24h of treatment using TGF-β1 (10 ng/mL), the protein expression of LC3 and Beclin1 was significantly elevated in the SGC7901 cells compared to those in the control group, but when

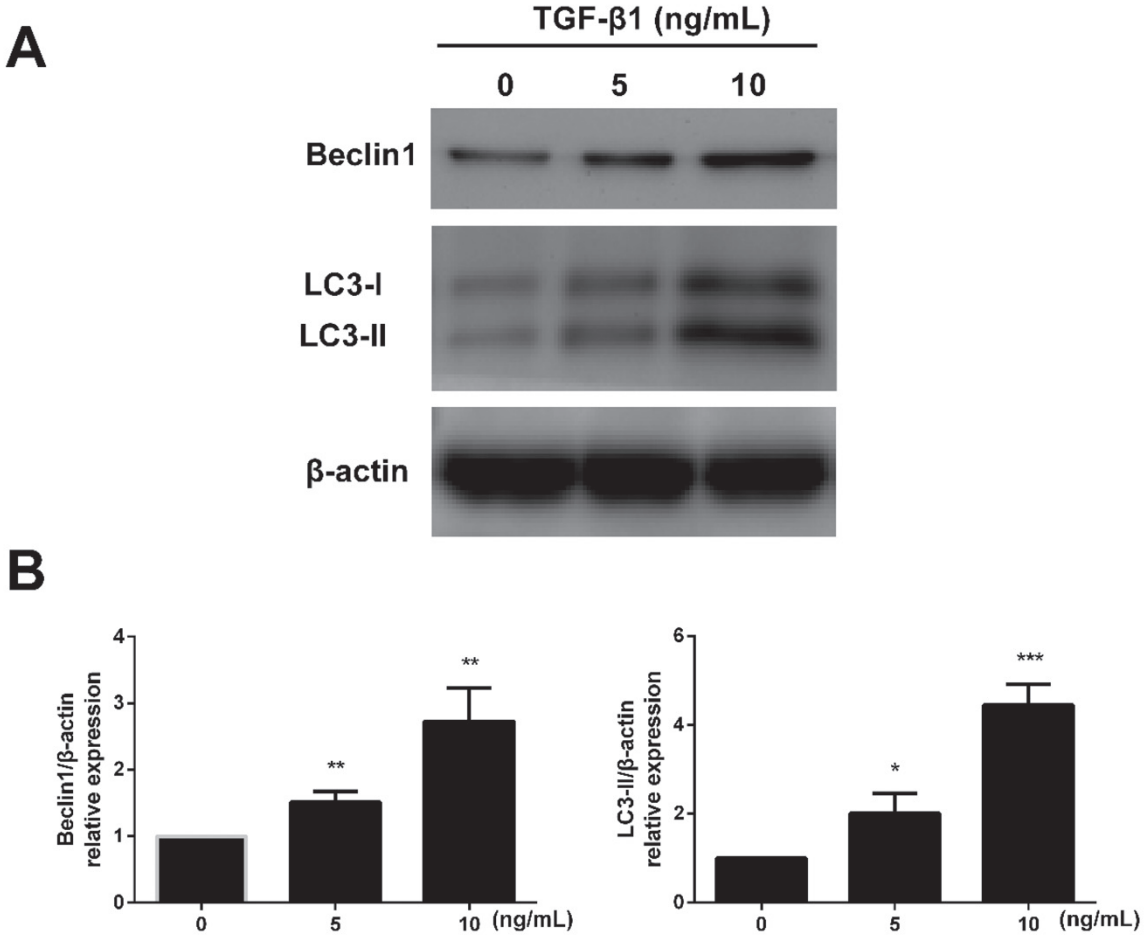

Figure 1. The protein expression level of LC3 and Beclin1 of SGC7901 cells induced by different concentrations (0 ng/mL, 5 ng/mL, 10 ng/mL) of TGF-β1 for 24 h.

3-MA (5 mmol/L, autophagic inhibitor) was added, the autophagic activity was significantly inhibited (Figure 2).

Enhancement Effect of TGF-β1 on the Invasion Capability of SGC7901 Cells

In the migration experiment of transwell chamber, we found that the invasion capability of SGC7901 cells in the TGF-β1 treatment group was remarkably enhanced compared to that in the control group (Figure 3).

Inhibition on Autophagy Reducing the Invasion Capability of SGC7901 Cells Induced by TGF-β1

In this study, we further verified whether autophagy could affect the invasion capability of cells induced by TGF-β1. In the invasion experiment of Transwell chamber, we found that after the cells had been simultaneously treated by TGF-β1 and 3-MA (5 mmol/L), a significant decrease was observed in the invasion capability of SGC7901 cells (Figure 4).

Discussion

Autophagy refers to a highly conservative metabolic regulation process, which is widely found in the eukaryote. Cells can maintain the protein metabolic balance and the homeostasis in cells through degrading the impaired organelles and biological macromolecules in the lysosome. Under the normal conditions, autophagy in cells

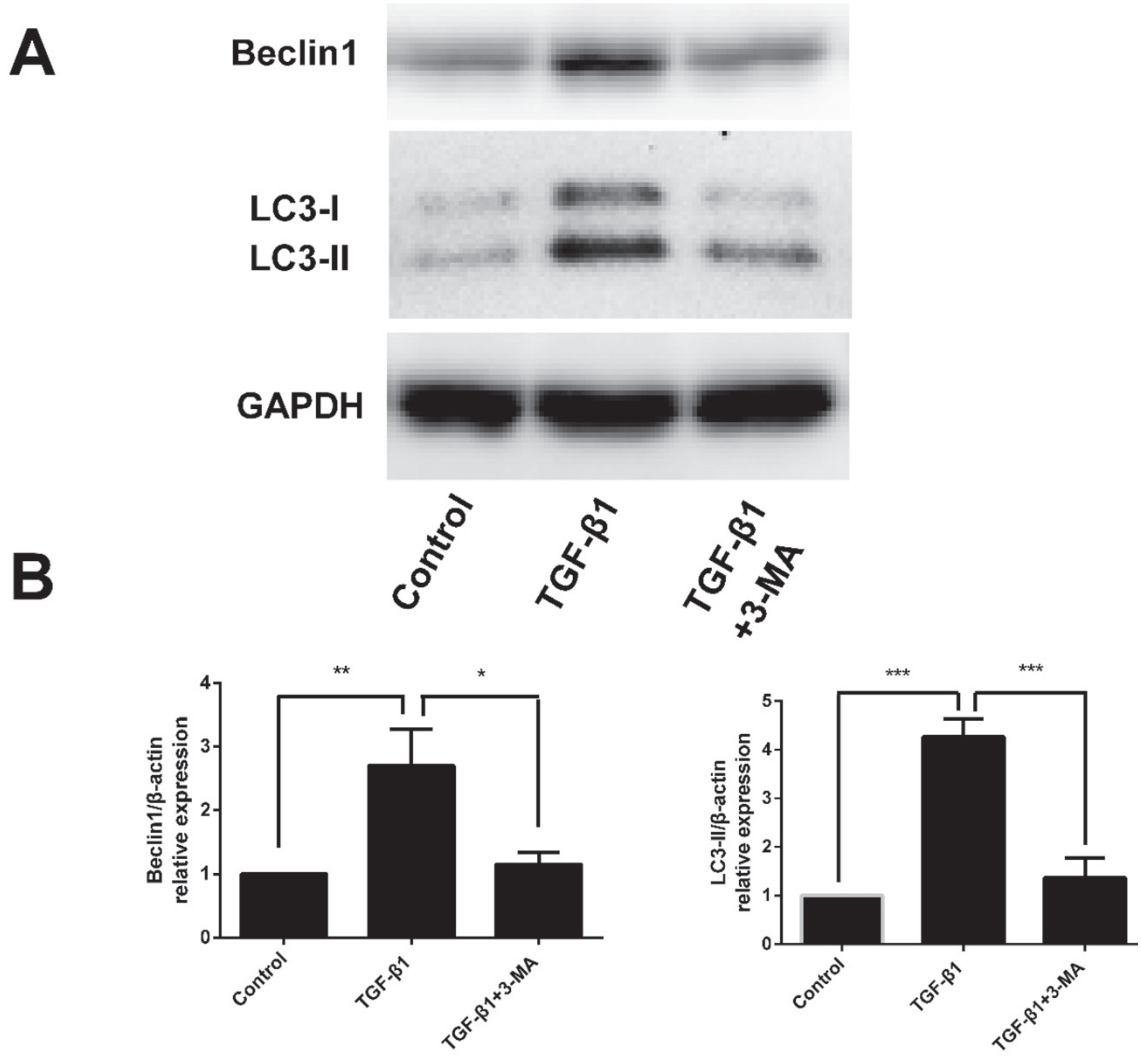

Figure 2. Autophagy inhibitor 3-MA inhibits TGF-β1 induced autophagy.

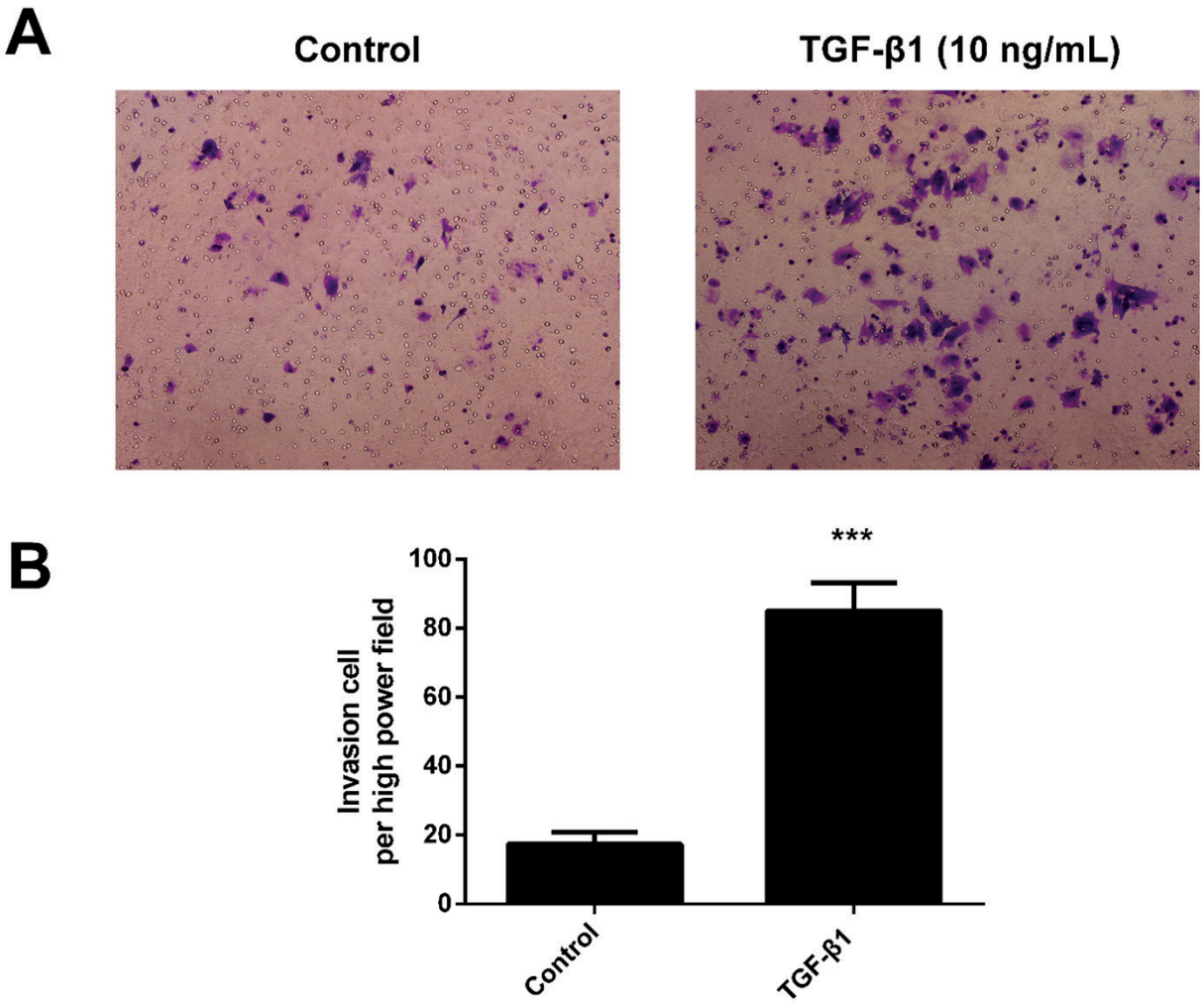

Figure 3. TGF-β1 enhances the invasion ability of SGC7901 cells (crystal violet staining × 200).

is kept at a baseline level; when cells are under stress, such as nutrition-deficiency, hypoxemia, high temperature, and differet hormone levels, autophagy will be activated to maintain the survival of cells until the stress is discharged[9]. More studies have shown that not only does autophagy participate in maintaining the homeostasis, growth, development, maturity and differentiation of normal cells, but also the autophagic activity is varied in different cells, indicating that autophagy also plays a role in the occurrence and development of tumor[10]. Wei et al[11] found that the expression of LC3B, a marker protein of autophagy, was significantly elevated in gastric cancer tissues, suggesting that the abnormally activated autophagy is key to the pathological changes and progression of gastric cancer[12].

Transformation growth factor β (TGF-β), as a kind of inhibitory factor of cell growth, can promote the formation of extracellular matrix, regulate cell proliferation and differentiation, cell apoptosis and immune regulatory responses. In the occurrence and development of malignant tumor, the role of TGF-β1 is just like a double-edged sword. In the early stage of the tumor, TGF-β1 can suppress the formation of tumor; but, in the existence of lesion of tumor, TGF-β1 can provide the nutrition support for the further growth of tumor through promoting the local angiogenesis surrounding the tumors, and enhance the ability of local infiltration and distant invasion and metastasis, thus accelerating the growth and development of tumors[13,14]. Literature reported that TGF-β1 can improve the survival of hepatic cells through activating the autophagy[8]. Hence, TGF-β1 and autophagy may constitute the survival and protection mechanism of cells in stress, especially the tumor cells, and investi-

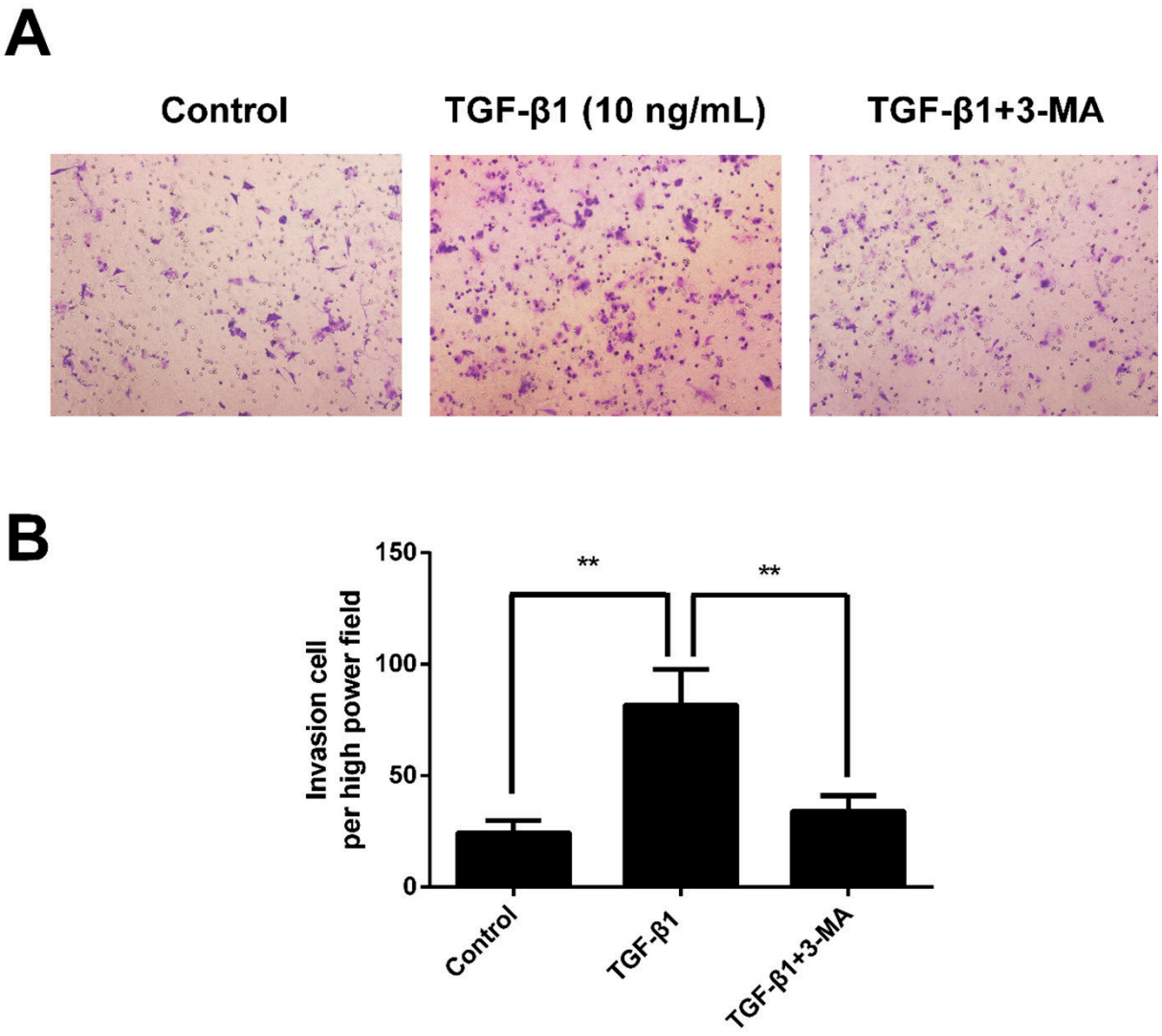

Figure 4. Autophagy inhibitor 3-MA attenuates TGF-β1 induced SGC7901 cell invasion (crystal violet staining × 200).

gation into the internal correlation between these two factors may clarify how cancer cells gain the invasion capability – the strong migration capability and the capability to acquire important malignant phenotype. In this work, we established the TGF-β1 model in gastric cancer cells to observe the influence of TGF-β1 on the autophagic activity and invasion capability of gastric cancer cell for investigating the correlation between the invasion capability of gastric cancer cells and autophagy, thus clarifying the induction mechanism of TGF-β1 for invasion capability of gastric cancer cells.

In this research, we firstly found that under the stimulation of TGF-β1 in varying concentrations, transformation from LC3-I to LC3-II in the LC3 (marker protein of autophagy) was promoted and the expression level of Beclin1 was significantly augmented in a dosage-dependent manner, indicating that TGF-β1 can sufficiently induce the increase in the autophagic activity of gastric cancer cells. Subsequently, we detected the influence of TGF-β1 on the autophagic activity through adding TGF-β1 inhibitor to antagonize the effect of TGF-β1, and found that TGF-β1 inhibitor can effectively reverse the increase of autophagic activity induced by TGF-β1. Further, we carried out the transwell invasion experiment to test the effect of TGF-β1 on the invasion capability of gastric cancer cells, and results revealed that TGF-β1 could enhance the invasion capability of gastric cancer cells.

However, when we used the 3-MA (inhibitor of autophagy) to block the autophagy, we found that TGF-β1 could not increase the invasion capability of gastric cancer cells, indicating that autophagy may be one of the major mechanisms for an increase of invasion capability of gastric cancer cells in stress. Enhancing the invasion capability may be another survival and protection mechanism of cancer cells conferred by autophagy. Recently, literature reported that Bafilomycin A1, an inhibitor of autophagy in the late period, can augment the sensitivity of gastric cancer to chemotherapy when it is used to inhibit the autophagic activity[15]. In addition, this effect has also been reported in the literature of breast cancer and lung cancer[16,17], which can be served as a potential target for treatment of the tumor.

Conclusions

We observed that TGF-β1 in the gastric cancer SGC7901 cell line could enhance the invasion capability of cancer cells through inducing the activation of autophagy, which will deepen understanding of the pathogenesis of gastric cancer. Thus, we should further carry out the research on the effect of autophagy in the occurrence and development of gastric cancer and the regulation mechanism of TGF-β1, blocking cell autophagy and reducing the invasion and metastasis of tumor cells to provide new ideas and reference for the individual treatment of gastric cancer, which is of great significance for clinical practice with a promising future.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA: Cancer J Clin 2015; 65: 5-29.
2) Brenner H, Rothenbacher D, Arndt V. Epidemiology of stomach cancer. Methods Mol Biol 2009; 472: 467-477.
3) Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. Nature 2008; 451: 1069-1075.
4) Kroemer G, Levine B. Autophagic cell death: the story of a misnomer. Nat Rev Mol Cell Biol 2008; 9: 1004-1010.
5) White E. Deconvoluting the context-dependent role for autophagy in cancer. Nat Rev Cancer 2012; 12: 401-410.
6) Katsuno Y, Lamouille S, Derynck R. TGF-β signaling and epithelial-mesenchymal transition in cancer progression. Curr Opin Oncol 2013; 25: 76-84.
7) Pickup M, Novitskiy S, Moses HL. The roles of TGFβ in the tumour microenvironment. Nat Rev Cancer 2013; 13: 788-799.
8) Fu MY, He YJ, Lv X, Liu ZH, Shen Y, Ye GR, Deng YM, Shu JC. Transforming growth factorbeta1 reduces apoptosis via autophagy activation in hepatic stellate cells. Mol Med Rep 2014; 10: 1282-1288.
9) Murrow L, Debnath J. Autophagy as a stress-response and quality-control mechanism: implications for cell injury and human disease. Annu Rev Pathol 2013; 8: 105-137.
10) Guo JY, Teng X, Laddha SV, Ma S, Van Nostrand SC, Yang Y, Khor S, Chan CS, Rabinowitz JD, White E. Autophagy provides metabolic substrates to maintain energy charge and nucleotide pools in Ras-driven lung cancer cells. Genes Dev 2016; 30: 1704-1717.
11) Liu B, Liu C, Zhao X, Shen W, Qian L, Wei Y, Kong X. Establishment of a cell line with stable expression of mCherry-EGFP tandem fluorescent-tagged LC3B for studying the impact of HIV-1 infection on autophagic flux. J Virol Methods 2014; 209: 95-102.
12) Zhang N, Wang AY, Wang XK, Sun XM, Xue HZ. GAS5 is downregulated in gastric cancer cells by promoter hypermethylation and regulates adriamycin sensitivity. Eur Rev Med Pharmacol Sci 2016; 20: 3199-3205.
13) Salvo E, Garasa S, Dotor J, Morales X, Peláez R, Altevogt P, Rouzaut A. Combined targeting of TGF-beta1 and integrin beta3 impairs lymph node metastasis in a mouse model of non-small-cell lung cancer. Mol Cancer 2014; 13: 112.
14) Wu YC, Tang SJ, Sun GH, Sun KH. CXCR7 mediates TGFbeta1-promoted EMT and tumor-initiating features in lung cancer. Oncogene 2016; 35: 2123-2132.
15) Li LQ, Xie WJ, Pan D, Chen H, Zhang L. Inhibition of autophagy by bafilomycin A1 promotes chemosensitivity of gastric cancer cells. Tumor Biol 2016; 37: 653-659.
16) Wen J, Yeo S, Wang C, Chen S, Sun S, Haas MA, Tu W, Jin F, Guan JL. Autophagy inhibition re-sensitizes pulse stimulation-selected paclitaxel-resistant triple negative breast cancer cells to chemotherapy-induced apoptosis. Breast Cancer Res Treat 2015; 149: 619-629.
17) Liu F, Liu D, Yang Y, Zhao S. Effect of autophagy inhibition on chemotherapy-induced apoptosis in A549 lung cancer cells. Oncol Lett 2013; 5: 1261-1265.