Translation of Chinese Text

Effect of TBL-12 on Proliferation, Migration, Invasion, and Apoptosis of Gastric Cancer Cell HGC-27

Abstract

Objective: To explore the effect of TBL-12 on proliferation, migration, invasion, and apoptosis of gastric cancer cell HGC-27 in vitro.

Methods: Gastric cancer cell HGC-27 was treated with TBL-12 of different concentrations. CCK8, scratch test, invasion assay and flow cytometry were adopted to investigate the effect of TBL-12 on HGC-27 in vitro.

Results: Dose-dependent inhibition of HGC-27 proliferation by TBL-12 with an IC_{50} value of 41.65 g/ml. TBL-12 significantly inhibited the migration and invasion of HGC-27. Flow cytometry analysis showed that TBL-12 induced apoptosis of HGC-27.

Conclusion: Inhibition of HGC-27 proliferation, migration, invasion, and apoptosis by TBL-12 in vitro was achieved.

Keywords: TBL-12; gastric cell HGC-27; proliferation; migration; invasion; apoptosis

Introduction

Gastric cancer is one of the most common gastrointestinal tumors[1]. In China, gastric cancer ranks the top three of morbidity and mortality of malignant tumors, and exhibits a rising trend over the past 30 years. The percentage of gastric cancer in China is high among the world[2]. Nowadays, the treatment of gastric cancer mainly relies on surgical resection followed by adjuvant chemotherapy and radiotherapy. However, this treatment did not show satisfactory results[3]. Therefore, the search for safe and effective anti-gastric cancer drugs is of prime importance. Marine life has been considered as a biologically versatile resource that can target many cellular pathways for biological purposes[4]. Sea cucumber, echinoderms from the class Holothuroidea, is a marine organism. It contains a variety of bioactive substances such as ginseng saponins, peptides, sea cucumber polysaccharides, active peptides, and sea cucumber ganglioside[5]. Pharmacological studies have shown the wide array of pharmacological activity, including anticoagulant, antithrombotic, antitumor, and immune regulation, of sea cucumber[6]. In recent years, research on the antitumor performance of sea cucumber has made noticeable progress. These studies mainly focused on the antitumor effects of single extracts from sea cucumbers. The antitumor effect of sea cucumbers as a whole has not yet been reported[7]. TBL-12 is an extract of sea cucumber. Recently TBL-12 has been shown to inhibit the growth of

different tumor cells such as myeloma, prostate cancer and cervical cancer[8]. However, investigation of the effect of TBL-12 on gastric cancer is comparatively limited. In this study, the effect of TBL-12 on gastric cancer cell HGC-27 was investigated in the context of proliferation, migration, invasion, and apoptosis level. This study provides the experimental basis for any follow-up study of TBL-12 and its clinical application of gastric cancer treatment.

1 Materials and Methods

1.1 Materials

Human gastric cancer cell HGC-27 was available in our laboratory. They were frozen and stored in the liquid nitrogen tank. TBL-12 was purchased from Unicorn Pacific Limited at a concentration of 1 mg/ml. Fetal bovine serum fetal bovine serum FBS, DMEM high glucose medium and Trypsin (0.25%) were purchased from Gibco, United States. Cell Counting Kit-8 (CCK8) was purchased from Dojindo, Japan. Transwell plates (8 μm aperture) were purchased from Coring, United States. Matrigel was purchased from BD, United States. Annexin-V/PI apoptosis double staining kit was purchased from the Invitrogen, United States.

1.2 Experiments

1.2.1 Cell Culture

The gastric cancer cell HGC-27 was inoculated in a single layer in DMEM growth medium containing 10% FBS and placed inside a 5% CO_2 incubator at 37℃. Subculturing was performed when the cells grew to 80-90%. The cells were washed with PBS twice before digested using 0.125% trypsin for 1 min. Digestion was stopped by adding DMEM growth medium with 10% FBS. The cells were subcultured every 2-3 days and the log-phase growth cells were taken for experiments.

1.2.2 Effect of TBL-12 on the proliferation of HGC-27 evaluated using CCK8 assay

Log-phase growth HGC-27 cells were harvested and trypsinized to prepare the single-cell suspensions. The cells were inoculated into a 96-well plate at a density of 2000 cells/well. The experiment was divided into blank group, control group and experimental group. The blank group contained the same amount of growth medium as the other groups. The control group contained the same amount of cells and growth medium as the other groups. The experimental group contained TBL-12 diluted using growth medium at concentrations of 15, 30, 45, and 75 μg/ml, respectively. Each group had 5 wells each 100 μl in volume. The surrounding wells were filled with PBS. After incubation for 24 h inside a 37℃ incubator, 10 μl CCK-8 solution was added to each well for a further 2 h incubation. The absorbance at 490

nm was measured using a microplate reader and the cell survival rate was evaluated following the formula provided by CCK-8 instruction manual.

$$\text{Cell survival rate (\%)} = \frac{\text{Absorbance value (experimental group)} - \text{Absorbance value (blank group)}}{\text{Absorbance value (control group)} - \text{Absorbance value (blank group)}} \times 100\%$$

1.2.3 Effect of TBL-12 on migration of HGC-27 evaluated using scratch assay
The back of the six-well plate was first marked to make a reference line. Two parallel horizontal lines were then drawn across the well. Single cell suspension was prepared using log-phase growth cell digested with 0.125% trypsin. At a concentration of $3x10^5$ / ml, the suspension was seeded into the six-well plate at 1 ml per well, and placed into 37℃ cell incubator at 5% CO_2 to culture for 24 h. The plate was then taken out from the incubator. A 10 μl pipette tip was used to mark a straight scratch perpendicularly to the reference line. The plate was then washed twice with PBS to remove any floating cells. The test was divided into the control group and the experimental group. The control group was fed with the same amount of growth medium. In the experimental group, TBL-12, diluted using growth medium at concentrations of 15, 30, 45, 60, and 75 g/ml, respectively, was added to each well to achieve a final volume of 2 ml per well. The plate was then placed into the incubator for continuous incubation. The plate was put under inverted fluorescence microscope (x100) at 0 and 12 h after the incubation. Mobility of the cells was evaluated using ImageJ. Each experiment was repeated three times.

1.2.4 Effect of TBL-12 on invasion of HGC-27 evaluated using Transwell invasion assay
Matrigel, which has been thawed overnight, was mixed with pre-chilled serum-free DMEM medium at 1:8. The solution was quickly added to the upper plate at 100 µl per well. This process was performed on ice and all tools were pre-chilled to avoid rapid solidification of the Matrigel. Matrigel was then solidified after 1 hour of incubation at 37℃. Log-phase growth cells were harvested and trypsinized to prepare single cell suspensions, which were seeded at Transwell upper plates at $3x10^4$ cells/well. The experiment was divided into the control group and the experimental group. An equivalent amount of serum-free medium was added to the control group. TBL-12 diluted using serum-free medium was added to the experimental group to make final concentrations of 30 and 60 µg/ml, respectively. The final volume was 100 µl per well. In the lower plate, 600 µl of growth medium containing 10% FBS was added and the cells were removed after culturing at 37°C

inside a 5% CO_2 incubator for 24 h. Cotton swabs were gently wiped off the cells that did not pass through the membrane. They were then fixed in 4% paraformaldehyde for 20 min, washed twice in PBS, stained with 0.1% crystal violet for 10 minutes, and washed twice in PBS again before inverted to dry naturally. The upper, lower, left, right, and middle fields of view were taken in a x200 inverted microscope to calculate the number of perforated cells where the mean value was reported. Each group of cells was set up with 3 replicates and the experiment was repeated 3 times.

1.2.5 Flow cytometry detection of apoptosis in HGC-27

The cells in the log-growth phase were taken and prepared by trypsin digestion to prepare single cell suspensions. They were inoculated into 6-well plates at a density of 2×10^5 cells/well. The experiments were divided into the control and the experimental groups. The control group was prepared by adding equivalent amount of growth medium. In the experimental group, TBL-12 diluted with growth medium achieving a final concentration of 15, 30, 45, 60, and 75 μg/ml, was added. The final volume of each well was 2 ml. Incubation was performed at 37°C for 24 h. Binding buffer was first diluted with deionized water at a ratio of 1:4. The diluted binding buffer was used to dilute PI at 1:9. The cells were washed twice with pre-chilled PBS and digested with trypsin to prepare the single cell suspensions. The cells were washed twice again with pre-chilled PBS, and the cell density was adjusted to 1×10^6 cells/ml. 100 μl was added inside the flow tubes. 5 μl of Annexin-V and 1 μl PI were added to each tube and well-mixed. The cells were incubated at room temperature in dark for 15 min, before measuring cell apoptosis rate by flow cytometry within 1 h.

1.3 Statistical analysis

The results were statistically analyzed and plotted using GraphPad Prism 5.0. Oneway ANOVA was used to compare the data between groups. Dunnett's test was undertaken to compare the data of each group with the control group. The difference was considered statistically significant at $p<0.05$.

2. Results

2.1 TBL-12 inhibiting the proliferation of HGC-27 in vitro

After treated with different concentrations of TBL-12 (15-75μg/ml) for 24h, TBL-12 showed significant inhibitory effect on HGC-27 as compared with the control group. The difference was statistically significant ($p< 0.05$). Compared with the control group, the proliferation activity was 74.2%, 66.4%, 48.9%, 42.2%, and 34.7%, respectively. As the concentration increased, the inhibitory effect of TBL-12 gradually increased exhibiting a dose-dependent response. TBL-12 inhibited the proliferation

of gastric cancer cell HGC-27 showing an IC_{50} of 41.65 μg/ml.

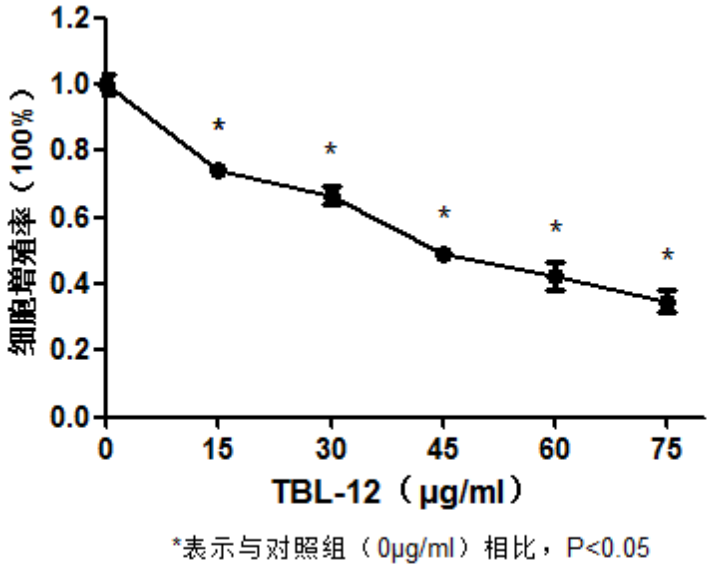
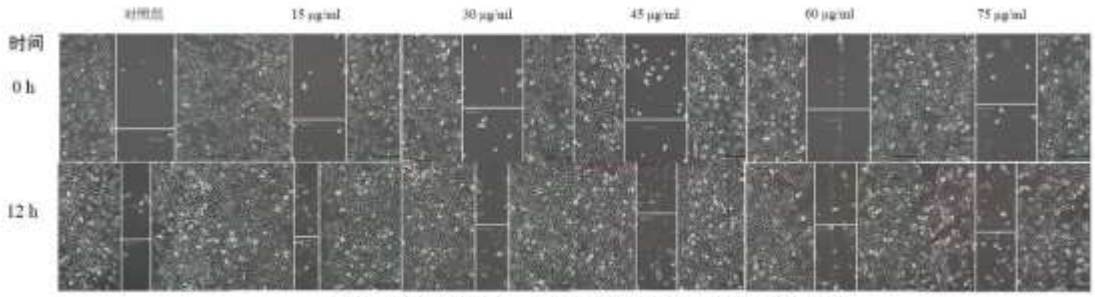


Figure 1. Influence of TBL-12 on HGC-27 proliferation in vitro

2.2 TBL-12 inhibiting the migration of HGC-27

Figure 2 shows the experimental results. Compared with the control group, TBL-12 showed an inhibitory effect on the migration of HGC-27 at a concentration of 30 μg/ml or higher, and the difference was statistically significant ($p<0.05$). With the increase of the concentration, the inhibitory effect of TBL-12 on HGC-27 cells gradually increased However, the difference was not statistically significant.



A.TBL-12对胃癌HGC-27细胞体外迁移的影响（100X）

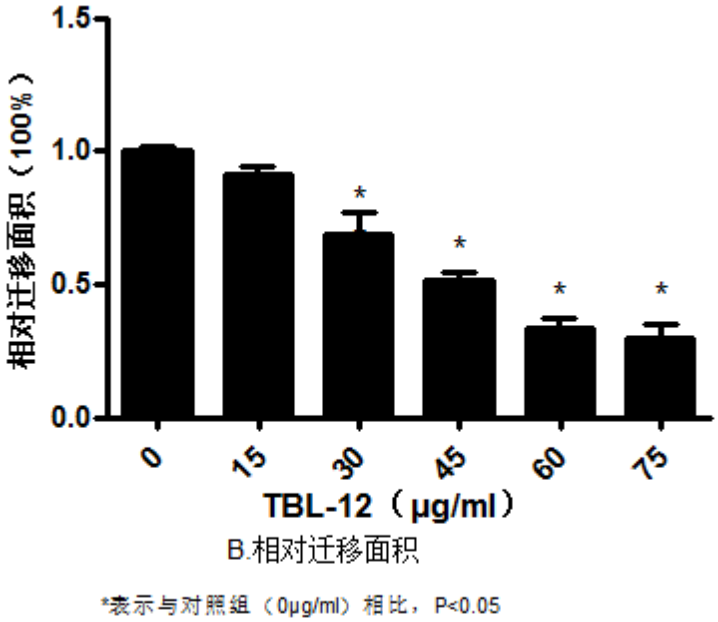
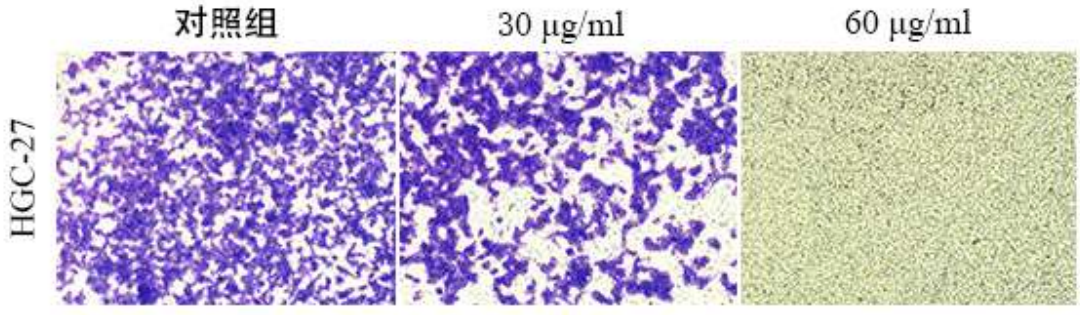


Figure 2. Influence of TBL-12 on HGC-27 migration

2.3 TBL-12 inhibiting the invasion of HGC-27

Figure 3 summarizes the experimental results of invasion. Two concentrations of low-dose treatment group (30 μg/ml) and high-dose treatment group (60 μg/ml) were selected as the experimental group. Compared with the control group, TBL-12 at both high and low doses inhibited the invasive ability of HGC-27, and the difference was statistically significant ($p<0.05$). With increasing concentrations, TBL-12 exhibited stronger ability to inhibit the invasion of gastric cancer cells. The dose-dependent response was found to be statistically significant ($p<0.05$).



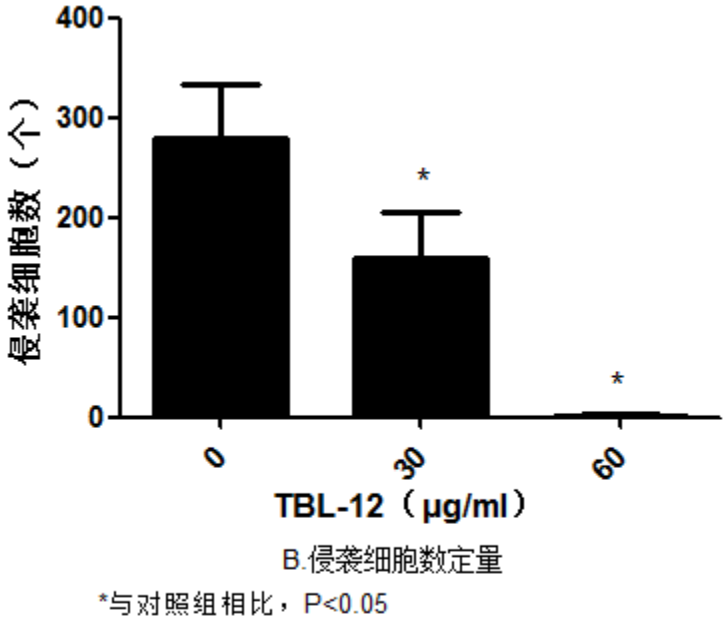


Figure 3. Influence of TBL-12 on HGC-27 invasion

2.4 TBL-12 inducing the apoptosis of HGC-27

The experimental results of apoptosis are shown in Fig. 4. The results showed that compared with the control group, TBL-12 could induce apoptosis of HGC-27 at a concentration of 30 μg/ml or higher, and the difference was statistically significant ($p < 0.05$). It suggested that TBL-12 has an apoptosis-inducing effect on gastric cancer HGC-27 cells.

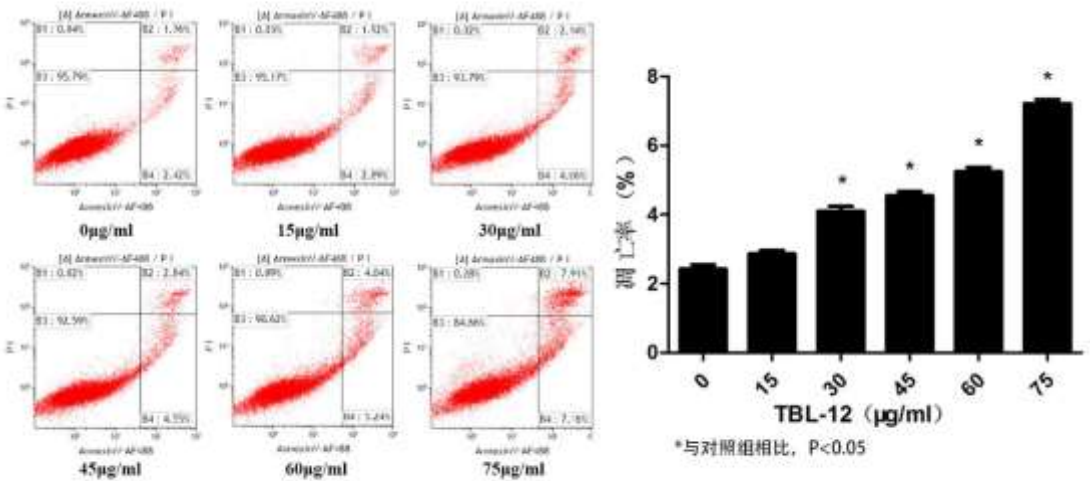


Figure 4. Inducing apoptosis of HGC-27 by TBL-12

3 Discussion and Conclusion

Gastric cancer is one of the most common tumors in the digestive system. In the world, the incidence of gastric cancer ranks fifth in malignant tumors, and the fatality

rate ranks third in all tumors. In China, the incidence of gastric cancer ranks second, and the mortality rate is in the third place. Besides, it shows a rising trend. For the treatment of gastric cancer, the benefits of conventional surgical treatment are limited, and the effects of radiotherapy and chemotherapy are not significant while the toxic and side effects are significant[9]. Therefore, the discovery of new, safe and effective therapeutic drugs to treat gastric cancer is imminent.

Sea cucumber is an important food resource in many Asian countries. It contains a variety of biologically active ingredients and has physiological functions such as immunity regulation, anticoagulation, anti-tumor, antiviral, anti-aging and anti-fatigue etc.[10]. Its pharmacological activity is very wide. Previous investigation on the anti-tumor effects of sea cucumbers had focused on single component studies. For instance, Gao Xiang and co-workers showed that sea cucumber sulphate mucopolysaccharide has a significant anti-tumor activity after treatment with melanoma B16 for 72 h[11]. Yuan Wenpeng and co-workers found that the monomeric component SC-2 could significantly inhibit the growth of mouse S180 solid tumors, and induce apoptosis of cancer cells to achieve anti-cancer activity[12]. However, the anti-tumor effect of the whole sea cucumber extract has been rarely studied.

TBL-12 is an extract of natural sea cucumber, which is currently sold as a health product without any side effects. However, there are few reports on its anti-tumor effect. Some studies have reported that TBL-12 could inhibit bone marrow, proliferate tumor and endothelial cells, and inhibit angiogenesis by down-regulating VEGFR2[13]. This study found that TBL-12 could effectively inhibit the proliferation of gastric cancer cell HGC-27. With increasing concentrations, the inhibition of proliferation became more obvious which exhibited a dose-dependent response. The results of cell scratch and Transwell invasion experiments showed that TBL-12 could significantly inhibit the migration and invasion of HGC-27 cells when the concentration of the drug reached 30 μg/ml. The results of flow double staining showed that the apoptosis rate of gastric cancer HGC-27 cells increased significantly after TBL-12 treatment, suggesting that probably through apoptosis TBL-12 inhibits the proliferation of HGC-27.

In summary, this study has demonstrated that TBL-12 not only reduces the migration and invasion of gastric cancer HGC-27 cells, but also inhibits cell proliferation and promoted apoptosis. The inhibitory capacity increases with increasing drug concentration. It is anticipated that TBL-12 not only has a role in promoting apoptosis but also has influence on the metastasis of gastric cancer, thus providing a theoretical

basis for the application of TBL-12 in the treatment of tumors. At present, further study is needed to investigate the mechanism of apoptosis of gastric cancer cells promoted by TBL-12, and its molecular mechanism of inhibiting migration and invasion ability.

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