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Basic Study

Effect of moxibustion on expressions of HSP70 mRNA and protein in gastric cancer-bearing rats

艾灸对胃荷瘤大鼠瘤体内HSP70 mRNA及其蛋白表达的影响

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Abstract

Objective: To observe the effect of moxibustion on the mRNA and protein expressions of heat-shock protein 70 (HSP70) in gastric cancer-bearing rats.

Methods: A total of 40 healthy Sprague-Dawley (SD) rats were adaptively fed for one week. The gastric cancer model was prepared by Walker-256 cancer tissue transplantation. After 7 d, 10 rats were randomly selected to verify the successful modeling, and the remaining 30 rats were divided into a model group, a moxibustion group and an infrared group by the random number table method, with 10 rats in each group. After enrollment, the moxibustion group received suspended moxibustion at Zhongwan (CV 12), Guanyuan (CV 4) and bilateral Zusanli (ST 36), (the first group of acupoints) on the 1st day, and suspended moxibustion at bilateral Pishu (BL 20) and Weishu (BL 21), (the second group of acupoints) on the 2nd day, 20 min each time, once a day. Moxibustion was alternately performed every other day at the two groups of acupoints for 21 d. From the day of enrollment, rats in the infrared group were irradiated with the infrared radiation at the stomach area on the 1st day, and at the T_{12}-T_{13} interspinous region on the 2nd day, 20 min each time, once a day, and the two locations were alternately irradiated every other day for 21 d. During the treatment, rats in the model group were intervened by grasping and fixation without treatment. At the end of the treatment, blood was collected from the inner eye orbit, and the HSP70 expression in peripheral blood was determined by enzyme linked immunosorbent assay (ELISA). Rats were sacrificed, the tumor volume and growth inhibition rate were measured. The position and changes of HSP70 in gastric cancer were observed by streptavidin-perosidase (SP); HSP70 protein expression was determined by ELISA; HSP70 mRNA expression in cancer tissues was determined by reverse transcription-polymerase chain reaction (RT-PCR) assay.

Results: In comparison of the model group, the volume growth of the gastric cancer in the moxibustion group was significantly restricted (P<0.01); the volume growth inhibition rate in the moxibustion group was 37.93%; the HSP70 expression in peripheral blood and the cancer tissues was significantly increased (both P<0.01); the expression of HSP70 mRNA and HSP70 content in gastric tumor were both obviously increased in the moxibustion group (P<0.01); and a large amount of HSP70 was released to the outside of cancer cells in the moxibustion group. In comparison of the model group, the volume growth of the gastric cancer in the infrared group was slightly restricted (P<0.05) with a volume growth inhibition rate of 15.89%; the HSP70 expression in the infrared group was increased significantly in peripheral blood (P<0.01) and in the gastric cancer tissues (P<0.05); more HSP70 was released outside of the cancer cells in the infrared group. In comparison of the infrared group, the volume growth of gastric cancer was more restricted in the moxibustion group (P<0.05), and the HSP70 expression in the gastric cancer tissues was also higher (P<0.05); more HSP70 was released outside of the cancer cells in the moxibustion group.

Conclusion: Moxibustion and infrared treatment inhibit the gastric cancer growth in the gastric cancer-bearing rats, up-regulate the HSP70 expression in gastric cancer tissues, and promote the production and extracellular release of HSP70, and the effect of moxibustion is more obvious.

Keywords: Acupuncture-moxibustion Therapy; Moxibustion Therapy; Moxa Stick Moxibustion; Neoplasms; Stomach; HSP70 Heat-shock Proteins; Rats

【摘要】目的：观察艾灸对胃荷瘤大鼠瘤体内热休克蛋白70 (HSP70) mRNA及其蛋白表达的影响。**方法**：四十只健康Sprague-Dawley (SD)大鼠适应性喂养一周后，采用Walker-256瘤组织胃部移植制备胃荷瘤模型，7 d后随机选取10只验证造模成功，剩余30只采用随机数字表法分入模型组、艾灸组和红外组，每组10只。自入组当日起，艾灸组第1日悬灸中脘、关元、双侧足三里(第一组穴位)，第2日悬灸双侧脾俞和胃俞(第二组穴位)，每次施灸20 min，每

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日1次，两组穴位隔日交替施灸，连续21 d。自入组当日起，红外组第1日用红外线照射胃脘部，第2日照射背部T_{12}-T_{13}棘突间区域，每次照射20 min，每日1次，两个部位隔日交替照射，连续21 d。治疗期间，模型组动物陪同抓取、固定，不予其他处理。治疗结束后，眶内采血，采用酶联免疫吸附试验(ELISA)测定外周血中HSP70含量。处死动物，测算肿瘤体积及生长抑制率。采用链霉菌抗生物素蛋白-过氧化物酶连结实验(S-P)观测胃瘤体中HSP70的表达位置及变化，ELISA测定HSP70含量，逆转录-聚合酶链反应(RT-PCR)测定瘤体内HSP70 mRNA表达水平。**结果**：与模型组比较，艾灸组胃部瘤体体积增长明显受限(P＜0.01)，体积增长抑制率为37.93%，外周血中HSP70含量明显增加(P＜0.01)，胃部瘤体内HSP70 mRNA表达及HSP70含量均明显增加(均P＜0.01)，大量HSP70释放到癌细胞外；与模型组比较，红外组胃部瘤体体积增长稍受限(P＜0.05)，增长抑制率为15.89%，外周血中HSP70含量明显增加(P＜0.01)，胃部瘤体内HSP70 mRNA表达及HSP70含量稍增加(均P＜0.05)，较多HSP70释放到癌细胞外；与红外组比较，艾灸组胃部瘤体体积增长受限更明显(P＜0.05)，胃部瘤体内HSP70 mRNA的表达量及HSP70含量更高(均P＜0.05)，释放到癌细胞外HSP70也更多。**结论**：艾灸及红外线治疗均能一定程度抑制胃荷瘤大鼠的瘤体增长，上调胃部瘤体内HSP70 mRNA的表达，促进HSP70的生成及细胞外释放，且艾灸的作用更明显。

【关键词】针灸疗法；灸法；艾条灸；肿瘤；胃；热休克蛋白 70；大鼠

【中图分类号】R2-03　　**【文献标志码】**A

Gastric cancer significantly threatens human health. There were 1 million new gastric cancer cases worldwide, and more than 780 000 people died from gastric cancer in 2018[1]. With the development of social economy and the improvement of people's living standard, the incidence of gastric cancer has declined in most countries worldwide. However, due to the large population and the increasing aged population, the burden of gastric cancer in China is still heavy[2].

Although surgery-based and chemotherapy and radiotherapy assisted treatment is the main cancer treatment principle, new technologies have been prompted to be explored due to its limitations and side effects. In recent years, because cancer cells are more sensitive to high temperature than normal cells, researchers have enhanced the body's immunity and inhibited cancer growth after increasing the temperature of cancer nests via infrared and microwave irradiation *in vitro*, which has become the fifth largest anticancer therapy[3]. As an important component of traditional Chinese medicine (TCM), moxibustion has made great contributions to the people's health for thousands of years. Modern research believes that the integrated light is mainly composed of infrared rays released when the wormwood leaves are burning[4]. The energy and the material composition in the tissues can be changed when these lights irradiate the acupoints, which will result in the sensory excitement and a response based on the neuro-endocrine-immune system, and in turn generate a multi-system comprehensive response through a complex regulatory network in the human body to achieve prevention and treatment of diseases. Our research team has been working on the prevention methods and mechanisms of moxibustion for stomach- related diseases. Previous studies have confirmed that moxibustion can protect the gastric mucosa in gastritis caused by different factors via up-regulating the expression of heat-shock protein 70 (HSP70) in gastric mucosa[5-7]. The latest research found that moxibustion could enhance the immune function of gastric cancer- bearing animals and improve their survival status[8-10]. HSP70 has an immunoadjuvant-like effect during the anti-cancer process since it is a heat stress protein[11]. Does moxibustion promote the expression of HSP70 in gastric cancer-bearing animals, and then inhibit the growth of tumor volume? The following exploration was made to answer these questions.

1 Materials and Methods

1.1 Laboratory animals and grouping

Six healthy male Sprague-Dawley (SD) rats, weighing 160-180 g, were used for solid tumor tissue preparation. Forty healthy male SD rats in SPF grade, weighing 200-240 g, were used for grouping and experiments.

Grouping method: After the 40 healthy SD rats were adaptively fed for one week, the gastric cancer model was prepared using gastric transplantation with Walker-256 cancer tissue. After 7 d, 10 rats were randomly selected to verify the success of modeling. After successful modeling, the remaining 30 rats were randomly divided into a model group, a moxibustion group and an infrared group by random number table method, with 10 rats in each group.

All rats were provided by the China Food and Drug Administration Research Institute [qualification number: SCXK (Beijing) 2014-0013], and kept in cages at the Animal Experimental Center of the Cancer Hospital of the Chinese Academy of Medical Sciences, with the feeding temperature at 20-25 ℃, humidity 50%-70%. Rats were treated in accordance with the relevant ethical regulations throughout the experiment.

1.2 Main instruments and reagents

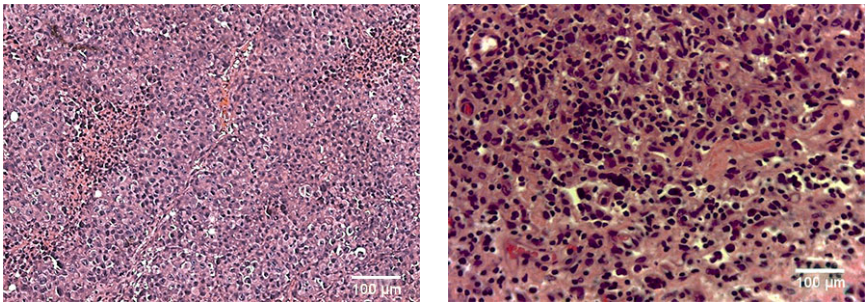
6002B infrared therapeutic instrument (Xuzhou Tianfei Electronic Equipment Co., Ltd., China); digital surface thermometer (Cixi Boshio Instrument Co., Ltd., China); ABI 3900 benchtop high throughput DNA synthesizer (Sangon, China); model 7300 fluorescence quantometer (ABI, China); C1000 thermal cycler polymerase chain reaction (PCR) instrument (BIO-RAD, USA); Scanspeed 1730R refrigerated centrifuge

(LaboGene, Denmark); moxa stick of 0.7 cm in diameter (Changsha Aiyi Biotechnology Co., Ltd., China).

Walker-256 cells (Cancer Hospital, Chinese Academy of Medical Sciences, China); sodium pentobarbital (Central South Chemical Industry, China); diethyl pyrocarbonate (DEPC) solution (Sigma, USA); trizol reagent for total RNA extraction (Invitrogen, USA); RR820A fluorescence quantification kit, RR047A reverse transcription kit (Takara, China); Ab2787 HSP70 antibody (Abcam, USA); CSB-E08308r HSP70 enzyme linked immunosorbent assay (ELISA) detection kit (Sino-American Biotechnology Co., Ltd., China).

1.3 Animal model preparation method

Animal models were prepared by orthotopic transplantation of cancer tissues according to the references[12-13]. In brief, three rats with a body mass of 160-180 g were intraperitoneally injected with 1 mL (6×10^6 cells/mL) Walker-256 cancer cells. After the formation of ascites, the rats were sacrificed, and the cancer cells in the ascites were collected. And 0.2 mL (5×10^6 cells/mL) ascites was injected subcutaneously into the anterior and hind limbs of three rats with a body weight of 160-180 g. After 7 d, a subcutaneous solid tumor (about 1.5 cm×1.5 cm×1.0 cm) at the injection site was formed. The rats were sacrificed; the fish-like cancer tissue of about 0.5 cm×0.5 cm×0.5 cm was isolated and transplanted into the junction between the dermatome area and the glandular area of the arcus major ventriculi in the 40 rats with the body weight of 240 g. After 7 d, 10 rats were randomly selected for laparotomy detection. Tumor formed at the original transplant site, with a diameter of about 1 cm, smooth surface, clear boundary, without adhesions and organ invasion, confirmed homologous histology to the subcutaneous solid tumor by microscopy (Figure 1), indicated the successful modeling. The random check results showed a 100% successful rate.



Subcutaneous cancer　　Gastric cancer

Figure 1. Cancer tissue [hematoxylin-eosin (HE) staining, ×100]

1.4 Intervention methods

1.4.1 Model group

Only received modeling with grasping during the intervention period without other treatment.

1.4.2 Moxibustion group

Acupoints: Acupoints were divided into two groups. The first group included Zhongwan (CV 12), Guanyuan (CV 4) and bilateral Zusanli (ST 36); the second group included bilateral Pishu (BL 20) and Weishu (BL 21). Acupoints were determined according to the commonly used animal acupoint positioning combined with anthropomorphic method[14]. The intersection of the lower 1/4 and the upper 3/4 on the line connecting the sternoclavicular joint and the pubic symphysis was defined as the umbilicus. Zhongwan (CV 12) was located at the midpoint of the line connecting the umbilicus and the ensiform process of sternum; Guanyuan (CV 4) was located at the intersection of the 3/5 and 2/5 on the line connecting the umbilicus and the upper edge of the pubic symphysis; Zusanli (ST 36) was located on the posterolateral side of the knee joint, approximately 5 mm below the capitulum fibulae; Pishu (BL 20) was located between the ribs both sides under the spinous process of the 12th thoracic vertebra; Weishu (BL 21) was located between the ribs both sides under the spinous process of the 13th thoracic vertebra.

Method: Moxibustion treatment began on the same day after enrollment. On the 1st day, rats were fixed in a supine position for moxibustion at Zhongwan (CV 12), Guanyuan (CV 4), and the bilateral Zusanli (ST 36); on the 2nd day, rats were fixed in a prone position for moxibustion at bilateral Pishu (BL 20) and Weishu (BL 21). When moxibustion was applied, a moxa stick of 7 mm in diameter was ignited vertically above the acupoints. The surface temperature was monitored by a surface thermometer, the ash was removed in time, the moxibustion distance was adjusted to ensure the skin temperature at (42±1) ℃. Moxibustion was maintained for 20 min each time, once a day for 21 d.

1.4.3 Infrared group

Location: In the stomach area and the T_{12}-T_{13} area on the back.

Methods: Infrared irradiation treatment was started on the same day after enrollment. On the 1st day, rats were fixed in a supine position to irradiate the stomach area; on the 2nd day, rats were fixed in a prone position

to irradiate the back area. The infrared wavelength was 0.4-3.0 μm with level 1 intensity, 3 cm diameter spot. The surface temperature was monitored by a surface thermometer, the irradiation distance was adjusted to ensure the skin temperature at (42±1) ℃. Irradiation was maintained for 20 min each time, once a day for 21 d.

1.5 Statistical processing method

All data were processed using SPSS 20.0 software. The measurement data were tested for normality and homogeneity of variance. The normal distribution data were expressed as mean ± standard deviation ($\bar{x} \pm s$). One-way analysis of variance was used for comparison among groups. The least significant difference (LSD) method was used for data with variance homogeneity, and the Dunnett T3 method was used for data with uneven variance. $P<0.05$ indicated that the difference was statistically significant.

2 Observation Items and Detection Methods

2.1 Serum HSP70 level (ELISA)

On the 22nd day of the experiment, the rats in each group were fasted for 12 h, and 3 mL of blood was collected from the inner orbit. The blood samples were kept in the coagulation tube for 2 h, and then centrifuged at 4 ℃ and 2 000 r/min for 20 min in a constant temperature centrifuge. Added samples into the ELISA plate and incubated for 2 h at room temperature. After washing the plate for 5 times, added 100 μL of enzyme-linked affinity, incubated for 1 h at room temperature; washed the plate, added 100 μL of substrate solution, incubated for 10 min at room temperature in the dark; and finally added 50 μL of stop solution to stop the reaction. The absorbance (A) at 450 nm was measured and 540 nm was set as the correction wavelength. A standard curve was made based on the concentration of the standards and the corresponding absorbance. The protein concentration of the interested sample was estimated from the optical density value.

2.2 Tumor growth inhibition rate

After the blood was collected, the rats were sacrificed, and the abdominal cavity was opened in a sterile environment; the pylorus and the cardia were ligated with a surgical line; the whole stomach was cut between the two ligatures, gently washed with the PBS solution, and the excess water was absorbed with sterile gauze. The stomach was spreaded on the shaped filter paper to expose the tumor site. The longest diameter (a) and the shortest diameter (b) of the gastric cancer were measured with an electronic vernier caliper, and the volume of the tumor was calculated according to the formula $V = (\pi/6) \times [(a+b)/2]^3$. The tumor growth inhibition rate was calculated according to the following formula: Inhibition rate (IR) = [1 − (Average tumor weight of the moxibustion group or the infrared group ÷ Average tumor weight of the model group)] × 100%.

2.3 HSP70 level in gastric cancer (immunohistochemistry S-P method)

Cancer tissues of about 5 mm×5 mm×5 mm on the stomach wall of each group were isolated. Conventional paraffin embedding, section, xylene dewaxing, gradient alcohol hydration were performed; followed by boiling for 15 min on the electric furnace in a citric acid buffer solution of 0.01 mol/L, pH 6.0; inactivated the endogenous enzyme at 37 ℃ with 3% H_2O_2 for 10 min, rinsed 3 times with PBS for 5 min; incubated with normal sheep serum (37 ℃, 10 min) and HSP70 mAb at 4 ℃ overnight; washed with PBS for 3 times and 5 min/time; added biotinylated secondary antibody in immunohistochemistry kit, incubated for 45 min at room temperature; washed 3 times with PBS for 5 min/time; finally applied DAB staining, hematoxylin counterstaining and neutral gum sealing. The positive signal was the appearance of yellow or brownish yellow particles in the cells. Images of three visual fields in each group of cancer tissues were collected under a 400-fold microscope using the Beihang Medical Image Analysis System, and the total number and density of the positive targets (total number of positive targets/total field of statistical fields) were analyzed.

2.4 HSP70 level in gastric cancer (ELISA)

The tumor tissue (100 mg) from the stomach wall was rinsed with PBS. After the water was absorbed, the lysate was added at a ratio of 10 mL/g to prepare a homogenate. Centrifuged for 15 min at 4 ℃ and 2 500 r/min, and collected the supernatant. Added 100 μL of the homogenate supernatant to the ELISA plate for incubation and reaction, and the instructions of the kit were strictly followed.

2.5 HSP70 level in gastric cancer (reverse transcription-polymerase chain reaction, RT-PCR)

Total RNA in 5 mm×5 mm×5 mm cancer tissues from the stomach wall of the model, the moxibustion and the infrared groups were isolated using RNase-free DNase® (Promega) kit, and the RNA integrity was checked. The target gene was amplified. HSP70 forward primer: 5′-ATCCAGTGTTCCGTTTCCAG-3′; HSP70 reverse primer: 5′-GTAGCTCACCTGCACCTTG-3′. The length of the amplified product was 384 bp. At the end of the PCR reaction, the amplified product was detected by 1.5% agarose gel electrophoresis. The images of the amplified bands were observed using an AlphaImager 2000 gel imaging system. The band gray of the electrophoresis images was analyzed with the Spot Density quantitative software in the system; the ratio of HSP70 to β-actin in the tissues was designated as the relative expression of HSP70 mRNA.

3 Results

3.1 Volume and growth inhibition rate of gastric cancer

No rat died during the intervention period. After the intervention, the rats were sacrificed followed by laparotomy detection. Collapsed and disappeared tumors were identified in rats of each group, including one in the model group, 3 in the moxibustion group, and 2 in the infrared group. Nine rats, 7 rats, and 8 rats of the model group, the moxibustion group and the infrared group respectively still showed gastric cancer at the end of the treatment.

After calculation, the volume growth inhibition rate of the gastric cancer in the moxibustion group was 37.93%, and in the infrared group was 15.89%. Compared with the model group, the volume of the gastric cancer in the moxibustion group was significantly reduced (P<0.01). The volume of the gastric cancer in the infrared group was slightly reduced, and the difference between the groups was statistically significant (P<0.05). Compared with the infrared group, the volume of the gastric cancer in the moxibustion group was decreased, and the difference between the groups was statistically significant (P<0.05), (Table 1).

Table 1. Comparison of tumor volume ($\bar{x}$ ±s, mm^3)

Group	n	Tumor volume
Model	9	1353.9±265.9
Moxibustion	7	840.4±167.8[2)3)]
Infrared	8	1138.7±294.1[1)]

Note: Compared with the model group, 1) P<0.05, 2) P<0.01; compared with the infrared group, 3) P<0.01

3.2 Serum HSP70 level (ELISA)

Compared with the model group, the serum HSP70 level in the moxibustion and the infrared groups was statistically significantly higher (P<0.01); there was no significant difference in the peripheral blood HSP70 level between the infrared group and the moxibustion group (P>0.05), (Table 2).

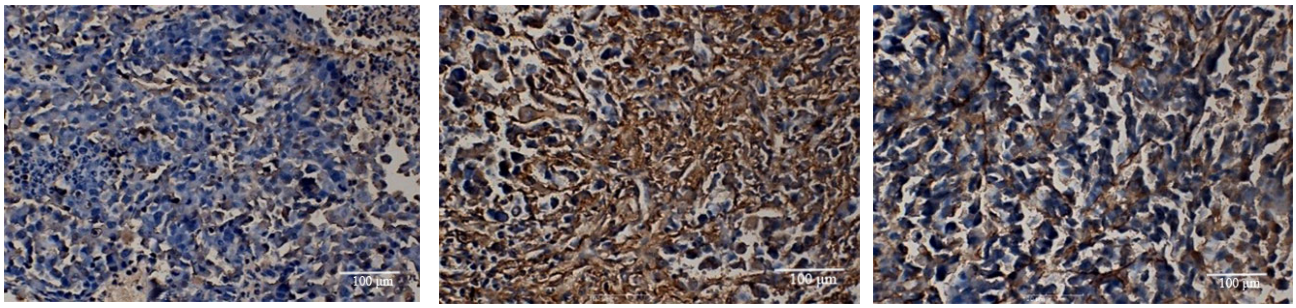
Table 2. Comparison of the serum HSP70 level of each group ($\bar{x}$ ±s, pg/mL)

Group	n	HSP70
Model	10	4375.27±3388.16
Moxibustion	10	18552.41±10218.52[1)]
Infrared	10	13237.04±5768.22[1)]

Note: Compared with the model group, 1) P<0.01

3.3 Expression of HSP70 in gastric cancer (S-P method)

In the model group, weakly positive expression of HSP70 was observed in the gastric cancer cells of the gastric cancer tissues, and the expression outside the cancer cells was less. In the moxibustion group, HSP70 was mainly expressed outside the cancer cells in the cancer tissues, and showed strong positive expression inside the cancer cells and significantly strong positive expression outside the cancer cells; the expression of HSP70 inside and outside the cancer cells was significantly stronger than that of the model group. HSP70 expression in the infrared group was more evenly distributed inside and outside the gastric cancer cells, and both were strong positive expression and slightly stronger than the model group (Figure 2).



Model group　　Moxibustion group　　Infrared group

Figure 2. Expression of HSP70 in tumor tissues (immunohistochemistry S-P method, ×100)

Compared with the model group, the total number and density of HSP70 positive cells in the gastric cancer tissues of the moxibustion group increased significantly (P<0.01); the total number and density of HSP70 positive cells of the infrared group increased (P<0.05). The total number and density of HSP70 positive cells in the gastric cancer tissues of the moxibustion group were higher than those of the infrared group (P<0.05), (Table 3).

3.4 HSP70 level in gastric tumor (ELISA)

Compared with the model group, the HSP70 expression in the gastric cancer of the moxibustion group was significantly increased (P<0.01). The HSP70 expression in the gastric cancer of the infrared group was increased slightly, and the inter-group difference was significantly (P<0.05). Compared with the infrared group, the HSP70 expression in the gastric cancer of the moxibustion group was higher, and the inter-group

difference was significant (P<0.05), (Table 4).

Table 3. Comparison of HSP70 positive expression in gastric tumors of each group ($\bar{x}$ ±s)

Group	n	Total number of positive cells	Density (positive cells/mm^2)
Model	27	941.11±111.2	675.8±79.8
Moxibustion	21	1543.78±231.5[2)3)]	1108.5±166.2[2)3)]
Infrared	24	1160.22±191.82[1)]	833.1±166.2[1)]

Note: Compared with the model group, 1) P<0.05, 2) P<0.01; compared with the infrared group, 3) P<0.01

Table 4. Comparison of HSP70 expression in gastric cancer of each group ($\bar{x}$ ±s, pg/mL)

Groups	n	HSP70
Model	9	15.22±4.98
Moxibustion	7	28.77±8.63[2)3)]
Infrared	8	22.18±3.54[1)]

Note: Compared with the model group, 1) P<0.05, 2) P<0.01; compared with the infrared group, 3) P<0.01

3.5 Expression of HSP70 mRNA in gastric cancer

Compared with the model group, the relative expression of HSP70 mRNA in the moxibustion group was statistically significantly increased (P<0.01); the relative expression of HSP70 mRNA was increased slightly in the gastric cancer of the infrared group, and the difference between the groups was statistically significant (P<0.05). Compared with the infrared group, the relative expression of HSP70 mRNA in the moxibustion group was higher, and the difference between the groups was significant (P<0.05), (Table 5).

Table 5. Comparison of HSP70 mRNA expression in gastric cancer of each group ($\bar{x}$ ±s)

Group	n	HSP70 mRNA
Model	9	2.378±0.387
Moxibustion	7	3.434±0.67[2)3)]
Infrared	8	2.824±0.432[1)]

Note: Compared with the model group, 1) P<0.05, 2) P<0.01; compared with the infrared group, 3) P<0.01

4 Discussion

Heat-shock proteins (HSPs) are special proteins that are induced under the stress conditions such as high fever and infection. HSPs are widely distributed in different populations and different cell parts. HSPs can be divided into high molecular weight families, HSP90, HSP70, HSP60 families and small molecular weight families according to their molecular weight and degree of homology. Among them, HSP70 is considered to be the most important one with the most in-depth investigation. HSP70 plays a role of double- edged sword in the oncogenesis and cancer treatment. According to the distribution in the tissues, HSP70 can be divided into the intracellular HSP70 and extracellular HSP70 (eHSP70). As a molecular chaperone, intracellular HSP70 is mainly involved in the folding, conformation, repair and transmembrane transport of proteins, and highly expressed during stress to protect the cancer cells[15]. As a danger signal of the immune system, eHSP70 plays an important role in tumor immune killing. In vitro studies have shown that eHSP70 can activate natural killer (NK) cells and macrophages to promote the release of anti-cancer cytokines, thus to enhance the body's non-specific killing of tumor cells[11,15]; activate the body's specific immunity against tumors by promoting the recognition of antigen-presenting cells and T lymphocytes[16]. *In vivo* experiments showed that the immune activation and anti-tumor effects of eHSP70 were more obvious. On one hand, eHSP70 can induce dendritic cell maturation, up-regulation of co-stimulating factor and histocompatibility complex expression to enhance the antigen presentation[17]; can activate macrophages and promote the secretion of cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1)[18]; can activate NK cells to promote their cytolysis, proliferation and migration abilities[19]; can stimulate the activation of antigen-dependent T cells and promote the secretion of interferon-γ (IFN-γ) to mediate tumor cell killing[20]. On the other hand, membrane-bound HSP70, as a tumor-specific antigen, is recognized by pre-activated NK cells and actively promotes the recognition of cytotoxic T cells and antigen-presenting histocompatibility complex Ⅰ, thereby activating cytotoxic T cells to kill the tumor cells; it also promotes the recognition of helper T cells and histocompatibility complex II, and promotes the uptake and treatment of antigen by CD4 helper T cells, thereby promoting specific killing of tumor cells[21]. However, under normal conditions, HSP70 is mainly located inside the tumor cells with very few in the extracellular space and blood. The promoted release of HSP70 from tumor cells can greatly advance the anti-cancer effect of autoimmune system.

This study found that after moxibustion and infrared treatment, the tumor growth of gastric cancer-bearing rats was inhibited significantly, and the HSP70 expression in peripheral blood increased significantly compared with that in the model group, suggesting that moxibustion and infrared treatment can increase the HSP70 expression in the peripheral blood, or it is the potential cause of previously observed activation of NK cells, helper T cells and cytotoxic T cells.

The expression location and level of HSP70 in gastric cancer were detected by immunohistochemistry, ELISA and RT-PCR, which indicated that moxibustion and infrared treatment could promote the expression and

extracellular release of HSP70, while the effect of the moxibustion group was more obvious. It is suggested that the inhibition of moxibustion and infrared treatment on the tumor growth of gastric cancer-bearing rats may be related to the promotion of intracellular synthesis and extracellular release of HSP70, but the specific pathway and mechanism involved remain unclear. In addition, whether the high HSP70 expression in the gastric cancer can inhibit the invasion of cancer cells into the gastric mucosa and its protective effect need further study. Furthermore, in this study, the cancer of one rat in the model group disappeared completely, which is worthy of further exploration that what relationship is between it and NK cells in the peripheral blood, helper T cells in the T lymphoid subpopulation, and cytotoxic T cells identified in the previous study.

Conflict of Interest

There was no potential conflict of interest in this article.

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Statement of Human and Animal Rights

The treatment of animals conformed to the ethical criteria in this experiment.

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