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Effect of Invigorating Spleen Herbs on Apoptosis of Transplanted Human Gastric Cancer Cells in Nude Mice

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Abstract: Gastric cancer is the most common disease among various malignant tumors in China. Moreover, the incidence rate of gastric cancer is obviously different from that of gender and age. The incidence of gastric cancer in the northwest and east coastal areas is significantly higher than that in the south. The incidence of gastric cancer is 50 years old, and the ratio of male to female is about 2:1. However, with the development of economy, many people's diet structure has changed, and the work pressure is increasing, which leads to the younger gastric cancer. Therefore, the study of gastric cancer cells is very important. In view of the current social situation, this paper proposed the possible relationship between spleen strengthening Chinese medicine and gastric cancer cells, and studied the nude mice as the experimental model of the experiment. In this paper, the spleen invigorating traditional Chinese medicine compound coix seed, Atractylodes macrocephala and Jianpi pill were used as the basis of spleen strengthening drugs. In this study, nude mice with human gastric cancer cells were used as the model, and the subcutaneous tissue of nude mice was injected into the tumor model. The apoptosis of human gastric cancer cells in nude mice was observed. The antitumor effect, apoptosis rate and morphological changes of tumor cells were observed by terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL). The content of VEGF protein was detected by ELISA, and the activities of Mg2 + - ATPase and G-6-Pase in lateral tissues were detected by chromogenic method. The results showed that the number of gastric cancer cells in nude mice induced by Jianpi traditional Chinese medicine was much less than that in nude mice without spleen invigorating Chinese medicine, which indicated that spleen strengthening Chinese medicine had a good inhibitory effect on gastric cancer cells, and inducing apoptosis of gastric cancer cells might be one of the mechanisms of anti-cancer effect of spleen strengthening Chinese medicine.

1. Introduction

Gastric cancer refers to the primary malignant tumor on the gastric mucosa, which is one of the most common malignant tumors in the world. Gastric cancer is one of the main causes of cancer related deaths worldwide. The incidence rate of gastric cancer ranks the first in the world, and the mortality rate is third in the world. The incidence rate and mortality rate of gastric cancer have become the second malignant tumors in China. However, the treatment of gastric cancer is still limited, and the emergence of gastric cancer drug resistance has led to the emergence of new anti-cancer drugs and combined chemotherapy. Surgery, radiotherapy and chemotherapy are commonly used methods for gastric cancer treatment at home and abroad, but surgical treatment is mainly for patients with early gastric cancer. Radiotherapy and chemotherapy will damage the immune function of human body and cause serious damage to the healthy qi of human body. The quality of life of patients and reduce the life rate of patients. At present, there are few chemoradiotherapy drugs for gastric cancer compared with other diseases, and only 5-fluorouracil (5-FU) and cisplatin are used in patients with advanced gastric cancer. The reason for gastric cancer is often unknown. The early clinical manifestations and incidence rate of gastric cancer are usually relatively stable. The main symptoms are stomach pain, nausea, vomiting, loss of appetite, weight loss and fatigue. At present, the pathogenic factors of gastric cancer cannot be determined clinically, but the main pathogenic factors are as follows: carcinogenic pollution of nitrosamines; formation of polycyclic aromatic hydrocarbons in smoked fish, smoked mutton and roasted fish; Helicobacter pylori infection; high salt content and excessive intake of salt in diet; alcoholism, smoking, food pollution; depression and family heredity. Therefore, for the sake of our own health and the health of our families, we should try our best to tell ourselves and the people around us that we should not smoke or drink, eat less fried and barbecue food, eat more light food and keep a happy mood.

At present, the basic research of gastric cancer mainly focuses on the molecular mechanism, pathogenicity and pathogenic factors of Helicobacter pylori, immune escape of gastric cancer stem cells and tumor cells, and genetic polymorphism. Among them, Helicobacter pylori infection is the most accurate risk factor for gastric cancer. However, cancer is characterized by proliferation of malignant cells, survival of cell division cycle, proliferation control center and prevention of genetic diseases. Factors such as apoptotic proteins are involved in various oncogene and protein molecule events, and p53 and MAPK signal transduction types activate multiple signal transduction pathways and accelerate malignant tumor relationship between abnormal expression and tumor cell proliferation, differentiation, apoptosis imbalance. Therefore, targeting cell cycle regulators (PS3, cyclin, CDK, cdc25, etc.), inducing proteins and signal transduction pathways regulating cell death, and inhibiting malignant proliferation of tumor cells have become important potential targets for the treatment of gastric cancer.

In the development of gastric cancer, the ancient Chinese medicine thought that spleen yang weakness, that is, the stomach of chronic disease due to spleen nourishing stomach failure, cannot be mild treatment of internal organs caused by pathological changes, in addition, Yang Qi is not good, resulting in heavy moisture in the body. According to the viewpoint of ancient Chinese medicine, we think that warming Yang method and invigorating spleen Chinese medicine have an important role in the treatment of gastric cancer. In the field of traditional Chinese medicine, after thousands of years of experience, traditional Chinese medicine has developed a strict compatibility principle based on the overall concept, regulating the function of internal organs through various aspects, and it is rich in resources and cheap in price, suitable for those who need long-term use, which can make up for the shortcomings of modern medicine. However, traditional Chinese medicine treatment of gastrointestinal malignant malnutrition is still in the research stage, has not yet formed a complete theoretical system. In the study of the occurrence and development of gastric

cancer, traditional Chinese medicine has played an important role in the prevention and treatment of gastric cancer. In addition to reducing toxins, enhancing physical fitness, stabilizing the number of cancer cells, and improving the medium and long-term survival period, Chinese herbal medicine and extracts are considered as potential important sources to inhibit gastric cancer cell mutation and replace radiotherapy and chemotherapy reagents.

Based on the treatment of gastric cancer in modern medicine, how to improve the level of nutritional treatment is a big problem for clinicians. Modern medicine mainly uses sports training and physical rehabilitation to treat malnutrition caused by malignant tumor. However, nutritional therapy mainly requires patients to use the right medicine. The high dependence and high cost of nutrition reagents bring great pressure to patients' psychology and economy. Therefore, the clinical application of this method is limited. First of all, we studied the spleen invigorating traditional Chinese medicine and the pathological basis of gastric cancer cells. The nude mice with gastric cancer cells injected subcutaneously were used to observe the DNA degradation of cell chromosomes, the protein status of human vascular endothelial growth factor (VEGF), and the activity of Mg2 + - ATPase and animal cell g-6-p enzyme. The results showed that the gastric cancer cells in nude mice treated with spleen invigorating herbs were necrotic to a certain extent. This experiment also proved that the spleen invigorating traditional Chinese medicine has a certain inhibitory effect on gastric cancer cells.

2. Theoretical Basis and Core Concepts

2.1. Brief Introduction of Spleen Strengthening Traditional Chinese Medicine

Invigorating spleen is a TCM term, which refers to a method of strengthening spleen and spleen. It is suitable for emphysema caused by weak temper, abdominal distension, and sparse stool, loss of appetite and fatigue of limbs. Commonly used prescription drugs such as Shenling Baizhu powder, Xiangsha Liujunzi Decoction, etc. The traditional Chinese medicine for invigorating the spleen is also one of the tonics, also known as tonifying the spleen and benefiting the spleen. Spleen deficiency is often manifested as yellowing, fatigue, fatigue, listlessness and laziness, loss of appetite, abdominal distension after eating, sparse stool, pale tongue coating and weak pulse. Common spleen strengthening herbs are Codonopsis pilosula, lotus seed, Atractylodes macrocephala, Poria cocos, yam, coix seed and so on. For those with spleen deficiency, it is suggested to eat food with spleen strengthening effect; to eat less or sweet food and nutritious and easily digestible food; to avoid eating natural cold food, otherwise it is easy to destroy the temper; to avoid eating thick and greasy food, otherwise it is easy to hinder the transmission function of temper; to avoid eating food that is beneficial to eating and easy to destroy the temper. Among the main foods suitable for people with spleen deficiency, they can choose to eat japonica rice, indica rice, corn, job's tears, sweet potato, bean curd, etc.; the meat, eggs and milk mainly include beef, chicken, rabbit meat, tripe, pig tripe, mandarin fish, black chicken, etc.; the vegetables available are lotus root, corn, yam, lentils, cowpea, carrot, potato, onion, Pleurotus ostreatus, etc. There are grape, jujube, peach, and apricot and so on.

In the diet, should avoid natural cooling and easy to damage the temper of food; should avoid eating a strong greasy taste, easy to hinder the function of the spleen; and should avoid eating good and easy to destroy the temper of food. For example, balsam pear, etc. The traditional Chinese medicines used in this experiment are coix seed, Atractylodes macrocephala and Jianpi pill. Coix lacryma jobI is the dry mature seed kernel of gramineous plant Coix. When the fruit matures in autumn, the plant is harvested and dried to remove the shell, yellowish brown seed coat and impurities, and the seed kernel is collected. The main medicinal part of Coix seed is the dry and

mature seed kernel of plant, which can promote water penetration and moistening, strengthen spleen and stomach and diarrhea, eliminate paralysis, expel pus, expel toxin and disperse knot. It is mainly used for edema, beriberi, urination, spleen deficiency, diarrhea, damp heat contraction, cancer. Atractylodes macrocephala Koidz is an important bulk Chinese herbal medicine, which has the functions of nourishing blood and spleen, strengthening stomach and dampness, stopping sweating and hemostasis. The rhizome of Atractylodes macrocephala contains volatile oil. The main components of the oil are atractylone, atractylol, atractylolide, etc. It is effective in the treatment of cirrhosis ascites, primary liver cancer, Meniere's syndrome, chronic low back pain, acute enteritis and leukopenia. Atractylodes macrocephala is widely used. In addition to drug formulations, it is also an important raw material for more than 40 kinds of Chinese patent medicine preparations. Jianpi pill is the name of traditional Chinese medicine. It has the effect of invigorating the spleen and eliminating food. It is mainly used to treat the symptoms of spleen deficiency and accumulation of food. The symptoms of eating spleen are difficult to eliminate, and the stomach is stuffy and the breath is short, the body is weak and the tongue is light and the pulse is weak. Jianpi pill is composed of ginseng, Atractylodes macrocephala, Pericarpium Citri Reticulatae, malt, and hawthorn with enucleation. Jianpi pill prescription treatment is spleen and stomach weakness, health decline due to health disorders, food stagnation, Qi deficiency, and spleen and stomach dyspepsia. In the prescription, orange peel and orange peel are vaporized and accumulated; hawthorn, malt, Shenqu digestion and stomach; ginseng and Atractylodes macrocephala have the effect of nourishing qi and nourishing spleen. All tonics and tonics are used together, and the specimens are treated together to treat the health of spleen.

2.2. Brief Introduction of Etiology and Pathogenesis of Gastric Cancer

Ancient medicine believed that the main cause of gastric cancer was the weakness of Spleen Yang, blood stasis and cancer toxin. "Plain questions" mentioned in the book "Yang Qi, Yin into line", and "Lingshu" also wrote: "Yang Qi is not good, blood coagulation but not scattered, body fluid astringent but not seeping, accumulated into gastric cancer." Some people think that the possibility of cancer in patients with ferritic Yang deficiency syndrome is higher than that in normal people. The cause of gastric cancer is long-term static, thermal anxiety and carcinogenic toxin. The normal operation of the stomach must rely on soft Yang Qi to be warm and bright. Weak Yang Qi is the decline of visceral function, the movement of essence and evil, and the growth of truth and evil. Therefore, we speculate that gastric cancer patients mainly exist in people with anxiety and yang deficiency. According to the ancient and modern medical system, there must be dead blood if you feel a slight pain when you feel a little pain when you eat it; and you can see that if you have a lump in your stomach, you must have visible blood. If qi stagnation lasts for a long time, if the blood is not unblocked, it will become blood stasis, which will accumulate in the body. If the blood stasis does not disperse, it will become cancerous and prevent the accumulation of viscera, meridians and limbs. Therefore, we believe that blood stasis is not only the pathological product of human body, but also an important cause of tumorigenesis.

In clinical practice, we found that patients with gastric cancer often show blood stasis, such as abdominal mass, abdominal pain, rejection and compression, and the tongue often has blood stasis, dark purple tongue and stringy pulse. In addition, the pathological products of blood stasis in the body, the most important reason is: because the internal organ function of the blood cannot be normal, blood retention in the blood vessels, due to trauma caused by massive bleeding, blood does not flow meristem, depression accumulation cannot dissipate, stay in the body for too long. Cancer toxin is a combination of various pathological factors, which can lead to toxicity to the body. Toxic pathogen is one of the important pathogenesis of gastric cancer. In clinical practice, attaching

importance to toxic substances and applying them to treatment can usually achieve unexpected therapeutic effects. Cancer is one of the special types, which plays an important role in the development of cancer. Internal pathological factors, visceral imbalance, blood, yin and Yang, diseases, cancer and cancer will accelerate the destruction of visceral function, the loss of Qi and blood in the body, yin and Yang produce more pathological products, such as water dampness, phlegm turbidity, and blood stasis. Cancer poisoning can cause tumors, while another poisoning can make the disease worse. Therefore, we believe that the key factor in the treatment of cancer is tumor resection.

2.3. Introduction to Apoptosis

Apoptosis refers to the spontaneous and orderly death of cells controlled by genes to maintain the stability of internal environment. Apoptosis is different from cell necrosis. Apoptosis is not a passive process, but an active process. It involves the activation, expression and regulation of a series of genes. Apoptosis is not a self-injuring phenomenon under pathological conditions, but a death process to actively strive for better adaptation to the living environment. Apoptosis is a basic biological phenomenon of cells. It plays an important role in the removal of harmful or abnormal cells by multicellular organisms. It plays an important role in biological evolution, internal environment stability and multi system development. Apoptosis is a special type of cell death, which has important biological significance and complex molecular biological mechanism. The concept of apoptosis was discovered and put forward by Australian scientists in 1956. The process of apoptosis includes the initiation of apoptosis, the formation of apoptotic bodies and the gradual phagocytosis of apoptotic bodies by adjacent cells or phagocytes in vivo. The residual substances after apoptotic cells are digested and reused.

Apoptosis usually occurs in a single cell or even asynchronously in several cells. First, cells shrink in size, their connections disappear, and they separate from surrounding cells. Then, the cytoplasmic density increased, mitochondrial membrane potential disappeared, permeability changed, cytochrome C was released into the cytoplasm, the nucleolus was concentrated, the nuclear membrane and nucleolus were destroyed, DNA was degraded into about 180 bp-200 BP fragments, the membrane was vesicles, phosphatidylserine exfoliated to the surface, and the cell membrane structure remained intact. The residue of apoptotic cells can be divided into several apoptotic bodies without spilling any contents. Therefore, apoptotic bodies can be quickly engulfed by the surrounding phagocytes. One of the most significant characteristics of apoptosis is the dissolution of DNA. In this study, we used terminal DEOXYNUCLEOTIDYLTRANSFERASE mediated dUTP nick end labeling method to observe the dissolution of DNA. Cell apoptosis changes not only the effective control of DNA, but also the expression of new genes and the synthesis of some biological macromolecules. In this experiment, we also used ELISA to detect the content of VEGF protein, and detected the changes of Mg^{2+} - ATPase and g-6-p enzyme activities in lateral tissues by chromogenic method.

3. Establishment of an Animal Model of Cadmium Exposure in Pregnancy

3.1. Experimental Animals and Conditions

In this experiment, a nude mouse model in which gastric cancer cells were injected into the subcutaneous tissue was used. Forty healthy nude mice, aged about 12W, weighed 250±15g. Divided into four groups, 10 in each group. The laboratory environment meets the requirements of the clean animal laboratory level, and the external environment is clean and tidy. Do not control diet and drinking water. Feed is standard feed. Drinking water is bottled tap water. Observe for 40 days.

3.2. Experimental Instruments

This experiment mainly uses 10 kinds of experimental instruments including vibrometer. The specific name, model and supplier are shown in Table 1.

Table 1. List of main experimental instruments for this experiment

Name and model	**S**upplier
Electric thermostatic water tank (HH. W21.CU600 type)	Shanghai Medical Device General Factory
Ultra-clean workbench (YJ-875)	Suzhou Purification Equipment Company
Inverted microscope, microscope camera (PM-10AD)	Olympus Corporation, Japan
RT-PCR PowerPac300 Nucleic acid quantitative analyzer	American Bio-Rad
Desktop High Speed Refrigerated Centrifuge (5417R)	Eppendorf, Germany
PCR Thermal Cycler (PE480)	American PE Company
Ultraviolet spectrophotometer (UV2401PC 13.0)	Tsushima Corporation, Japan
Gel imaging processing system	Kodak Digital-Science system
Ice Machine (AF10)	Scot man Company, Italy
Low temperature refrigerator (-86℃)	Thermo Forma
Low temperature refrigerator (-30℃) MDF-U333 2. 20	Japan Sanyo Corporation

3.3. Experimental Reagents and Drugs

A total of 12 main reagents and drugs including cadmium chloride were used in this experiment. The specific names and suppliers are shown in Table 2.

Table 2. Main experimental agents and drugs in this experiment

Name	**S**upplier
Human Vascular Endothelial Growth Factor (VEGF)	ELISA Kit American R&D Company
Animal cell G-6-P enzyme activity colorimetric quantitative detection kit	Shanghai Ruicong Laboratory Equipment Co., Ltd.
Mg^{2+}-ATPase kit	Genmed

3.4. Spleen Injection of Traditional Chinese Medicine

The first group is the co-seed group, and 0.5 mL ix seed reagent is fed to nude mice every day, the second group is trachylids macrocephaly, and the nude mice are given 0.5 mL adactylies reagent every day, and the third group is trachylids macrocephaly. The spleen invigorating herbs group was given gavage spleen invigorating herbs (0.5g) to the nude mice by gavage, and the fourth group was the control group. Through ELISA kit, TUNEL detects the change of terminal deoxyribonucleic acid in tumor tissue, G-6-P enzyme activity colorimetric quantitative detection kit, monitors the change of body weight and appetite throughout the process, and detects the content of human vascular endothelial growth factor protein to detect the activity of G-6-P enzyme, and use Mg2+-ATPase kit to detect the change of Mg2+-ATPase activity.

3.5. Weight Gain and Appetite of Nude Mice.

In the first 10 days after the injection of gastric cancer cells, the nude mice of the first three experimental groups and the control group all lost weight and lost their appetite, but with time, the first three groups all of the nude mice are lowly regaining appetite, the weight also returned to the

weight before the experiment. However, compared with the situation in the first three groups, the spirit of the nude mice in the control group continued to languish, and the appetite also decreased day by day.

3.6. DNA Degradation

Every 10 days, the gastric cancer fine tissue sections of the nude mice of the four experimental groups were taken, and the tumor tissue apoptosis was detected using the TUNEL detection kit. The operation was carried out according to the kit's operating manual, and the positive cells were detected under the light microscope, count and analyze. Take 10 fields from each section to calculate the positive rate.

Observation results show that the tumor volume of the nude mice in the experimental group on day 10 was $1.99 \pm 0.11cm^3$, $1.88 \pm 0.10cm^3$, $1.93 \pm 0.08cm^3$, nude mice The weights are $207 \pm 10g$, $205 \pm 10g$, $208 \pm 10g$, and the tumor volume and weight of the nude mice in the control group are $2.01 \pm 0.07cm^3$,$205 \pm 10g$, respectively.

The tumor volume of the nude mice in the experimental group on day 20 was $1.66 \pm 0.11cm^3$, $1.62 \pm 0.10cm^3$, $1.58 \pm 0.08cm^3$, and the weight of the nude mice was $230 \pm 10g$, $235 \pm 10g$, $228 \pm 10g$, the tumor volume and weight of nude mice in the control group were $2.51 \pm 0.07cm^3$,$175 \pm 10g$, respectively.

The tumor volume of the nude mice in the experimental group on the 30th day was $1.06 \pm 0.11cm^3$, $0.98 \pm 0.10cm^3$, $0.77 \pm 0.08cm^2$, the weight of the nude mice were $240 \pm 10g$, $245 \pm 10g$, $248 \pm 10g$, the tumor volume and weight of the nude mice in the control group were $3.01 \pm 0.07cm^2$,$155 \pm 10g$, respectively.

The tumor volume of the nude mice in the experimental group on day 40 was $0.46 \pm 0.11cm^2$,$0.40 \pm 0.10cm^2$, $0.30 \pm 0.08cm^2$, the weight of the nude mice were $245 \pm 10g$, $250 \pm 10g$, $255 \pm 10g$, the tumor volume and weight of the nude mice in the control group were $351 \pm 0.07cm^2$,$155 \pm 10g$.

From the above data, we can see that the tumor tissue volume of the nude mice in the experimental group is getting smaller and smaller, and the weight is restored to before the injection of gastric cancer cells. Compared with the experimental group, the tumor volume of the nude mice in the control group is increasing. The weight of nude mice is also getting lighter.

3.7. Changes in VEGF Protein Content

Every 10 days, 10 mg tumor tissue samples of the nude mice of the four experimental groups were washed with PBS to remove excess blood, homogenized and placed overnight in 1 ml PBS solution at -20 ℃. The next day, the homogenate was centrifuged at 5000xg for 5 minutes, and the supernatant was tested according to the instructions of the kit.

3.8. Changes in Mg2+-ATPase and G-6-P enzyme Content

10 mg tumor tissue samples of nude mice in four experimental groups were taken every 10 days. The samples were cleaned according to the requirements of the kit. The activities of Mg2 + -ATPase and G-6-Pase in gastric cancer tissues were detected according to the instructions of the kit.

3.9. Data Processing

The experimental data was analyzed using SPSS22.0 statistical software. The results of this part of the experiment are expressed as "mean + standard deviation". The data are first tested for

normality and homogeneity of variance. ANOVA is used for comparison between multiple groups, and the homogeneity of variance is compared by the LSD method. P<0.05 is considered significant.

4. Experimental Results and Analysis

4.1. Analysis of DNA Degradation Data in Nude Mice

According to the results in Figure 1, when TUNEL detection kit was used to detect the apoptosis of tumor tissue, the data of Coix seed group, trachylids macrocephaly group and spleen invigorating herbs group in the experimental group showed a decreasing way, while the tumor volume of the control group was increasing. From the data, we can see that on the 10th day of the experiment, the tumor volume in the experimental group and the control group was almost the same, with the volume of about 2cm3. However, on the 20th day of the experiment, the tumor volume of the three groups of nude mice in the experimental group was reduced to 1.5. However, the tumor volume of nude mice in the control group changed to about 2.5cm3. On the 30th day of the experiment, the changes in the two groups were the same. On the 40th day, the tumor volume of nude mice in the experimental group gradually decreased to about 0.5cm3, but the tumor volume in the control group increased to about 3cm3. In the experimental group using three different kinds of spleen invigorating herbs, we can also see that the different spleen strengthening Chinese medicine has different apoptosis. The effect of spleen invigorating herbs is better than the former two groups, which shows that spleen invigorating herbs is a very good choice for the treatment of gastric cancer. This is because spleen invigorating herbs is a mixture of different kinds of Chinese herbal medicine, which can help each other and treat gastric cancer together.

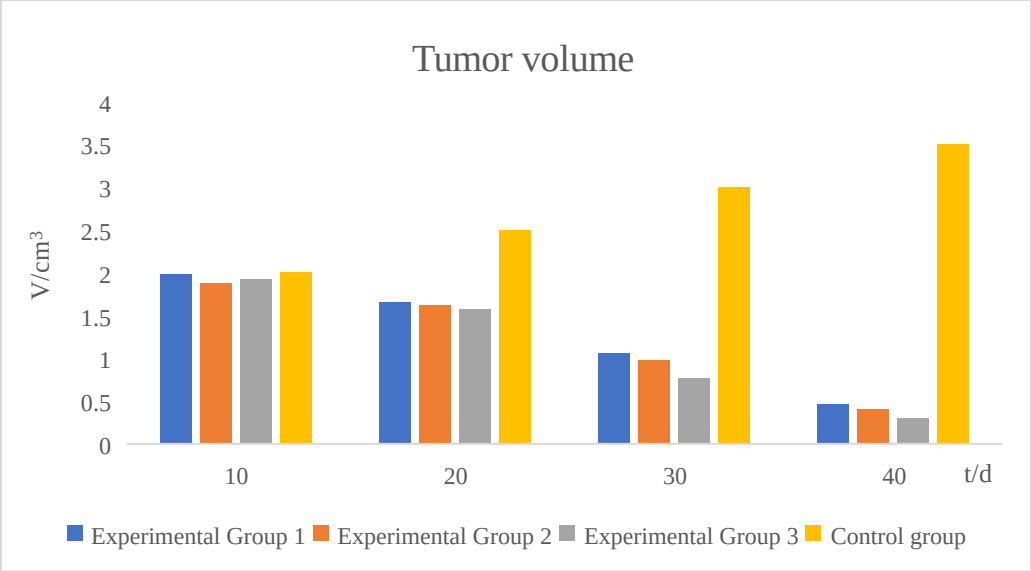

Figure 1. DNA degradation data analysis

4.2. Data Analysis of Protein Content in Nude Mice

According to the above method, the protein standard curve was drawn with time as abscissa and VEGF protein content as ordinate, as shown in Figure 2. The protein content of the first and second groups in the experimental group decreased in the experiment, but eventually recovered to the content before the experiment, and the protein content in the third group remained basically unchanged. However, the protein content of nude mice in the control group decreased linearly, until

the 40th day of the experiment, the VEGF protein content in nude mice decreased to 110ng/g.

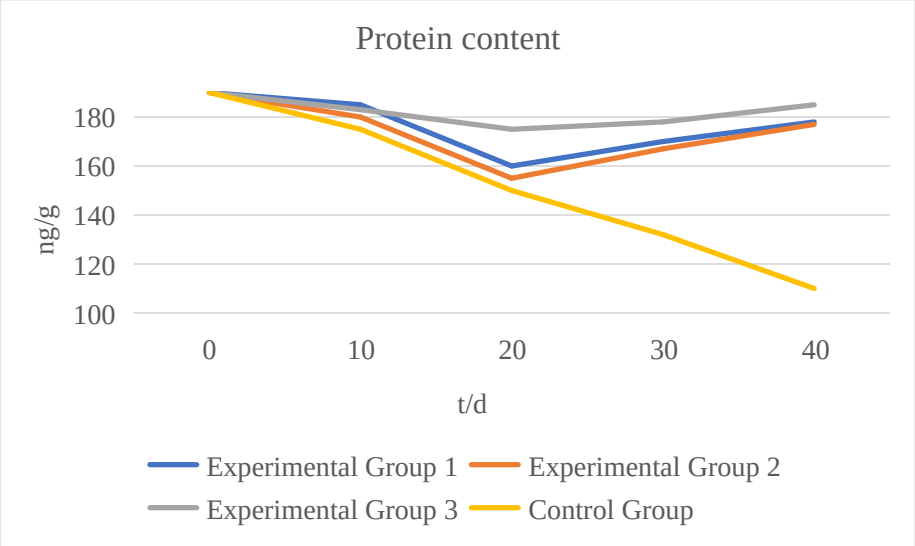

Figure 2. Protein content

According to Figure 2, the protein loading amount of PBS buffer can be calculated, and the effect of proteins related to apoptosis pathway of mitochondria can be observed. The results showed that: the expression of Box protein increased with the increase of pipeline concentration in gastric cancer cell line BGC-823 for 24 hours. The expression of anti-apoptotic protein Bcl-2 decreased with the increase of pipeline concentration, while cleave-caspase-9 increased with the increase of concentration of spleen strengthening Chinese medicine ($P > 0.05$).

4.3. Mg^{2+}-ATPase and G-6-Pase Activities

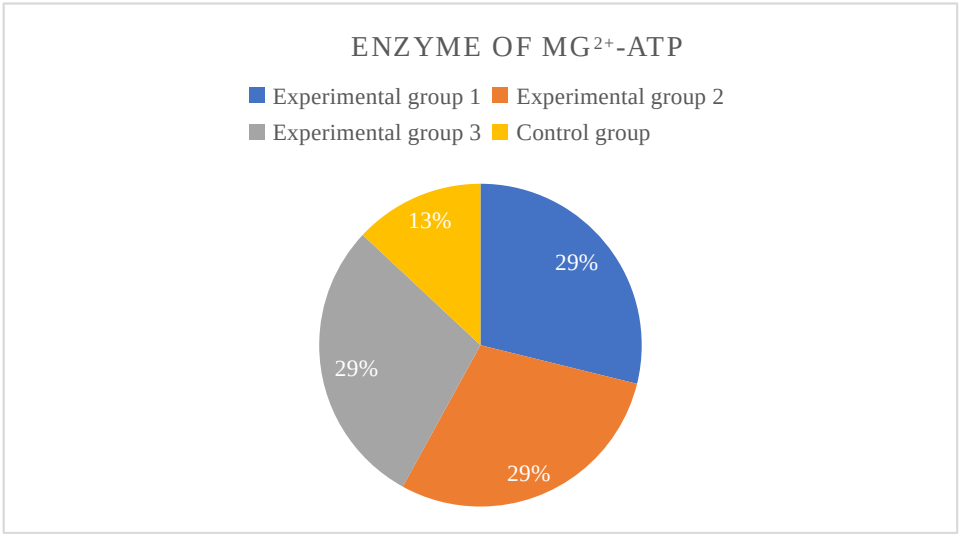

Figure 3. Enzyme of Mg^{2+}-ATP

After the animals were killed, the tumor mass was weighed and the tumor inhibition rate was calculated. Tumor inhibition rate = (tumor mass in saline control group, tumor mass in medication group) / tumor mass X in normal saline control group was 100%. In this experiment, the data in Figure 3 is the content of Mg^{2+} - ATPase in nude mice on the 40th day of the experiment. It can be

seen from the data that the content of Mg^{2+} - ATPase in the three groups of the experimental group accounts for the same proportion, which is 29%, while the content of Mg^{2+} - ATPase in the control group is much lower, accounting for only 13%. The proportion of Mg^{2+} - ATPase content in this part showed that the activity of Mg^{2+} - ATPase in the three groups of the experimental group was very high, while the content of Mg^{2+} - ATPase in the control group became very low.

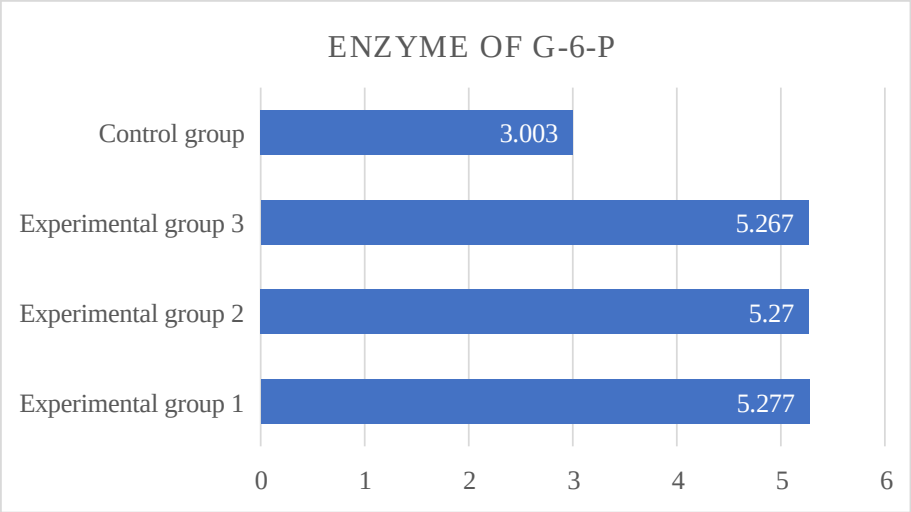

Figure 4. Enzyme of G-6-P

In this experiment, the data in Figure 4 is the content of g-6-p enzyme in nude mice on the 40th day of the experiment. It can be seen from the data in Figure 4 that the content of g-6-p enzyme in coix seed group in experimental group 1 was 5.2777, that in trachylids macrocephaly Kodi group in Experiment 2 was 5.27, and that in spleen invigorating herbs group in Experiment 3 was 5.267, but that in control group was only 3.003. According to the data in Figure 4, it can be seen that the spleen invigorating Chinese medicine has inhibitory effect on gastric cancer cells.

According to previous studies, it can be found that multiple genes are involved in the process of tumorigenesis. If the correct regulatory genes can inhibit the rapid growth and reproduction of tumor cells and promote the recovery of patients' health, the wrong regulatory genes can promote the growth and reproduction of tumor cells, making the patient's physical condition worse.

In conclusion, the spleen invigorating herbs can inhibit the growth of human gastric cancer xenografts and subcutaneous xenografts in nude mice, and inhibit the growth of orthotopic and subcutaneous xenografts of human gastric cancer in nude mice. The results showed that spleen invigorating traditional Chinese medicine had effect on apoptosis of subcutaneous transplanted tumor of human gastric cancer in nude mice. The results of this study support that a new vascular inhibitor can increase the effect of chemotherapeutic drugs at a non-toxic dose. This experiment also confirmed that the spleen strengthening Chinese medicine can significantly inhibit the growth of gastric cancer in nude mice.

5. Conclusion

A large number of experimental results show that the spleen strengthening Chinese medicine can control the tumor to varying degrees, prolong the life time of patients, and improve the survival level of patients. There are many components in the spleen strengthening Chinese medicine that can inhibit the development of gastric cancer cells and promote cell necrosis. This experiment also proves that the spleen strengthening Chinese medicine has a certain inhibitory effect on gastric cancer cells; especially the effect of spleen invigorating herbs of the experiment group is the most

obvious, because there are many active ingredients in spleen invigorating herbs. The treatment of serum cells containing medicine can affect many aspects of cell cycle. Cell apoptosis should be the result of multiple regulation and comprehensive effects. It shows that traditional Chinese medicine can realize the adjustment of overall function and disperse the micro effect of micro management through the implementation of multiple websites, the diversity and unity of body regulation balance is the advantage of TCM syndrome differentiation and treatment. In conclusion, the inhibitory effect of spleen strengthening Chinese medicine on gastric cancer cell proliferation is time concentration dependent. The results showed that spleen invigorating herbs could induce cell cycle G2 / M arrest and cell death, and inhibit cell proliferation. Now we analyze the modern pharmacological effects of spleen strengthening Chinese medicine. Zoonosis piously polysaccharide can promote humoral immunity, trachylids microcephalia volatile oil can prevent cancer cachexia, Peoria Cocos carboxymethyl polysaccharide fermentation sugar can enhance the immunity of patients, Amomum villous can enhance the function of stomach, promote the secretion of digestive juice, hawthorn, malt, Shaniqua can increase the secretion of gastric juice, promote digestion, increase appetite, and so on. Generally speaking, the spleen strengthening Chinese medicine not only has the functions of anti-tumor, improving immunity, promoting appetite and digestion, and improving nutritional status. The increase of food intake and nutrient absorption can be reflected in clinical objective indicators, and the correlation has been described previously. From the above analysis, spleen strengthening Chinese medicine can not only effectively improve the symptoms of malnutrition, but also has a good effect on the improvement of gastric function related indicators. In this paper, the mechanism of stomach cancer induced by spleen invigorating traditional Chinese medicine was discussed from the perspective of molecular mechanism for the first time, which provided a certain reference value for the extensive application of spleen strengthening Chinese medicine in clinical practice in the future.

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Data Availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Conflict of Interest

The author states that this article has no conflict of interest.

References

[1] Zhao Aiguang, Yang Jinkun, Zhao Hailei. Experimental study of spleen-invigorating Chinese medicine on apoptosis of transplanted tumor cells of human gastric cancer nude mice. World Chinese Journal of Digestology, 201/0, 8(007):737-740.

[2] Ning Yan, Luo Songping. Experimental study on the kidney-spleen-invigorating and spleen-invigorating Chinese herbal compound on the model of inhibiting abortion in pregnant rats with kidney-deficiency corpus luteum. Chinese Journal of Traditional Chinese Medicine, 2018, 15(6):28-30. DOI: 10.3969/j.issn.1673-1727.2000.06.008

[3] Wang Xiaoyun, Zhang Chunling, Mo Lili, et al. The effect of Chinese herbs for nourishing the kidney and spleen on bone metabolism and estrogen in perimenopausal women. Journal of

Guangzhou University of Traditional Chinese Medicine, 2017, 17(003):230-232. DOI: 10.3969/j.issn.1007-3213.2000.03.013

[4] Chai Xiqing, Wen Jinkun, et al. Genes of Yiqi Jianpi traditional Chinese medicine on vascular smooth muscle cell apoptosis and gene expression related to apoptosis. Chinese Journal of Integrated Traditional Chinese and Western Medicine, 2019, 17(010):23-26.

[5] Xing X. Inhibitory Effect of Invigorating Spleen and Replenishing Qi Formula on Transplanted Gastric Cancer in Nude Mice. Chinese Journal of Clinical Oncology, 2019, 7(004):25-28.

[6] Xie R, Gao C C, Yang X Z, et al. Combining TRAIL and liquiritin exerts synergistic effects against human gastric cancer cells and xenograft in nude mice through potentiating apoptosis and ROS generation. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 2017, 93:948-960. DOI: 10.3969/j.issn.1000-8179.2011.22.003

[7] Guozhuang S, Youjun C, Haiyan M, et al. Experimental study on apoptosis of gastric cancer cell line SGC-7901 induced by coix seed oil. Journal of Clinical Medicine in Practice, 2019, 7(013):13-18.

[8] Zhu Jinshui, Shen Bo, Chen Jinlian, et al. Mechanism of Apoptosis of Human Gastric Cancer Cells Induced by NM-3 in Nude Mice. Chinese Clinical Medicine, 2005, 012(002):221-223.

[9] Shishido T, Yasoshima T, Denno R, et al. Inhibition of liver me tastasis of human pancreatic carcinoma by angiogenin esis inhibitor T NP470 in combination with cisplatin. Jpn J Cancer Res. 2018,89:963-969 DOI: 10.1111/j.1349-7006.1998.tb00655.x

[10] Wei pinlu, Xu Ling, Qin Zhiwei, et al. Mechanism and clinical study of treating gastric cancer from phlegm. Chinese Journal of basic medicine of traditional Chinese medicine, 2017.13 (3): 18-20.

[11] Li Xiangyong, Wei Pinkang, Jinlongshe oral liquid in the treatment of advanced Putian cancer. Hubei Journal of traditional Chinese medicine, 2017, 23 (11): 35-38.

[12] Objective to investigate the effect of Jinlongshe oral liquid combined with Shenmai Injection on tumor markers and immune function in patients with advanced gastric cancer. Chinese Journal 2017.42 (10); 60-66.

[13] Xu Ling, Chen Yalin, Liu YONGYING, et al. Jinlongshe oral liquid combined with cinobufacini injection. Clinical observation of Astragalus Injection in the treatment of stage IV Putian cancer. Journal of Chengdu University of traditional Chinese medicine, 2015, 28 (1): 79-82.

[14] Xu Ling, Wei Pinkang, Chen Yalin, et al. Xiaotan Sanjie Decoction inhibits the growth and metastasis of human cancer SGE 7901 orthotopic transplantation in nude mice. World Chinese Journal of Gastroenterology, 2016, 12 (5): 12-15.

[15] Xiao Yan, Wei pinlu, Xu Ling, et al, Xiaotan Sanjie Decoction on the expression of MMP2 in MKN45 human gastric cancer in nude mice. Journal of Chengdu University of traditional Chinese medicine, 2015,25 (4): 32-33. DOI: 10.11569/wcjd.v12.i5.1015

[16] Xu Ling, Su Xiaomei, Chen Yalin, et al. Effect of Xiaotan Sanjie Recipe on vege and KDR mRNA expression in orthotopic transplantation of human cancer in nude mice. World Chinese Journal of Gastroenterology, 2015 1 (4): 95-99.