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Effects of AZT and RNA-protein complex (FA-2-b-β) extracted from Liang Jin mushroom on apoptosis of gastric cancer cells

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Abstract

AIM: To investigate the synergistic effects of 3'-azido-3'-deoxythymidine (AZT) and FA-2-b-β extracted from Ling Jin mushroom on apoptosis of gastric cancer cells MKN45 *in vitro*.

METHODS: MTT analysis was made to examine the inhibition rate of MKN45 cells treated with AZT (2.5, 5, 10 and 20 mg/L) and FA-2-b-β (5, 10, 20 and 40 mg/L) singly and combinatively for 24, 48 and 72 h. Apoptotic effects were evaluated by morphological methods, DNA agarose gel electrophoresis and flow cytometry, respectively. Telomerase activity was estimated by TRAP-ELISA. The mRNA expression of caspase-3 and Bcl-2 were detected by RT-PCR.

RESULTS: AZT and FA-2-b-β could significantly inhibit MKN45 cell proliferation and induce its apoptosis. MKN45 cells were inhibited in dose- and time- dependent manner. The inhibition effect of AZT combined with FA-2-b-β was obviously better than that used singly (0.469 ± 0.022 *vs* 1.075 ± 0.055, $P < 0.05$, 0.325 ± 0.029 *vs* 0.469 ± 0.022 $P < 0.01$). AZT used singly and combination of FA-2-b-β could decrease the activity of tumor cell telomerase, and AZT has synergistic function with FA-2-b-β. A certain concentration of AZT could up-regulate the expression of caspase-3 mRNA ($r = 0.9969$, $P < 0.01$), which was positively related to apoptosis rate, and could down-regulate the expression of Bcl-2 mRNA, which was negatively related to apoptosis rate ($r = 0.926$, $P < 0.01$). Furthermore, the effect of AZT combined with FA-2-b-β was significantly higher than that used singly.

CONCLUSION: Combination of AZT and FA-2-b-β has an obviously synergetic effect in the gastric cancer cells MKN45, which has provided a new approach to the treatment of gastric cancer clinically.

Key words: AZT; FA-2-b-β; Apoptosis; Telomerase; Bcl-2; Caspase-3; MKN45 cell

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INTRODUCTION

Gastric cancer is the most common malignant tumor in China. At present, there is still lack of effective treatment for the cancer. The search for effective drugs or treatment methods has been well concerned. Apoptosis is an active, programmed cell death following genetic procedure, which is a physiological phenomenon. It is believed that cellular malignant proliferation and differentiation or apoptosis inhibition are important pathological basis for malignant tumor development. Clinically, killing the malignant cells and inducing cell differentiation or apoptosis have become the basic strategies for treating malignant tumors. Up to now, many anti-tumor drugs have been discovered to induce apoptosis of tumor cells. Intensity of anti-tumor effect in some anti-tumor drugs is parallelled to the activity of inducing tumor cell apoptosis[1]. Telomerase is a nuclear ribonuclear protein (RNP) that constantly produces telomeric repeats which are added to the terminal end of the chromosome to sustain the stability of the chromosome, and to confer the ceil infinite proliferating capacity. Previous research showed that there was a high and strong positive expression of telomerase in almost all malignant tumor tissues, however, the expression of telomerase in normal somatic cells (except germ cells) was negative. So the telomerase has become the most specific and common marker of malignant tumors[2]. Telomerase activity is high in gastric cancer tissues, and it is not only the marker for specific malignant tumor, but also the ideal target for tumor treatment. The inhibition of telomerase

activity provides a new way to treat malignant tumors[3]. 3'-azido-3'-deoxythymidine (AZT) is an inhibitor of reverse transcriptase mainly used in the AIDS therapy. In recent years, AZT has been used in tumor treatment, whose mechanism is to competitively inhibit reverse transcription process such as nucleoside analog, thus inhibiting the activity of telomerase and blocking the elongation of telomere[4]. Gomez[5] found that the long-term use of AZT could shorten the telomere sequence of cultured HeLa cells and reduce the activity of telomerase. The inhibitor of telomerase may enhance the sensibility of tumor cell to DNA detriment agent[6]. Sun *et al*[7] reported that AZT induces gastric cancer SGC-7901 cell apoptosis. *Agaricus blazei* is mycelium of fungi, which belongs to Basidiomycete and is originated from Brazil. The artificial cultivation of *Agaricus blazei* was established in 1978 at the Iwade Institute in Japan, and since 1988 *Agaricus blazei* has been widely cultivated in Japan and Indonesia. In recent years, many experts have isolated and purified steroids, polysaccharides, glycoprotein complex and nucleic acids from *Agaricus blazei*. Its effective components and pharmaceutical functions have been studied thoroughly. *Agaricus blazei* has been found to possess anti-tumor functions[8-10], and increase immunity[11,12], anti-mutation[13], and sterilization[14] In 1995, Professor Liang Zheng in the Institutes of Botany, Chinese Academy of Sciences, successfully cultivated it with wild strain from virgin forest in Brazil, and named it Liang Jing mushroom. Our research discovered that Liang Jing mushroom has the anti-tumor and promoting haematopoiesis functions in mice[15,16]. Although chemotherapy now plays an important role in late-stage gastric cancer treatment, it has the shortcoming of selective inhibition effects, intensive side-effect, narrow anti-tumor span and easy drug tolerance. We therefore, have tried to discover such natural products, which have minimal or none side-effects and are more effective than that of the existing natural products for tumor treatment. We isolated further a novel type of RNA-protein complex (FA-2-b-β), a water-soluble extractive constituent from Liang Jing Mushroom. It is reported that the inhibition rates of Sarcoma 180/ICR-JCL mice by FA-2-b-β were 85.8% and 74.5% through intravenous injection and orally respectively 3 wk after the administration, and complete regression rates of the tumor were 33.3% and 35.2% respectively 6 wk after the administration[17].

The purpose of this study is to use the combined AZT and RNA-protein complex (FA-2-b-β) extracted from Liang Jing mushroom to treat gastric cancer cell MKN45 and examine its effects on inducing cell apoptosis so as to explore the mechanism of the drugs and to provide experimental data for new anti-tumor drug development targeted on telomerase.

MATERIALS AND METHODS

Drugs and reagents

Liang Jing mushroom was provided by Hua Sheng Food Limited Company in Fuzhou, China. The active RNA-protein complex, FA-2-b-β, was extracted and identified by School of Chemistry and Chemical Engineering, Lanzhou University, China. The previously reported methods of extraction were used[17-19]. Briefly, dried plant material 500 g was powdered with hot water and fractionated, and the extract was purified by ethanol precipitation, gel filtration, Deae-cellulose, and Sephadex G-200 column chromatograph. FA-2-b-β of 4.86 g was obtained, consisting of a RNA molecule, whose molecular weight was estimated to be 10 000 by gel filtration method. This fraction contained 15.7% protein and the major constitutive sugar was D-ribose. Its [α] D value was +58.6° (H_2O) and a UV-spectrum showed a pattern specific to nucleic acids. As nucleic acid base components, A, Ap, Cp, Gp and some other modified bases were detected by two dimensional TLC (thin-layer chromatography). The RNA-protein complex, FA-2-b-β was diluted into required concentration with double steamed water, then sterilized and stored at 4℃. AZT was purchased from Sigma Company, USA. AZT was diluted into required concentration with saline. RNA extraction, RT-PCR and telomerase activity kits were purchased from Roche Company, USA. Bcl-2 protein monoclonal antibody was purchased from the Promega Company, USA. Caspase-3 primers were supplied by Shanghai Biological Technique Service Company, Ltd, China.

Cell culture

Gastric cancer cells MKN45 were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences. The cells suspension was adjusted to 2×10^5/mL and inoculated into RPMI 1640 (Sigma, USA) within or without drugs (containing 10% fetal calf serum, 100 U/mL penicillin and 100 μg/mL streptomycin) at 37℃, 50 mL/L CO_2 for culture. The experimental cells were in logarithmic growth phase.

Detection of drug sensitivity (MTT assay)

MKN45 cell suspension was inoculated into 96-well culture plate with 100 μL in each well. The cells were divided into AZT (2.5, 5, 10 and 20 mg/L) group, FA-2-b-β (5, 10, 20 and 40 mg/L) group, AZT (2.5, 5, 10 and 20 mg/L) + FA-2-b-β (5 mg/L) group and control group. Each group had 8 ambi-wells maintained in a incubator with saturated humidity at 37℃, 10 mL/L CO_2 and MTT 10 μL was added into each well at 24, 48 and 72 h, respectively, and each group continued to be cultivated for 4 h. Before termination of cultivation, 100 μL of dimethyl sulfoxide was added and kept at 37℃ overnight. ELISA was used to examine the absorbance (A570 nm) value. The inhibition rate (IR) was calculated as follows:

IR (%) = [(Mean A of control) - (Mean A of experiment)] /(Mean A of control) × 100%.

To calculate the q value according the following formula helped us judge whether the combined use of AZT and FA-2-b-β had a synergistic effect: q =I (A + B) / [IA + (1 - IA × IB)]. IA:A, inhibition rate of single agent A; IB: B, the inhibition rate of single agent B; I (A + B), the inhibition rate of the combination of both agents A and B. q = 0.85-1.15, the use of the two were summed; q > 1.15, the use of the two were synergized; and q < 0.85, the two agents were antagonized.

Acridine orange staining

The method of Acridine orange staining was used to observe the various apoptotic morphology of MKN45 cells. Cells were suspended when exposed to FA-2-b-β and AZT for 72 h, fixed on the slide, and added with 10 μL acridine orange to stain and observe the morphological changes of the MNK45 cells under an fluorescence Olympus BH-2 microscope and photographed by an Olympus camera, and a total of 1000 cells were counted.

DNA fragmentation assay

Apoptosis was detected with fragmentation of chromosomal DNA by the classic DNA ladder method. Briefly, 1×10^6/mL cells were immersed in cytolysis buffer (Tris-HCl, 1 mmol/L, pH 8.0; edetic acid,10 mmol/L, pH 8.0; proteinase K, 200 mg/L; 0.5% SDS) and incubated for 3 h at 50℃. The DNA was extracted with phenol-chloroform, precipitated in one-tenth volume of NaAc, 2 mol/L, and two volumes of ethanol at 20℃ overnight, recovered by centrifugation at 12000 r/min for 30 min at 4℃, and then resuspended in TE buffer. RNase A was added at a concentration of 200 mg/L, and the treated extract was incubated at 37℃ for 30 min and electrophoresed on a 1.2% agarose gel.

Apoptosis rate analysis by flow cytometry

For analysis of DNA content, cells treated with different concentrations of AZT and FA-2-b-β were harvested after 72 h and fixed with 70% cold ethanol for 4 h. The cell apoptosis rate was detected by flow cytometry (FACSalibur, B.D). Briefly, 1×10^6/mL cells were suspended in 0.2% Triton-X-100/PBS solution containing 1 g/L ribonuclease A. After incubation for 20 min, DNA was stained with 50 mg/L of propidum iodide (PI), the flow cytometric analysis was performed to determine the apoptosis rate.

Assay of telomerase activity

Telomerase activity was determined by TRAP-ELISA method modified by Kim[20] using a telomerase PCR ELISA Kit. All the procedures were performed following the description in the kit. Briefly, 1×10^6/mL cells from all groups were harvested, and washed once with PBS. The cells were centrifuged at 4℃ with 10000 r/min for 1 min, the sediment was washed by 150 μL lotion and then centrifuged for 1 min. The liquid was removed. Fifty μL splitting liquid, was added, suspended and fixed up well for 30 min, and then centrifuged at 4℃ with 14000 r/min for 20 min. The 2 μL supernatant was taken as TRAP reaction template. Forty-five μL reaction mixture was added into the reaction tube and 2 μL into prepared sample tube, and mixed well. Thirty μL liquid paraffin was added and water bathed at 25℃ for 30 min and circulated in PCR at 94℃ for 120 s, 94℃ for 30 s, 48℃ for 30 s, 72℃ for 90 s, and 72℃ for 300 s. All the above procedure was done for 35 cycles. After circulation, hybridization liquid was added into micro-plate, then 25 μL amplified products were added and mixed. Negative, positive and blank control groups were set up. They reacted at 37℃ for 60 min and were added colorant, avoiding light at 37℃ for 10 min and added terminating reagent to stop the reaction. Absorbance values were read at 450 nm.

Telomerase inhibition rate (%) = [(average A value of control group-average A value of experimental group)/ average A value of control group] × 100%.

Detection of Caspase-3 and Bcl-2 expression

Caspase-3 and Bcl-2 mRNA expression was determined by reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted with RNA extraction kit. The mRNA of Caspase-3 in RNA was amplified by RT-PCR kits from Roche Company, USA. Caspase-3 primer sequences: the upper stream primer: 5'-GGTATTGAGACAGACAGTGG-3', the down-stream primer: 5'-GATGGGATCTGTTTCTTTGC-3'. PCR product was 288 bp. The condition of RT-PCR was as follows: reverse transcription at 37℃ for 60 min and pre-denaturation at 94℃ for 4 min and then 30 circles were carried out at 94℃ for 30 s, 55℃ for 15 s, 72℃ for 45 s, and finally at 72℃ for 7 min to elongate the primer. The primer sequences of Bcl-2 were 5'-CTTCAGAGACACCCAC-3', 5'-GATGGGATCTGTTTCTTTGC-3', and 535 bp of amplified product. The above sequences were synthesized by Shanghai Bioengineering Technology Service Co. Ltd. China. Image analyzer was used to analyze semi-quantitatively the specific amplification segments. PCR product was obtained through gel electrophoresis (2%) with voltage of 5 v/cm and 0.5 mL/L EB staining. After 1-2 h, the observation and photography were taken on ultraviolet reflective analyzer.

Statistical analysis

Data were expressed as mean ± SD. For most experiments, statistical difference was evaluated using Student's *t* test. A statistically significant difference was considered at $P < 0.05$.

RESULTS

Effect of drugs on tumor cell proliferation in vitro

AZT and FA-2-b-β had inhibition effects on human MKN45 cell proliferation in a dose-dependent and time-dependent manner (Table 1). At the same time, as showen in Table 2, the combination of AZT and FA-2-b-β had higher inhibition rate than that used alone, and q value was above 1.15, the killing capacity can be increased when AZT and FA-2-b-β were used in combination, and the latter has a synergistic effect on AZT.

Morphology of cell apoptosis

After treatment for 72 h with FA-2-b-β and AZT at different concentrations, MKN45 cells showed cell shrinkage, membrane blebbing, cell volume reduction, nuclear fragmentation and formation of apoptotic body under light fluorescent microscope in all treatment groups, except the the groups treated with AZT 2.5 mg/L and 5 mg/L (Figure 1).

DNA fragmentation

Apart from the morphological changes, apoptosis induction by FA-2-b-β and AZT were analyzed by using the DNA

Table 1 The effect of FA-2-b-β and AZT on proliferation of MKN45 cells (mean ± SD)

	Concentration	24 h		48 h		72 h	
	(mg/L)	A	IR (%)	A	IR (%)	A	IR (%)
FA-2-b-β	0	0.659 ± 0.022	0	1.073 ± 0.033	0	1.078 ± 0.058	0
	5	0.633 ± 0.022[a]	3.94	1.018 ± 0.022[a]	5.13	0.998 ± 0.034[b]	7.42
	10	0.561 ± 0.017[b]	14.87	0.885 ± 0.022[b]	17.52	0.868 ± 0.035[b]	19.48
	20	0.497 ± 0.020[b]	24.5	0.743 ± 0.017[b]	30.75	0.650 ± 0.032[b]	39.7
	40	0.464 ± 0.018[b]	29.59	0.668 ± 0.013[b]	37.74	0.545 ± 0.025[b]	49.44
AZT	0	0.660 ± 0.020	0	1.070 ± 0.030	0	1.075 ± 0.055	0
	2.5	0.635 ± 0.018[c]	3.79	1.017 ± 0.022[c]	4.95	0.988 ± 0.035[d]	8.09
	5	0.562 ± 0.018[d]	14.85	0.855 ± 0.022[d]	20.09	0.826 ± 0.030[d]	23.16
	10	0.458 ± 0.020[d]	30.61	0.657 ± 0.015[d]	38.59	0.557 ± 0.038[d]	48.19
	20	0.434 ± 0.208[d]	34.24	0.589 ± 0.013[d]	44.95	0.469 ± 0.022[d]	56.37

IR: inhibition rate. FA-2-b-β at different concentrations *vs* FA-2-b-β at 0 mg/L, [a]$P < 0.05$, [b]$P < 0.01$; AZT at different concentrations *vs* AZT at 0 mg/L, [c]$P < 0.05$, [d]$P < 0.05$.

Table 2 The synergistic effect of AZT and FA-2-b-β on proliferation of MKN45 cells (mg/L)

Groups	IR (%)	*q* value
0 (control group)	-	-
AZT (2.5) + FA-2-b-β (5)	18.3	1.22
AZT (5) + FA-2-b-β (5)	45.8	1.60
AZT (10) + FA-2-b-β (5)	49.9	1.17
AZT (20) + FA-2-b-β (5)	72.7	1.26

Table 3 Synergistic effects of FA-2-b-β and AZT on telomerase of MKN45 cells

Drug (mg/L)	IR (%)
FA-2-b-β (5)	8.2
AZT (2.5)	12.4
AZT (5)	28.6[a]
AZT (10)	60.6[b]
AZT (20)	80.5[b]
AZT (2.5) + FA-2-b-β (5)	26.4[a]
AZT (5) + FA-2-b-β (5)	42.9[b]
AZT (10) + FA-2-b-β (5)	79.1[b]
AZT (20) + FA-2-b-β (5)	94.7[b]

Combined AZT groups at different concentrations *vs* corresponding single groups, [a]$P < 0.05$, [b]$P < 0.01$.

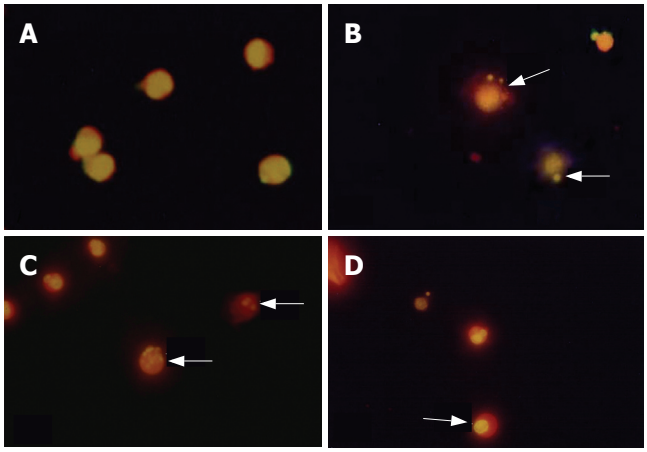

Figure 1 MKN45 cells, treated or not treated with FA-2-b-β and AZT. **A**: Untreated cells; **B-D**: FA-2-b-β (5 mg/L) + AZT 5, 10 and 20 mg/L for 72 h exposure, apoptotic body (**B**), nuclear fragmentation (**C**) and chromatin condensation (**D**) (Acridine orange staining, × 100).

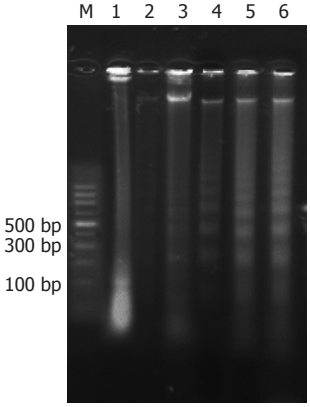

Figure 2 Agarose gel electrophoresis of DNA of MKN45 cells. M: marker, Lane 1: control; Lane 2-5: MKN45 cells treated by FA-2-b-β (5 mg/L), AZT (2.5 mg/L), FA-2-b-β (5 mg/L) + AZT 5, 10 and 20 mg/L for 72 h, respectively.

fragmentation assay. FA-2-b-β and AZT treatment at different concentrations for 72 h led to typical DNA laddering in MKN45 cells by agarose gel electrophoresis (Figure 2).

Cell apoptotic rate detected by flow cytometry

Single use of AZT and combined use of AZT and FA-2-b-β for 72 h increased the apoptosis rate of gastric cancer MKN45 cells with higher concentrations accordingly under the FCM. The combined use of the AZT had a higher cell apoptosis rate than single use of AZT, the statistical analysis indicated that FA-2-b-β had a synergistic effect in apoptosis with AZT ($P < 0.05$) (Table 3, Figure 3).

Expression of Caspase-3 and Bcl-2 detected by RT-PCR

As seen in Figures 4 and 5, 72 h after the MKN45 cells were treated by the single AZT and combination with FA-2-b-β (5 mg/L), the expression of Caspase-3 mRNA was increased, and the expression of Bcl-2 was decreased. The analysis by the image analyzer (Alphalmager™ 2200, USA) showed that difference was significant between the treatment groups and the control groups. The expression of caspase-3 mRNA was positively correlated to the apoptosis rate ($r = 0.9969$, $P < 0.01$) while the expression

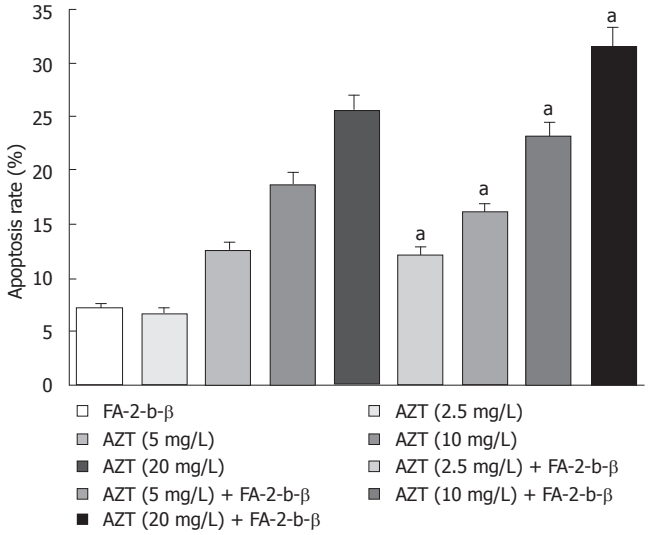

Figure 3 Effect of FA-2-b-β (5 mg/L) and AZT on apoptosis rate in MKN45 cell for 72 h ($^aP < 0.05$).

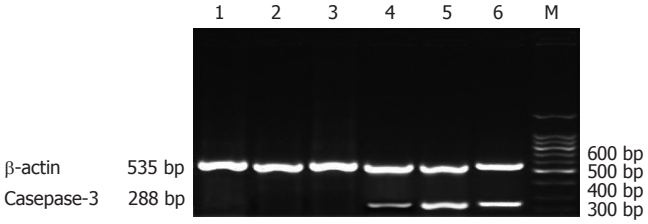

Figure 4 Expression of Caspase-3 mRNA in MKN45 cells treated with AZT and FA-2-b-β. M: marker; Lane 1: control; Lane 2-6: MKN45 cells treated by FA-2-b-β (5 mg/L), AZT (2.5 mg/L), FA-2-b-β (5 mg/L) + AZT 5, 10, 20 mg/L respectively for 72 h.

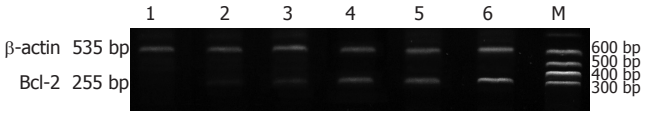

Figure 5 Expression of Bcl-2 mRNA in MKN45 cells treated with AZT and FA-2-b-β. M: marker; Lane 1-5: MKN45 cells treated by FA-2-b-β (5 mg/L) + AZT 20, 10 and 5 mg/L, AZT (2.5 mg/L), FA-2-b-β (5 mg/L), respectively for 72 h; Lane 6: control.

of Bcl-2 was negatively correlated to apoptosis rate ($r = 0.926$, $P < 0.01$).

DISCUSSION

To study the molecular mechanism of apoptosis in tumor cells is one of the important issues in oncology. Inducing tumor cell differentiation and promoting its apoptosis are often regarded as the marker for screening anti-tumor drugs. Telomerase is a ribonucleoprotein composed of RNA and proteins, which maintains the length of telomere in order to keep the constant cell division so as to immortalize the cells, which is also called malignant tumor cells. Immortalization is the key step for cell canceration. About 85.0%-90.9% of the tumor cells have high expression of telomerase and the activation of telomerase are commonly found during the period of normal cells transiting towards tumor cells[21]. The activation of telomerase may be the key step in immortalization and malignant tumor cell formation[22]. It was discovered recently that the formation and the development of gastric cancer are tightly associated with telomere and telomerase[23,24]. Gastric cancer is one of the most common malignancies in China with a considerable high mortality. Gastric cancer formation is a process involved in multiple factors, multiple steps and multiple genes. The activation of telomerase is a necessary step for formation of many tumors, and there is concordance in RNA structure of telomerase of human tumors, making telomerase as a target to rationally select the drug to treat malignant tumors like gastric cancer, which may lead to a revolution in gene therapy against tumors. As a reverse transcriptase inhibitor, the main function of AZT is to interrupt reverse transcription of cells to block the cell cycle, interfere with normal differentiation, terminate the cell replication and inhibit the cell growth[25,26]. AZT has been found to inhibit many kinds of enzymes in tumor cells. The telomerase activity is closely related to abnormal proliferation of tumor cells, indicating that AZT may be an effective anti-tumor drug[27,28]. Since telomerase does not express itself in normal human tissues, telomerase has little effect on normal tissues. We discovered that both AZT and FA-2-b-β of different concentrations have obvious inhibiting effects on gastric cancer cells, and the inhibiting effects increased in a dose- and time-dependent manner. We also found that AZT can inhibit the activity of telomerase in MKN45 cells, and FA-2-b-β from *Agaricus blazei* combined with AZT had a significant higher inhibition rate to gastric cancer than that used alone, and the concentration of AZT had a negative correlation with the activity of telomerase. This combined administration can lower the dose of AZT so as to reduce the side effects of AZT, which will greatly extend the clinical use of AZT.

Apoptosis is controlled by genes. There are many genes involved in apoptosis process, in which Bcl-2, p53, Caspase-3 and C-myc play important roles of regulation. Caspase is one of the family members containing a cysteine in cytoplasma[29]. All factors inducing apoptosis will go through caspase system, in which Caspase-3 expression is the final key step for apoptosis of many cells[30-32]. Bcl-2 is an apoptosis inhibiting gene located in 18q21 of human chromosome, which is a membrane protein at the outer membrane of mitochondrion, regulating the activity of telomerase. When Bcl-2 expression increased, telomerase activity increased. Vice versa, when Bcl-2 expression decresed, the activity of telomerase decreased[33]. It is reported that Bcl-2 can lower the level of cytochrome C and apoptosis inducing factor (AIF), e.g. when Caspase was released from mitochondrion to cytoplasms, it would inhibit apoptosis and promote the formation of cancer[34]. Caspase-3 has been implicated in the development of gastric cancer, and played an important role in the transformation from normal gastric mucosa to gastric cancer. Our results indicated that certain concentration of AZT can up-regulate the expression of Caspase-3 mRNA, which was positively correlated to the apoptosis

rate ($r = 0.926$, $P < 0.01$) and it could also down-regulate the expression of Bcl-2, which was negatively correlated to apoptosis rate ($r = 0.9969$, $P < 0.01$). These might be one of the molecular mechanisms of the apoptosis induction effect of AZT and FA-2-b-β on gastric cancer cells. And the efficiency of combined FA-2-b-β and AZT was obviously higher than that use alone. FA-2-b-β has a synergistic effect with AZT.

Although chemotherapy is a common treatment for malignant tumors, the chemo-drugs kill not only cancer cells but also many normal cells like lymphocytes, hemopoietic blood stem cells, *etc*, and greatly affect the life qualities of the patients and therapeutic effect. With the bio-characteristics of malignant tumor, the effect of any single use of drugs is limited, however, increasing the dose of the drug may be of no benefit to improving the treatment at all. So to develop combinations of drugs for multiple target treatment to improve the effect, to decrease the toxicity and to resist the drug tolerance has become major aspects of treatment of tumors. The RNA protein compound (extracted from Liang Jing mushroom), FA-2-b-β, not only has certain inhibition effect of cancer cells, but effect of decreasing the toxicity of the chemo-drugs. When used in combination, it may decrease both the dose and toxicity of chemo-drugs without reducing its effects. For all the reasons above, as a ancillary agent of chemotherapy, FA-2-b-β might be a promising agent in clinical practice. Our study has provided the experimental data for further researches. And the synergistic effect of AZT and FA-2-b-β can be expected as a new approach to the treatment of gastric cancer.

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