

Effects of radioactive ^{125}I on apoptosis of HGC-27 gastric cancer cells

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Abstract. Effects of radioactive ^{125}I particles at different doses on apoptosis of HGC-27 gastric cancer cells were investigated. HGC-27 gastric cancer cell suspension was used to establish a tumor-bearing mouse model. The model was reared for approximately 3 weeks and then divided into the control group (implanted with blank particles), the low dose group (implanted with 1.48×10^{-7} Bq ^{125}I particles), the medium dose group (implanted with 2.22×10^{-7} Bq ^{125}I particles) and the high dose group (implanted with 2.96×10^{-7} Bq ^{125}I particles) ($n=15$ per group). Six nude mice were randomly sacrificed to collect the tumor tissue and measure tumor volume and mass. TUNEL (TdT-mediated dUTP nick-end labeling) was used for detecting apoptosis of tumor cells, and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) for detecting the relative expression of Bax, caspase-3 and caspase-8. On the 28th day after implantation, the apoptotic rate in the low, medium and high dose groups was significantly higher than that in the control group, which in the medium and high dose groups was significantly lower than that in the low dose group ($P<0.05$). On the 28th day after implantation, the relative expression of Bax, caspase-3 and caspase-8 mRNA in the control group was significantly lower than that in the low, medium and high dose groups ($P<0.05$), which in the low dose group was significantly higher than that in the medium and high dose groups ($P<0.05$). ^{125}I particles can inhibit the growth of HGC-27 gastric cancer cell transplants and promote the expression of Bax, caspase-3 and caspase-8 mRNA in the tumor tissue. Low-dose ^{125}I particles are significantly more effective than medium- or high-dose ^{125}I particles.

Introduction

Malignant tumors are recognized worldwide as public health and safety issues. Global cancer statistics in 2012 showed that gastric cancer was clinically common, and cancer statistics in China showed that there were 679,100 new patients and 498,000 deaths in 2015. The high incidence and mortality rates of the disease need to be urgently reduced (1-3). Operation, the best way to clinically treat cancer, improves conditions through excising the focal zone. However, early gastric cancer shows no obvious abnormalities, and patients are admitted to hospital after they feel unwell. As a result, most patients miss the best treatment time and can only be conservatively treated to alleviate conditions (4,5).

Therapeutic regimens for cancer have been increasing with the improvement of medical levels and technology. External radiotherapy combined with chemotherapy which has been widely used in the treatment of the middle and advanced stages of cancer effectively prolongs the survival time of patients. However, toxic and side effects easily occur during the treatment, and the patients may even suffer from organ failure (6,7). According to a recent study, as a new and effective adjuvant treatment for tumors and a new option especially for patients with poor efficacy of traditional chemotherapy regimens, interstitial irradiation causes a small trauma to the body and has an appropriate radiation radius to accurately treat the local area of lesions (8). ^{125}I is the main particle of internal radiotherapy and effective in the treatment of lung, prostate, pancreatic, rectal and gastric cancer (9-11). However, its specific mechanism on inhibiting tumor growth remains unclear. Apoptosis, which is an important means of the clinical treatment of tumors, refers to the autonomous and orderly death of cells controlled by genes in order to maintain homeostasis (12).

Therefore, the mechanism of ^{125}I on anti-apoptosis of gastric cancer HGC-27 cells was explored in this study to provide a reference for clinical treatment.

Materials and methods

Animal sources. Sixty male SPF (specific pathogen free) nude mice BALB/c (Beijing Vital River Laboratory Animal Technology Co., Ltd., weighing 20 ± 2 g and aged 4 weeks,

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Key words: interstitial irradiation, ^{125}I , gastric cancer, apoptosis, dose, tumor-bearing mice

Table I. Primer sequences.

Genes	Upstream primers	Downstream primers
Bax	5'-GGGTTGTGCGCCCTTTTCTAC-3'	5'-GGTGAGGAGGCTTGAGGAGT-3'
Caspase-3	5'-AAGGCAGAGCCATGGACCAC-3'	5'-CTGGCAGCATCATCCACACATAC-3'
Caspase-8	5'-GTTCTCTCAGTTGCCTTTC-3'	5'-AGCTGTAACTGTGCGCCGAGTCCC-3'
GAPDH	5'-GCACCGTCAAGGCTGAGAAC-3'	5'-TGGTGAAGACGCCAGTGGA-3'

Table II. Comparison of tumor volume (mm³).

Groups	Before implantation (Day 0)	7th day after implantation	14th day after implantation	21st day after implantation	28th day after implantation
Control group (n=6)	548.7±24.5	580.1±24.5	605.4±16.2 ^a	609.5±22.7 ^a	608.8±24.5 ^a
Low dose group (n=6, 1.48x10 ⁻⁷ Bq ¹²⁵ I)	551.4±24.1	475.4±20.1 ^{a,e}	357.1±16.7 ^{a,b,e}	325.6±15.1 ^{a,b,e}	230.1±14.2 ^{a-e}
Medium dose group (n=6, 2.22x10 ⁻⁷ Bq ¹²⁵ I)	550.1±24.4	461.2±17.3 ^{a,e}	361.5±20.1 ^{a,b,e}	329.1±20.2 ^{a-c,e}	270.2±19.2 ^{a-f}
High dose group (n=6, 2.96x10 ⁻⁷ Bq ¹²⁵ I)	557.1±21.6	540.1± 19.3 ^{e-g}	330.1±22.2 ^{a,b,e,g}	331.0±17.5 ^{a,b,e}	261.3±15.7 ^{a-f}

^aP<0.05 indicates a difference compared with before implantation, ^bP<0.05 indicates a difference compared with the 7th day after implantation, ^cP<0.05 indicates a difference compared with the 14th day after implantation, ^dP<0.05 indicates a difference compared with the 21st day after implantation, ^eP<0.05 indicates a difference compared with the control group, ^fP<0.05 indicates a difference compared with the low dose group, ^gP<0.05 indicates that there is a difference compared with the medium dose group.

were reared by professionals for 1 week after being purchased. The indoor temperature was 21±2°C, the indoor humidity was 50-70%, with 12-h light/dark cycles and free access to food and water.

The study was approved by the Ethics Committee of Huai'an TCM Hospital Affiliated to Nanjing University of Chinese Medicine (Huai'an, China).

Main reagents and instruments. HGC-27 cells (Elabscience Biotechnology Inc., CL-0107), ¹²⁵I particles (Tianjin Xiehe Medicine Technology Group Co., Ltd.), RPMI-1640 medium culture solution, 10% fetal bovine serum (FBS), penicillin-streptomycin (antibiotics) and TRIzol extraction reagent [Thermo Fisher Scientific (China) Co., Ltd., 61870044, 10099141, 15070063, 16096020], TransScript Two-Step RT-PCR SuperMix kit (TransGen Biotech, AT411-02). Primers for Bax, caspase-3, caspase-8 and GAPDH PCR (Table I) were designed and synthesized by Sangon Biotech (Shanghai) Co., Ltd. TUNEL apoptosis detection kit (Beyotime Biotechnology, C1091), PCR instrument (Applied Biosystems; Thermo Fisher Scientific, Inc., 7500).

Cell culture. HGC-27 cells were resuscitated, transferred to the RPMI-1640 medium culture solution (containing 10% FBS, penicillin-streptomycin), and then cultured in an incubator (37°C, 5% CO₂) for 2-3 days. After that, passage was performed. Cells in logarithmic growth phase were prepared into 1x10⁶ cells/ml cell suspension using PBS buffer in order to establish an animal model.

Animal modeling and grouping. The cell suspension was inoculated into the subcutaneous side of the left back near the

head of nude mice, reared by professionals for 3 weeks under SPF conditions. Subsequent experiments were performed after neoplasia. Sixty nude mice were randomized into the control group (blank particles), the low dose group (1.48x10⁻⁷ Bq ¹²⁵I particles), the medium dose group (2.22x10⁻⁷ Bq ¹²⁵I particles) and the high dose group (2.96x10⁻⁷ Bq ¹²⁵I particles) (n=15 per group). According to the principles of aseptic technique, a needle for particle implantation was implanted into the central region of the tumor based on the tumor diameter. After 4 weeks of implantation under SPF conditions, the nude mice in groups were separately reared in cages to avoid mutual biting.

Detection of tumor volume and mass. Six nude mice were randomly anesthetized with 10% chloral hydrate (350 mg/kg) by intraperitoneal injection and then sacrificed by cervical dislocation before implantation (day 0) and on the 7th, 14th, 21st and 28th day after implantation, respectively, in order to collect the tumor tissue and measure tumor volume and mass [Tumor volume = long diameter (mm) x short diameter (mm²)/2].

Detection of apoptotic rate. After being anesthetized with 10% chloral hydrate (350 mg/kg) and then sacrificed by cervical dislocation, on the 28th day after implantation, the tumor tissue of nude mice was collected, fixed with 4% paraformaldehyde, dehydrated with gradient ethanol, embedded with paraffin, and finally sectioned. The TUNEL (TdT-mediated dUTP nick-end labeling) apoptosis detection kit was used for detecting apoptosis of tumor cells, with the operation carried out in strict accordance with the kit instructions. After staining, the apoptotic cells in the visual field were observed and counted using an optical microscope (x400; Olympus). The apoptotic rate = total number of apoptotic

Table III. Comparison of tumor mass (g).

Groups	Before implantation (Day 0)	7th day after implantation	14th day after implantation	21st day after implantation	28th day after implantation
Control group (n=6)	2.032±0.044	2.125±0.073	2.131±0.051	2.134±0.051	2.137±0.066
Low dose group (n=6, 1.48x10 ⁻⁷ Bq ¹²⁵ I)	2.030±0.061	1.968±0.060 ^e	1.488±0.069 ^{a,b,e}	1.015±0.041 ^{a,c,e}	0.686±0.050 ^{a,e}
Medium dose group (n=6, 2.22x10 ⁻⁷ Bq ¹²⁵ I)	2.035±0.049	1.987±0.077 ^e	1.815±0.062 ^{a,b,e,f}	1.435±0.052 ^{a,c,e,f}	1.061±0.048 ^{a,f}
High dose group (n=6, 2.96x10 ⁻⁷ Bq ¹²⁵ I)	2.044±0.052	1.944±0.051 ^{a,e}	1.752±0.070 ^{a,b,e-g}	1.384±0.067 ^{a,c,e,f}	1.185±0.052 ^{a,c,e-g}

^aP<0.05 indicates a difference compared with before implantation, ^bP<0.05 indicates a difference compared with the 7th day after implantation, ^cP<0.05 indicates a difference compared with the 14th day after implantation. ^dP<0.05 indicates a difference compared with the 21st day after implantation, ^eP<0.05 indicates a difference compared with the control group, ^fP<0.05 indicates a difference compared with the low dose group, ^gP<0.05 indicates that there is a difference compared with the medium dose group.

cells / total number of cells x 100%. After nuclear staining, the nuclei of normal tumor cells were blue, but those of apoptotic tumor cells were yellow or brownish yellow. Five visual fields of each section were randomly observed.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) detection. After being anesthetized with 10% chloral hydrate (350 mg/kg) and then sacrificed by cervical dislocation, the tumor tissue of nude mice on the 28th day after implantation was collected. The TRIzol extraction reagent was used for extracting total RNA, ultraviolet spectrophotometer (BioRad) and agarose gel electrophoresis for detecting its purity, concentration and integrity. Of the total RNA, 1 µg was reverse transcribed using 5X TransScript® II All-in-One SuperMix for PCR, with operations carried out in strict accordance with the manufacturer's kit. PCR amplification experiments were performed using the TransScript Two-Step SYBR-Green RT-PCR SuperMix kit, and the system was as follows: 1 µl of cDNA, each 0.5 µl of upstream and downstream primers, 12.5 µl of 2X TransTaq® HiFi PCR SuperMix II, and finally Nechelase-free Water was added to total of 25 µl. Conditions were as follows: pre-denaturation at 94°C for 5 min, denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, extension at 72°C for 30 sec, for 40 cycles. Three identical wells were set for each sample and the experiment was performed 3 times. GAPDH was used as an internal reference, and 2^{-ΔΔCq} was used to analyze the data (13).

Statistical analysis. In this study, SPSS20.0 software package (Guangzhou Pomine Information Technology Co., Ltd.) was used for statistically analyzing the data, GraphPad Prism 7 (Cabit Information Technology Co., Ltd.) for plotting the figures. Measurement data were expressed as mean ± standard deviation (mean ± SD), and comparison between two groups was tested by independent samples t-test and represented by t. Analysis of variance was used for comparison between groups, LSD-t test for pairwise comparison after that. P<0.05 was considered to indicate a statistically significant difference.

Results

Modeling. Sixty nude mice were subjected to tumorigenicity experiments, and no mouse died during the modeling.

Comparison of tumor volume. In this study, there was no significant difference in the tumor volume between the groups before implantation (P>0.05). On the 7th day after implantation, the tumor volume in the low, medium and high dose groups was significantly lower than that in the control group (P<0.05); there was no difference between the low and medium dose groups (P>0.05); the tumor volume in the low and medium dose groups was significantly lower than that in the high dose group (P<0.05). On the 14th day after implantation, the tumor volume in the low, medium and high dose groups was significantly lower than that in the control group (P<0.05), which in the medium dose group was significantly higher than that in the high dose group (P<0.05); there was no significant difference between the other groups (P>0.05). On the 21st day after implantation, the tumor volume in the low, medium and high dose groups was significantly lower than that in the control group (P<0.05); there was no difference between the other groups (P>0.05). On the 28th day after implantation, the tumor volume in the low, medium and high dose groups was significantly lower than that in the control group (P<0.05), which in the low dose group was significantly lower than that in the medium and high dose groups (P<0.05); there was no difference between the other groups (P>0.05). The comparison in the group showed that the tumor volume in the control group gradually increased with time, while that in the low, medium and high dose groups gradually decreased with time. In the control group, there was a significant difference between day 0 and on the 14th, 21st, 28th day (P<0.05); there was no significant difference between the other two time-points (P>0.05). In the low dose group, there was no significant difference between the 14th day and the 21st day (P>0.05); there was a significant difference between the other time-points (P<0.05). In the medium dose group, there was a statistically significant difference between each time-point (P<0.05). In the high dose group, there was no difference between day 0 and the 7th day, as well as between the 14th day and 21st day (P>0.05); there was a significant difference between the other time-points (P<0.05) (Table II).

Comparison of tumor mass. There was no significant difference in the tumor mass between the groups before implantation (P>0.05). On the 7th day after implantation, the tumor mass in the low, medium and high dose groups was significantly lower

Table IV. Apoptotic rates on the 28th day.

Groups	Apoptotic rate on the 28th day (%)
Control group (n=6)	7.24±1.78
Low dose group (n=6, 1.48x10 ⁻⁷ Bq ¹²⁵ I)	45.22±5.97 ^a
Medium dose group (n=6, 2.22x10 ⁻⁷ Bq ¹²⁵ I)	35.12±4.22 ^{a,b}
High dose group (n=6, 2.96x10 ⁻⁷ Bq ¹²⁵ I)	40.54±4.35 ^{a,b}
F value	92.188
P-value	<0.001

^aP<0.05 indicates a difference compared with the control group, ^bP<0.05 indicates a difference compared with the low dose group.

than that in the control group (P<0.05); there was no difference between the other groups (P>0.05). On the 14th day after implantation, the tumor mass in the low, medium and high dose groups was significantly lower than that in the control group, which in the medium and high dose groups was significantly higher than that in the low dose group, and which in the high dose group was significantly lower than that in the medium dose group (P<0.05). On the 21st day after implantation, there was no difference in the tumor mass between the medium and high dose groups (P>0.05); the tumor mass in the low, medium and high dose groups was significantly lower than that in the control group, which in the medium and high dose groups was significantly higher than that in the control group (P<0.05). On the 28th day after implantation, the tumor mass in the low, medium and high dose groups was significantly lower than that in the control group, which in the medium and high dose groups was significantly higher than that in the low dose group, and which in the high dose group was significantly higher than that in the medium dose group (P<0.05). The comparison in the group showed that the tumor mass in the control group gradually increased with time, while that in the low, medium and high dose groups gradually decreased with time. In the control group, there was no significant difference between each time-point (P>0.05). In the low dose group, there was no significant difference between day 0 and on the 7th day (P>0.05); there was a significant difference between the other time-points (P<0.05). In the medium dose group, there was no significant difference between day 0 and the 7th day (P>0.05); there was a significant difference between the other time-points (P<0.05). In the high dose group, there was no significant difference between the 21st and 28th day (P>0.05); there was a significant difference between the other time-points (P<0.05) (Table III).

Comparison of apoptosis. On the 28th day after implantation, there was a significant difference in the apoptotic rate between the groups (P<0.001). The apoptotic rate in the low, medium and high dose groups was significantly higher than that in the control group, which in the medium and high dose groups was significantly lower than that in the low dose group (P<0.001). There was no difference between other groups (P>0.05) (Table IV, Fig. 1).

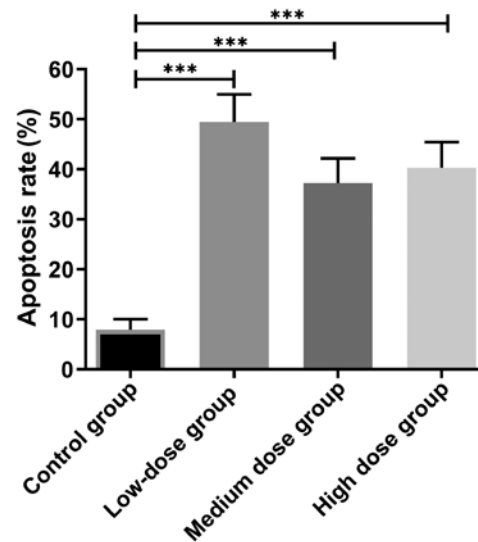


Figure 1. Tumor cell apoptosis on the 28th day. Apoptotic rate in the low, medium and high dose groups were higher than that in the control group. ***P<0.001.

Comparison of Bax, caspase-3 and caspase-8 mRNA. On the 28 day after implantation, the relative expression of Bax, caspase-3 and caspase-8 mRNA in the control group was significantly lower than that in the low, medium and high dose groups (P<0.05), which in the low dose group was significantly higher than that in the medium and high dose groups (P<0.05). There was no significant difference between the medium and high dose groups (P>0.05) (Table V, Fig. 2).

Discussion

Gastric cancer is a common malignant disease in the clinical department of gastroenterology and oncology. A cancer statistics report in the United States showed that there were 28,000 new patients and 15,700 deaths in 2017 (14). The disease is usually caused by changes in dietary habits, *Helicobacter pylori* infection, genetics and socioeconomic status among which the first one is the most common cause. Irregular diet, alcohol abuse, and lack of trace elements in daily diet lead to an increase in the incidence rate of the disease (15,16). At present, gastric cancer is clinically treated by operation, but most patients after admission are in the middle and advanced stages of the disease and have missed the best treatment time, so they cannot be treated by operation (17). Radiotherapy, as an important means of the clinical treatment of cancer, has been widely used. According to a study, the survival time of patients with local advanced esophageal and gastric cancer after radiotherapy was significantly increased (18). However, traditional radiotherapy leads to necrosis in the normal tissue other than the lesion tissue during the treatment, and excessive radiation increases adverse reactions.

Internal radiotherapy has been gradually recognized by clinicians as medical technology continues to be improved, and its adjuvant treatment of malignant tumors has been widely used in clinical practice. ¹²⁵I is a synthetic isotope and has a long half-life (60.1d). Compared with traditional radiotherapy, it has a more appropriate radiation radius to accurately treat

Table V. Comparison of Bax, caspase-3 and caspase-8 mRNA on the 28th day.

Groups	Bax	Caspase-3	Caspase-8
Control group (n=6)	1.035±0.041	1.024±0.032	1.028±0.030
Low dose group (n=6)	1.892±0.174 ^a	2.522±0.301 ^a	2.122±0.170 ^a
Medium dose group (n=6)	1.641±0.120 ^{a,b}	1.891±0.260 ^{a,b}	1.715±0.121 ^{a,b}
High dose group (n=6)	1.705±0.081 ^{a,b}	1.755±0.217 ^{a,b}	1.658±0.148 ^{a,b}
F value	62.461	43.962	73.853
P-value	<0.001	<0.001	<0.001

^aP<0.05, a difference compared with the control group, ^bP<0.05, a difference compared with the low dose group.

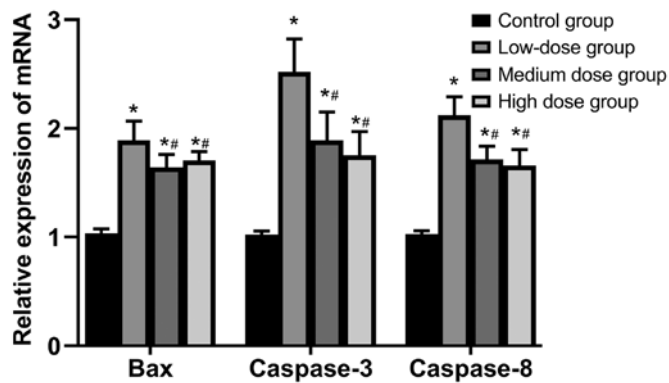


Figure 2. Expression levels of Bax, caspase-3 and caspase-8 in tumor tissues. The relative expression of Bax, caspase-3 and caspase-8 mRNA in the control group was significantly lower than those in the low, medium and high dose groups. The expression levels of Bax, caspase-3 and caspase-8 mRNA in the low dose group were significantly higher than those in the medium and high dose groups (P<0.05). *P<0.05, vs. the control group; #P<0.05, vs. the low dose group.

the local area of lesions, and causes less trauma and damage to the normal tissue adjacent to lesions (19). In a study by Shi *et al* (20), the implantation of ¹²⁵I particles in patients with recurrent colorectal cancer significantly prolonged the survival time of the patients (21). In a study by Jiang *et al* (21), 56 lesions were treated through the implantation in patients with gastrointestinal cancer with pulmonary metastasis, with an overall local control rate of 91.1%. These findings indicate that the transplantation of ¹²⁵I particles effectively inhibits tumor growth. Currently, clinical radiotherapy and chemotherapy affect tumor growth mainly through promoting tumor apoptosis (22). How do ¹²⁵I particles affect apoptotic factors in gastric cancer? Effects of radioactive ¹²⁵I on apoptosis at different doses in gastric cancer cells were investigated. In this study, a tumor-forming mouse model was established to observe the effects of radioactive ¹²⁵I particles at different doses on the tumor tissue, so as to provide a reference for clinical practice.

The proliferation of tumors is an important factor determining their growth rates. A tumor-forming mouse model was successfully established. The tumor volume and mass of nude mice in the low, medium and high groups gradually decreased with time, suggesting that ¹²⁵I particles can effectively inhibit the growth of gastric cancer tumors, probably because ¹²⁵I radiation directly destroys the DNA double

strands of tumor cells and activates the expression of apoptotic factors in cells, thereby inducing apoptosis (23). Therefore, the apoptotic factors of gastric cancer tumors in nude mice were detected on the 28th day. Bax is a classical pro-apoptotic factor in the Bcl-2 family and lowly expressed in cells, and its expression is significantly increased when cells undergo apoptosis (24). Caspase-3 and caspase-8 belong to the Caspase family; Caspase-8 plays an important role in the activation of apoptosis and caspase-3 playing an important role during apoptosis (25). A study shows that the activation of them promotes apoptosis (26). In this study, on the 28th day after implantation, the relative expression of Bax, caspase-3 and caspase-8 mRNA in the low, medium and high groups was significantly higher than that in the control group, which indicates that ¹²⁵I particles can promote expression of Bax, caspase-3 and caspase-8 mRNA in gastric cancer tumors and thereby promote apoptosis. According to TUNEL, apoptosis in the low, medium and high groups was significantly higher than that in the control group. Sun *et al* (27) treated patients with stage IIIB-IV lung cancer by EGFR-TKI combined with the implantation of ¹²⁵I particles, and the expression of Bax in the tissue of the patients was significantly increased 1 month later. In a study by Wang *et al* (28), compared with high-dose ⁶⁰Co, the expression of Bax and caspase-3 was significantly increased after low-dose ¹²⁵I radiation to lung cancer cells A549 and H1299.

In a study by Chen and Wang (29), the expression of caspase-8 was significantly increased through ¹²⁵I irradiation to a rat model of glioma. In HGC-27 gastric cancer cell transplants in nude mice, ¹²⁵I particles effectively increased the expression of Bax, caspase-3 and caspase-8 mRNA in gastric cancer tumors, indicating that ¹²⁵I particles can increase the expression of Bax, caspase-3 and caspase-8 mRNA in tumor cells and promote apoptosis. However, on the 28th day after implantation, the tumor volume and mass in the low dose group were significantly lower than those in the medium and high dose groups, while the expression of Bax, caspase-3, caspase-8 mRNA and apoptosis were significantly higher than those in the medium and high dose groups. These findings suggest that low-dose ¹²⁵I particles are significantly more effective than medium- and high-dose ¹²⁵I particles in inhibiting the growth of gastric cancer tumors. In a study by Lehmann and Dröge (30), the apoptotic rate of HeLa cells after 5 Gy ¹²⁵I irradiation was significantly higher than that after 10 Gy ¹²⁵I irradiation. The above is similar to the results

of our study, possibly because tumor cells increase their own adaptability and protection during the growth in order to avoid the toxic effects of radiation, resulting in resistance which is often referred to as the inverse dosage effect (31).

The present study showed that low-dose ¹²⁵I was significantly more effective than medium- and high-dose ¹²⁵I in inhibiting the growth of HGC-27 gastric cancer cell transplants. However, there are still deficiencies. This animal experiment was not clinically verified, and the expression of Bax, caspase-3 and caspase-8 mRNA at other time-points was not detected. Thus further confirmational studies are required.

In summary, ¹²⁵I particles can inhibit the growth of HGC-27 gastric cancer cell transplants, and promote the expression of Bax, caspase-3 and caspase-8 mRNA in the tumor tissue. Low-dose ¹²⁵I particles are significantly more effective than medium- and high-dose ¹²⁵I particles.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YY wrote the manuscript. YY and AC were responsible for animal modeling. JM and AW performed PCR and TUNEL assay. FX contributed to detection of tumor volume and mass. All the authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Huai'an TCM Hospital Affiliated to Nanjing University of Chinese Medicine (Huai'an, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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