Research on quercetin in inhibiting the epithelial–mesenchymal transition of gastric cancer

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1. Introduction

Gastric cancer has not only already posed a severe threat to the people’s health, but also brought a heavy load on the society. Therefore, studying on the pathogenesis of gastric cancer, detection of the important regulating and controlling molecule in the genesis and development of gastric cancer, and the early intervention is a pressing task for the medical workers in the tumor field. Epithelial-mesenchymal transition (EMT) is an initial factor for the remote metastasis of malignant tumors[1]. Studying on the molecular basis and action mechanism of EMT is not only conducive to an early diagnosis of gastric cancer, but also can indicate a new direction for the treatment of gastric cancer. Reversion of EMT process can inhibit the progression of gastric cancer, and even cure gastric cancer. Tracing the references, it is found that quercetin has a certain role in resisting the metastasis of malignant tumors in vitro and in vivo. Mechanism research demonstrates that reversion of EMT is probably one of the mechanisms[2-5]. Therefore, the study is aimed to deeply explore the role of quercetin in inhibiting EMT of gastric cancer cells and its mechanism, which can broaden a comprehending of the pathogenesis of gastric cancer and provide a theoretical support for the research and development of therapeutic drugs.

2. Materials and methods

2.1. Reagents and instruments

Human gastric cancer BGC-803 and quercetin were purchased
from the Cell Bank of Chinese Academia of Sciences; TGFβ-1 from R&D Company, fetal bovine serum and DMEF medium from Thermo Fisher, anti-mouse E-cadherin (C0510), anti-rabbit Phospho-Akt (9271S), anti-mouse Akt (2920S), and anti-mouse Vimentin from Santa Cruz Biotechnology Inc, inverted microscope from Nikon, carbon dioxide incubator from NuAire, ELIASA from Thermo, and Odyssey-fluorescence imaging system from LI-COR.

2.2. Method

Human gastric cancer BGC-803 cells were placed in DMEM medium with 10% fetal bovine serum at 37 °C with a saturation humidity of 5% CO2. The technique of 0.25 trypsin enzyme digesting was used for subculturing. When there was a 30% adherence fusion of cell walls, a cell model was started to establish. As requested, the gastric cancer cells were divided into three groups, i.e. normal control group, TGFβ-1 group, TGFβ-1 + quercetin group. TGFβ-1 (5 μg/L) was added to the three groups. No treatment was given in the normal control group. After an evenly shaking of the cells, quercetin was added in the TGFβ-1 + quercetin group, an equal volume of PBS was added in the normal control group, while no processing was done in the TGFβ-1 group. After 5 d culturing, the morphological changes of cells in the three groups were observed under a microscope.

2.3. Evaluation indicators

Western blot was used to detect the expressions of Vimentin, E-cadherin, and N-cadherin. MTT was used to evaluate the cell proliferation ability. The cell scratch repairing experiment was used to evaluate the migration ability. Cells in a logarithmic phase were placed in a 24-pore plate according to 1×105 cells in each pore. DMEM medium containing 10% fetal bovine serum was used for culturing. After 24 h, the micro pipette tip was used to scratch in a straight line in the 24-pore plate. Then the nutrient liquid was changed and the exfoliative cells were removed. The corresponding reagents were added according to the different processing methods in each group. After a continuous 24 h culturing and twice PBS washing, the changes of cell healing were observed under a microscope. The degree of cell healing was used to reflect the migration ability of cells.

3. Results

3.1. Cell proliferation and migration abilities

Figure 1 showed that the cell proliferation abilities from the highest to the lowest were successively in the TGFβ-1 group, the normal control group, and the TGFβ-1 + quercetin group. After 72 h culturing, OD value was 1.113 in the normal control group, 1.243 in the TGFβ-1 group, and 0.506 in the TGFβ-1 + quercetin group. The above results in the study showed that quercetin had a strong inhibition effect on the cell proliferation of gastric cell BGC-803 induced by TGFβ-1. Moreover, cell scratch repairing experiment results showed that the best cell healing degree was in the TGFβ-1 group, the normal was in the normal control group, while the worst was in the TGFβ-1 + quercetin group, indicating that the cell migration abilities from the biggest to the smallest were successively in the TGFβ-1 group, the normal control group, and the TGFβ-1 + quercetin group.

3.2. Expressions of E-cadherin, N-cadherin and Vimentin in each group

The expression levels of Vimentin and N-cadherin were up regulated in the TGFβ-1 group and the TGFβ-1 + quercetin group when compared with those in the normal control group, but the expression levels in the TGFβ-1 + quercetin group were lower than those in the TGFβ-1 group. The expression level of E-cadherin was the highest in the normal control group, the expression levels in the TGFβ-1 group and the TGFβ-1 + quercetin group were significantly down regulated, but a more down regulation was in the TGFβ-1 group.

3.3. Effect of quercetin on the activity of PI3K/Akt pathway

Figure 3 showed that AKT and p-AKT in the TGFβ-1 group was up showing that PI3K/Akt pathway activity in the TGFβ-1 group was higher than that in the control group. Expressions of AKT and p-AKT in the TGFβ-1 + quercetin group were significantly lower than those in the TGFβ-1 group, showing that PI3K/Akt pathway activity in the TGFβ-1 + quercetin group was higher than that in the TGFβ-1 group.
Gastric cancer is one the most common global malignant tumors and the second pathogenic factor for the mortality due to malignant tumors in the world, and can occur in patients at various ages, mostly in the middle-aged and elderly patients. Clinical statistical data show that about 2/5 patients can receive an operation after an attack. Even though the operation succeeds, postoperative 5-year survival rate was 30%, and metastasis will occur in 80% patients[6]. Gastric cancer severely affects the patients’ living qualities, and brings seriously economic and psychological burdens on the family. Invasion and metastasis are the main reasons for the recurrence and are closely associated with the prognosis. Recent research demonstrates that EMT is an initial factor for the metastasis of malignant tumors, and plays a vital role in the invasion and metastasis of malignant tumors. Moreover, research verifies that TGF-β-1 is a main inducer of EMT[7]. Quercetin has a certain role in resisting the metastasis of malignant tumors in vivo and in vitro, but the effect on EMT of gastric cancer and its mechanism are not reported[9]. In the study, TGF-β-1-induced gastric cancer cell lines are used to establish EMT model of gastric cancer cells, and quercetin is used for the intervention, in order to explore the effect of quercetin on EMT and its mechanism.

The results in the study showed that quercetin has a strong inhibition effect on the proliferation of TGF-β-1-induced gastric cancer cell BGC-803, and can also significantly reduce their migration abilities, indicating that quercetin can inhibit the metastasis and invasion of gastric cancer, and enhance the clinical therapeutic effect of gastric cancer patients. Vimentin and N-cadherin are the marker genes of mesenchymal cells, and the amount of expressions can represent the increase or establishment of the number of mesenchymal cells in a certain degree. E-Cadherin is one of the main components for the adherent junction of epithelial cells. When it is redistributed in other regions from the joint of epithelial cells due to some reasons, there will be an increase of cell scattering due to the loss of mutual adhesion between the epithelial cells[9,10]. Related researches testify that the expression levels of N-cad and Vimentin are elevated in the gastric cancer cells with a relatively high expression of high mobility group protein, while the expression level of E-cad is significantly reduced[11]. The results in the study showed that quercetin can up regulate the expression of E-cad, and meanwhile down regulate the expressions of N-cad and Vimentin, probably manifesting that quercetin can inhibit EMT in TGF-β-1-induced gastric cancer cell BGC-803 in a certain degree. AKT, also called protein kinase B, is a direct target protein of the downstream of PI3K, whose activation can promote the combination of AKT and PI3P generated due to a phosphoinositide phosphorylation in the membrane surface, and the phosphorylated AKT will lead to an activation of PI3K/AKT cell survival pathway. Some researches verify that PI3K/AKT signal transduction pathway can promote the tumor metastasis through EMT[12]. The results in the study showed that quercetin can down regulate AKT, and inhibit its phosphorylation, indicating that quercetin can regulate and control PI3K/AKT cell survival pathway probably through affecting AKT phosphorylation and down regulating AKT, so that EMT in the gastric cancer cell BGC-803 can be inhibited.

In conclusion, quercetin can inhibit EMT process in the TGF-β-1-induced gastric cancer cell lines, whose pathogenesis mechanism is probably associated with the regulating and controlling of PI3K/AKT signal transduction pathway.

4. Discussion

References