ORIGINAL ARTICLES

Expressions of apoptosis inducing factor and heat-shock protein 70 in gastric polypss and gastric cancer and their correlation with gastric cancer

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Abstract

Objective: To explore the effects of apoptosis inducing factor (AIF) and heat-shock protein 70 (HSP70) in the occurrence and development of gastric cancer by detecting their mRNA levels in gastric polypss and gastric cancer, and provide a new way and insight for the early diagnosis and treatment of gastric cancer.
Methods: Gastric biopsy samples of 120 patients in the Third Hospital of Inner Mongolia Medical University were collected. According to their pathological characteristics, the samples were divided into four groups as followed: normal gastric mucosa group (n = 30), gastric hyperplastic polypss group (n = 30), gastric adenomatous polypss group (n = 30) and gastric cancer group (n = 30). The expressions of AIF and HSP70 mRNA were detected by real-time PCR.
Results: The expressions of AIF and HSP70 mRNA were gradually increased in gastric hyperplastic polypss, gastril adenomatous polypss and gastric cancer, the difference was significant ($p < .05$). In the tissues of adenomatous polypss and gastric cancer, the expression of AIF mRNA was positively correlated with HSP70 mRNA ($p < .01$).
Conclusions: AIF and HSP70 are correlated with the development of gastric cancer. AIF and HSP70 may act synergistically in the development of gastric cancer. Both of them can be used as markers for the diagnosis of gastric cancer.

Key Words: AIF, HSP70, Gastric polypss, Gastric cancer

In addition to abnormal cell proliferation and differentiation, abnormal apoptosis is another important cause of gastric cancer. Apoptosis inducing factor (AIF) is an apoptosis-effector molecule,[1] which mediates apoptosis independently of Caspase pathway.[2] Under normal physiological conditions, AIF is located in the mitochondrial membrane gap with oxidoreductase activity. After the cell is stimulated by apoptosis, AIF translocates from mitochondria into cytoplasm and nucleus, and combines with chromosomes in the nucleus to induce apoptosis.[3,4] Heat-shock protein 70 (HSP70) is the most important heat-shock protein family member and carries the role of molecular chaperone. Under stress, HSP70 binds to proteins, protects the protein, participates in its folding and assembly and mediates the localization and transport of the protein. HSP70 also participates in protein hydrolysis in order to prevent protein accumulation. HSP70 can participate in tumorigenesis and development through the interaction with oncogene or tumor suppressor gene protein.[5] As a chaperone, HSP70 protects tumor cells from a wide range of apoptosis and regulates tumor cell proliferation and differentiation by interacting with proto-oncogenes and tumor suppressor genes and their corresponding proteins. HSP70 is highly expressed in many tumor tissues and cells, and is closely related to the malignancy of the tumor. HSP70 and tumor cell proliferation and apoptosis are closely related. There is no literature on the

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combined detection of AIF mRNA and HSP70 mRNA in gastric and gastric polypss. The purpose of this experiment was to detect the expression of AIF and HSP70 in gastric cancer and gastric polypss, and to explore their effects on the occurrence and development of gastric cancer, so as to provide some new methods and ideas for early diagnosis and treatment of gastric cancer.

1 Data and methods

1.1 General information

A total of 90 cases of gastroscopy and biopsy were performed and selected in the Third Affiliated Hospital of Inner Mongolia Medical University (Inner Mongolia Baotou Steel Hospital) from July 2013 to July 2014. The patients were divided into 3 groups: 30 cases in normal gastric mucosa group, 11 males and 19 females. The age was (41.0 $\pm$ 8.8) years old (35-52 years old, only healthy examiners were chosen or patients with abdominal distention and abdominal discomfort were cured. *H. pylori* infection was treated by ^{13}C breath test. No proton pump inhibitor, antibiotics, bismuth and non-steroidal anti-inflammatory drugs were taken within 4 weeks of treatment, and no alcohol consumption history). 30 cases in the gastric hyperplastic polypss group, among them 9 cases were male and 21 cases were female. The age was (26.6 $\pm$ 11.2) years old (26-60 years old). The adenomatous polypss group consisted of 30 cases, 17 males and 13 females, aged (42.4 $\pm$ 6.8) years old (40-53 years old). 30 cases of gastric cancer patients who underwent gastroscopy biopsy and general surgery and tumor surgery at the same time in our hospital were enrolled. There were 14 males and 16 females, aged (52.2 $\pm$ 12) years (44-70 years).

1.2 Experimental methods

1.2.1 Collection of specimens

The specimens were obtained by biopsy or surgical resection under the gastroscope. All tissue specimens need to be approved and informed consent was signed by patients before they were collected. The specimens were divided into two parts, one was sent to the Department of Pathology, and the other was classified as pathological specimen.

1.2.2 Specimen storage

The specimen was placed in the cryopreservation tube (pre-equipped with protective agent) and frozen in -196°C liquid nitrogen.

1.2.3 Real-time PCR detection of AIF and HSP70 mRNA expression

The cryopreserved frozen tissue samples were promptly transferred to a mortar which was pre-cooled with liquid nitrogen. During pestle grinding, liquid nitrogen was constantly added until the sample was ground to powder shape, fully homogenized. Then the homogenate was transferred to the centrifuge tube. At 4°C, after centrifugation at 12,000 r/min for 10 min, the supernatant was discarded, and 1 ml of 750 g/L ethanol was added to wash the pellet. After centrifugation at 12,000 r/min for 5 min again at 4°C, RNA was fractionated from the obtained RNA solution. 5 μL of RNA was used to determine the concentration and purity by NanoDrop nucleic acid protein analyzer Nd 1000. The remaining RNA was stored at -80°C for subsequent experiments. After that, the DNA was removed from the RNA on ice and then reverse-transcribed. The conditions of the reverse transcription were: incubated at 37°C for 15 minutes and then heated at 85°C for 5 seconds. The obtained cDNA template was stored at -20°C. Then the real-time PCR relative quantitation was carried out with the modified cDNA as the template, and the expression level of AIF and HSP70 mRNA was detected.

1.3 Statistical analysis

The experimental results were analyzed statistically using SPSS 19.0 statistical software, measurement data with $\bar{x} \pm$ s, analysis of variance was used for multiple comparisons between groups, *t*-test was used for the comparison between the two groups, correlation analysis was taken by Pearson two gene, α = 0.05 for the standard test, $p < .05$, the difference was statistically significant.

2 Results

2.1 AIF mRNA expression in each group

The amplification curve of AIF mRNA in each group showed that the peak time of AIF gene in different tissues was different, indicating that there was difference in the expression of the gene in different tissues. AIF mRNA expression in hyperplastic polypss, gastric adenomatous polypss and gastric cancer group was higher than that in normal control group, the difference was statistically significant ($p < .05$), and AIF mRNA expression in gastric cancer group was higher than that in gastric adenomatous polypss group, the difference was statistically significant ($p < .05$, see Figure 1 and Table 1).

2.2 HSP70 mRNA expression in each group

HSP70 mRNA amplification curve in each group showed that HSP70 mRNA in different tissues had different peak time, indicating the differences in expression of the gene

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in different tissues, HSP70 mRNA in hyperplastic polypss group, gastric adenomatous polyps group and gastric cancer group were higher than that in the control group, the differences were statistically significant ($p < .05$), HSP70 mRNA expression in gastric cancer group was higher than that in gastric adenomatous polyps group, and the difference was statistically significant ($p < .05$, see Figure 2 and Table 1).

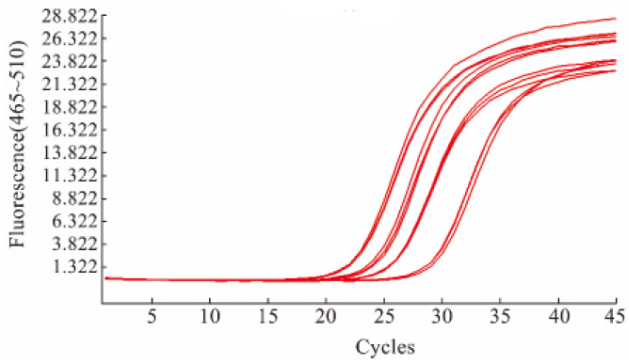

Figure 1: AIF amplification curve in normal gastric mucosa, gastric hyperplastic polypss, gastric adenomatous polypss and gastric cancer tissues. The curves follow the order from right to left

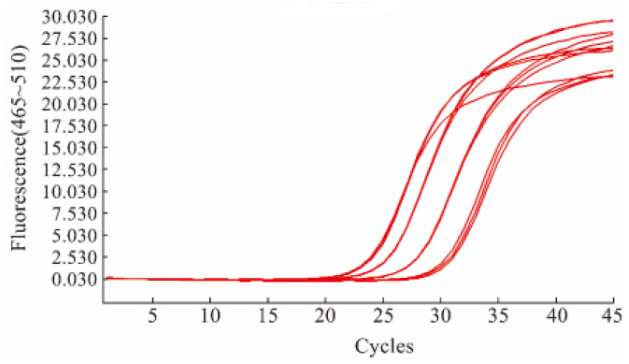

Figure 2: HSP70 amplification curve in normal gastric mucosa, gastric hyperplastic polyps, gastric adenomatous polyps and gastric cancer tissues. The curves follow the order from right to left

Table 1: Comparison of expressions of AIF mRNA and HSP70 mRNA in each group ($\bar{x} \pm s$)

Groups	AIF mRNA	HSP70 mRNA
Normal mucosa group	0.011 ±0.002	0.045 ±0.007
Gastric hyperplastic polypss group	0.043 ±0.011	0.088 ±0.012
Gastric adenomatous polypss group	0.074 ±0.013	0.229 ±0.028
Gastric cancer group	0.142 ±0.040	0. 280 ±0.068

2.3 Correlation of AIF and HSP70 mRNA expression in gastric adenoma and gastric cancer tissues

The correlation between AIF and HSP70 in gastric adenoma and gastric cancer was tested by Pearson correlation analysis. In gastric adenomatous polyps group, the expression of AIF mRNA was positively correlated with the expression of HSP70 mRNA ($r = 0.672$, $p < .01$). In gastric cancer group, there was a positive correlation between the expression of AIF mRNA and HSP70 mRNA ($r = 0.741$, $p < .01$, see Figure 3).

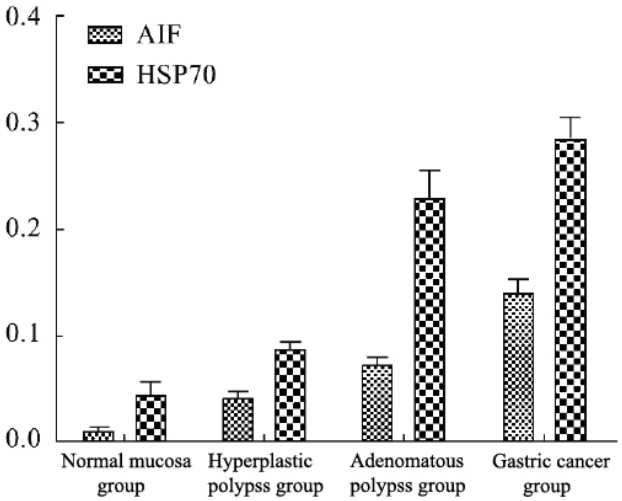

Figure 3: Correlation between the expressions of AIF mRNA and HSP70 mRNA in each group

3 Discussion

Gastric cancer is one of the most common digestive system malignancies, and it is one of the high incidence areas of gastric cancer in China. In our country, the incidence of gastric cancer ranks the second in malignant tumors and the death rate accounts for the third in malignant tumors,[6] which seriously affects the quality of life and safety of our citizens. Although the diagnosis and treatment technology of gastric cancer is progressing, the incidence and mortality of gastric cancer is still increasing year by year,[7] according to the recent 20 years' survey, there is a trend of younger age of onset. Due to the lack of typical symptoms at the early stage of gastric cancer, most of them are found in the middle and late stages. The quality and efficiency of surgery, radiotherapy and chemotherapy are relatively low, and the treatment effect is poor. Therefore, finding a way to diagnose gastric cancer in the early stage is particularly important. Gastric polyps are protrudes of the gastric mucosa to the gastric cavity. Currently, studies have shown that gastric hyperplastic polyps and gastric adenomatous polyps are cancerous tendencies and belong to precancerous lesions of gastric cancer. Therefore, the mechanism of canceration of

gastric polyps is of great importance for the diagnosis and treatment of early gastric cancer.[8,9] The occurrence of gastric cancer is caused by abnormal cell proliferation and differentiation and abnormal process of apoptosis. Apoptosis is a process of programmed cell death induced by gene control. Under normal circumstances, cells proliferation and apoptosis maintain a dynamic balance. Once the balance is broken, it can lead to the occurrence of malignancies. Much of human apoptosis is mediated and performed by the caspase family of enzymes, both extrinsic and endogenous.[10] AIF is a apoptosis-effector molecule that is independent of Caspase pathway-mediated apoptosis. Under normal physiological conditions, AIF is an oxidoreductase, located only in the mitochondrial membrane space. When the cells are stimulated by apoptotic signals, AIF is released from the mitochondria to the cytoplasm, after stimulated by apoptotic signals in the cytoplasm, AIF moves to the nucleus through complex localization process, which directly causes chromosome agglutination and DNA fragmentation in large fragments (50 kb), leading to apoptosis.[11] The study found that AIF mRNA expression in each tissue was gradually increased. Compared with the normal control group, the expression in gastric hyperplastic polypss group, adenomatous polypss group and gastric cancer group was higher, the difference was statistically significant. This is consistent with the results of the detection of AIF mRNA expression in 60 cases of gastric adenocarcinoma tissues by Lee et al.,[11] and matches the results of the expression level of AIF mRNA in gastric cancer tissues and corresponding normal tissues adjacent to the carcinoma by Zhao Y et al.[12]

HSP70 is a type of heat-shock proteins with a molecular weight of about 70 kD. It is a highly conserved cytoplasmic protein widely found in various human tissues. HSP70 is closely related to the proliferation, differentiation, invasion, metastasis, immune recognition and apoptosis of tumor cells. In the heat-shock protein family, HSP70 is the most conservative, but also the most important one, with the most intense reaction after cell stress. Therefore, it plays a very important role in the maintenance of cell structure and the tolerance of cells to stress. As a molecular chaperone, HSP70 is preferred to be expressed in tumor cells. By interacting with its client proteins, it plays an important role in cell signal transduction, cycle regulation, differentiation and apoptosis, so as to protect tumor cells from widespread apoptosis. HSP70 can also interact with the proto oncogene and tumor suppressor gene to regulate the cell cycle. At the same time, the protein products of the above gene are regulated by each other so that the malignant tumor is continuously proliferating. It was studied[13] that the proliferation of malignant tumor cells with selective removal of HSP70 was significantly slower. The over expression of HSP70 interferes with the signal transduction process of phospholipase A2 in tumor cells, which greatly reduces its cytotoxicity, thereby reducing the killing effect of tumor necrosis factor on specific tumor cells and preventing tumor cells from being cleared by the immune system. HSP70 is involved in the process of antigen presentation, which can control cells presenting antigen, antigen specific peptide decomposition and provide with proteins involved in antigen processing, cooperative transport and immunoglobulin assembly. The expression in various tumor tissues and cells in human body increases significantly. Over expression in breast, cervical, endometrial and bladder cancer cells makes the antitumor treatment less effective.[14] Selective down-regulation of HSP70 in tumor cells can inhibit the rapid proliferation and even induce apoptosis of tumor cells, suggesting that HSP70 may be an important gene to control the proliferation and apoptosis of tumor cells. We found that the expression of HSP70 mRNA in normal polyps group, gastric adenomatous polyps group and gastric cancer group was higher than that in normal control group, and the difference was statistically significant. The expression level in gastric cancer group was higher than that in gastric adenomatous polyps group, and the difference was statistically significant, which is basically the same with that of Liu ZY et al.[15] and Zuo DS et al.[16]

Study[17] showed that HSP70 could bind to AIF molecules and inhibit non-Caspases-dependent apoptotic pathway, and participate in the regulation of apoptosis pathway by inhibiting the formation of apoptotic bodies. Because AIF mediated apoptosis does not depend on Caspase pathway, its pro apoptotic activity is not inhibited by Caspase inhibitors or Bcl-2 overexpression. However, there are multiple horizontal crossovers between AIF and Caspases pathway. Activated Caspase-2, 8 and t-Bid (after Caspase stimulation) can trigger mitochondria to release AIF molecule.[18] HSP70 is another important intersection point between AIF pathway and Caspase pathway.[19] HSP70 Caspase plays a role through Apaf-1 as one of the inhibitors of activation pathway. Since AIF competes with Apaf-1 for binding to HSP70, theoretically, binding of AIF to HSP70 may indirectly release inhibition of Caspase cascade activation, Therefore, Caspase-dependent apoptosis and AIF-dependent apoptosis are both independent and interdependent, stimulating the different signals that determine their contribution to apoptosis, the same cell types also determine the apoptosis way. AIF promotes apoptosis, but the apoptosis of tumor cells is selective. The possible mechanism may be due to the resistance effect of tumor cells to AIF apoptosis. Another possibility is that AIF plays the role of eliminating free radicals and scavenging peroxidation toxicity in cells so that cells avoid peroxidation and continue to survive. In this study, the expression of AIF was positively correlated with the expression of HSP70 mRNA in the gastric adenomatous polyps group. In the gastric cancer group, the expression of AIF is positively correlated with the expression of HSP70 mRNA, suggesting that the AIF and HSP70 genes may be related to the formation of gastric cancer. HSP70 may impose a synergistic effect on AIF.

Conflicts of Interest Disclosure

The authors have no conflicts of interest related to this article.

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