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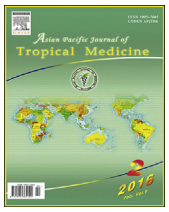

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Inhibiting effect of Endostar combined with ginsenoside Rg3 on breast cancer tumor growth in tumor-bearing mice

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ABSTRACT

Objective: To study the inhibiting effect of Endostar combined with ginsenoside Rg3 on breast cancer tumor growth in tumor-bearing mice.
Methods: Female mice were selected as experimental animals, and breast cancer tumor-bearing mouse models were established and then divided into groups A, B, C and D that respectively received saline, recombinant human endostatin, ginsenosides Rg3 and recombinant human endostatin combined with Rg3 intervention; 7 d, 14 d and 21 d after intervention, tumor tissue volume was measured; 21 d after intervention, mice were killed, tumor tissue was collected, and mRNA contents of angiogenesis molecules, invasion molecules, autophagy marker molecules and autophagy signaling pathway molecules were detected.
Results: At 7 d, 14 d and 21 d after intervention, tumor tissue volume of groups B, C and D was lower than that of group A, and tumor tissue volume of group D was lower than that of groups B and C; mRNA contents of *VEGFA*, *VEGFB*, *VEGFC*, *MMP2*, *MMP9*, *p62*, *mTOR*, *PI3K*, *Akt*, *JNK* and *Beclin-1* in tumor tissue of groups B, C and D were significantly lower than those of group A, and *LC3-II/LC3-I* was significantly higher than that of group A; mRNA contents of *VEGFA*, *VEGFB*, *VEGFC*, *MMP2*, *MMP9*, *p62*, *mTOR*, *PI3K*, *Akt*, *JNK* and *Beclin-1* in tumor tissue of group D were significantly lower than those of groups B and C, and *LC3-II/LC3-I* was higher than that of groups B and C.
Conclusions: Endostar combined with ginsenoside Rg3 has stronger inhibiting effect on breast cancer tumor growth in tumor-bearing mice than single drug, and it can inhibit angiogenesis and cell invasion, and enhance cell autophagy.

1. Introduction

Breast cancer is the malignant tumor with highest incidence in women, and its incidence increases in recent years. Cancer cell proliferation, invasion and local angiogenesis are the malignant biological behaviors closely related to the occurrence and development of breast cancer. Regulation of malignant biological behaviors of tumors is a process involving multiple targets and multiple genes, and simultaneous use of drugs against different targets can more effectively inhibit the development of malignant tumors. Recombinant human endostatin (Endostar) is a kind of targeted drug with anti-angiogenesis effect, and ginsenoside Rg3 is an important component with effect of inhibiting cell proliferation and invasion [1,2]. The two drugs can target different links of malignant biological effect and exert antitumor effect, and are expected to be able to achieve synergistic and additive effect. In the following research, the inhibiting effect of Endostar combined with ginsenoside Rg3 on breast cancer tumor growth in tumor-bearing mice was analyzed.

2. Materials and methods

2.1. Experimental materials

A total of 32 Female C57 mice were purchased from Shandong University Laboratory Animal Center; breast cancer MCF-7 cell lines were purchased from the cell bank of Chinese

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Academy of Sciences; recombinant human endostatin (Endostar) was purchased from Shandong Simcere Medgenn Biological Pharmaceutical Co., Ltd.; ginsenoside Rg3 was purchased from Liaoning Bio-medical Technology Co., Ltd., and RNA extraction as well as PCR amplification kits were purchased from Beijing ComWin Company.

2.2. Experimental methods

2.2.1. Establishment of tumor-bearing mouse models

MCF-7 cell lines were recovered and cultured with RPMI-1640 media that contained 10% fetal bovine serum, 100 IU/mL penicillin and 100 IU/mL streptomycin; after 2–3 subcultures, cells that were digested with pancreatin were collected, density was adjusted to 5×10^7 mL and 0.1 mL of cells were inoculated under right mammary gland; 2–3 wk after inoculation, tumor volume grew to 150–200 mm^3, and mice were used for subsequent study and randomly divided into groups A–D, each group with 8 mice.

2.2.2. Medication of tumor-bearing mice

Group A received intraperitoneal injection of same volume of saline as groups B–D; group B received subcutaneous injection of 10 mg/kg recombinant human endostatin, 1 time/2 d; group C received subcutaneous injection of 5 mg/kg ginsenoside Rg3, 1 time/2 d; group D received subcutaneous injection of 10 mg/kg recombinant human endostatin and 5 mg/kg ginsenoside Rg3. Medication was 10 times in a row.

2.2.3. Measurement of tumor volume

At 7 d, 14 d and 21 d after medication, major diameters and minor diameters of tumor tissue of four groups were measured, and the following formula was used to calculate tumor volume: volume = major diameter × minor diameter^2 × 0.5.

2.2.4. Collection of tumor tissue and detection of related indexes

At 21 d after medication, tumor-bearing mice were killed after measurement of tumor volume was completed; tumor tissue was collected, washed with saline and then rapidly frozen with liquid nitrogen; then RNA extraction kits were used to obtain RNA in the tissue and reverse-transcribe it to cDNA for PCR reaction, and amplified genes included *vascular endothelial growth factor (VEGF)A*, *VEGFB*, *VEGFC*, *matrix metalloproteinase (MMP)2*, *MMP9*, *LC3-II*, *LC3-I*, *p63*, *mTOR*, *PI3K*, *Akt*, *JNK* and *Beclin-1*. Amplification curve was obtained, and then mRNA contents of related genes in tumor tissue of group A were set to 100 to calculate relative values of mRNA contents of corresponding genes in tumor tissue of groups B, C and D. Mice were killed and incinerated together after materials were collected.

2.3. Statistical process

SPSS18.0 software was used to input above data, measurement data of four groups were processed by variance analysis and $P < 0.05$ was standard of statistical significance in differences.

3. Results

3.1. Trend of tumor tissue volume

At 21 d after intervention, tumor tissue volume of four groups showed increasing trend, increasing trend of tumor tissue volume of groups B, C and D was weaker than that of group A, and increasing trend of tumor tissue volume of group D was weaker than that of groups B and C; tumor tissue volume of groups B, C and D at various points in time was lower than that of group A, and tumor tissue volume of group D was lower than that of groups B and C (Table 1).

3.2. Expression levels of VEGFs and MMPs molecules in tumor tissue

Expression levels of *VEGFA*, *VEGFB* and *VEGFC* as well as *MMP2* and *MMP9* in tumor tissue of groups B, C and D were significantly lower than those of group A; expression levels of *VEGFA*, *VEGFB* and *VEGFC* as well as *MMP2* and *MMP9* in tumor tissue of group D were significantly lower than those of groups B and C (Table 2).

3.3. Expression levels of autophagy marker molecules

LC3-II/LC3-I in tumor tissue of groups B, C and D was higher than that of group A, and mRNA contents of *p62* were significantly lower than that of group A; *LC3-II/LC3-I* in tumor tissue of group D was higher than that of groups B and C, and mRNA content of *p62* was lower than those of groups B and C (Table 3).

3.4. Autophagy signaling pathway function

mRNA contents of *mTOR*, *PI3K*, *Akt*, *JNK* and *Beclin-1* in tumor tissue of groups B, C and D were lower than those of group A, and mRNA contents of *mTOR*, *PI3K*, *Akt*, *JNK* and *Beclin-1* in tumor tissue of group D were lower than those of groups B and C (Table 4).

Table 1
Trend of tumor tissue volume of four groups (mm^3).

Group	Before intervention	7 d After intervention	14 d After intervention	21 d After intervention
Group A	158.52 ± 16.15	314.36 ± 36.78	498.37 ± 51.28	723.67 ± 81.51
Group B	160.23 ± 15.38[a]	244.44 ± 24.34[a]	333.63 ± 37.55[a]	485.29 ± 61.17[a]
Group C	157.39 ± 12.92[a]	250.34 ± 27.42[a]	338.57 ± 35.34[a]	492.33 ± 47.22[a]
Group D	161.29 ± 17.88[a,b,c]	194.28 ± 22.14[a,b,c]	257.35 ± 27.14[a,b,c]	337.28 ± 42.78[a,b,c]
F	0.182	6.967	8.485	11.339
P	>0.05	<0.05	<0.05	<0.05

Compared with group A, [a]$P < 0.05$; compared with group B, [b]$P < 0.05$; compared with group C, [c]$P < 0.05$.

Table 2
Expression levels of VEGF and MMPs molecules in tumor tissue of four groups.

Group	mRNA contents of *VEGFs*			mRNA contents of *MMPs*	
	VEGFA	*VEGFB*	*VEGFC*	*MMP2*	*MMP9*
Group A	100.00 ± 12.58	100.00 ± 15.10	100.00 ± 13.16	100.00 ± 11.48	100.00 ± 13.22
Group B	68.18 ± 7.37[a]	63.51 ± 7.24[a]	70.36 ± 8.17[a]	52.15 ± 6.32[a]	57.41 ± 6.23[a]
Group C	71.67 ± 7.11[a]	68.24 ± 6.91[a]	72.73 ± 7.78[a]	53.73 ± 6.75[a]	61.27 ± 7.35[a]
Group D	28.41 ± 2.95[a,b,c]	19.33 ± 2.14[a,b,c]	39.22 ± 4.28[a,b,c]	22.36 ± 3.95[a,b,c]	15.33 ± 1.86[a,b,c]
F	18.948	26.844	15.482	20.183	17.785
P	<0.05	<0.05	<0.05	<0.05	<0.05

Compared with group A, [a]$P < 0.05$; compared with group B, [b]$P < 0.05$; compared with group C, [c]$P < 0.05$.

Table 3
Expression levels of autophagy marker molecules in tumor tissue of four groups.

Group	*LC3-II/LC3-I*	*p62*
Group A	1.00 ± 0.11	100.00 ± 12.91
Group B	1.89 ± 0.13[a]	53.42 ± 6.31[a]
Group C	1.77 ± 0.20[a]	55.38 ± 5.72[a]
Group D	3.23 ± 0.38[a,b,c]	27.37 ± 3.12[a,b,c]
F	14.922	22.108
P	<0.05	<0.05

Compared with group A, [a]$P < 0.05$; compared with group B, [b]$P < 0.05$; compared with group C, [c]$P < 0.05$.

Table 4
Expression levels of autophagy signaling pathway-related molecules in tumor tissue of four groups.

Group	*mTOR/PI3K/Akt*			*JNK/Beclin-1*	
	mTOR	*PI3K*	*Akt*	*JNK*	*Beclin-1*
Group A	100.00 ± 12.78	100.00 ± 15.91	100.00 ± 13.18	100.00 ± 15.47	100.00 ± 13.48
Group B	58.95 ± 7.12[a]	63.94 ± 7.22[a]	61.35 ± 6.94[a]	82.26 ± 9.14[a]	55.68 ± 5.84[a]
Group C	62.28 ± 6.88[a]	66.20 ± 6.96[a]	60.17 ± 5.35[a]	77.33 ± 7.97[a]	58.15 ± 6.24[a]
Group D	35.22 ± 4.12[a,b,c]	21.39 ± 3.25[a,b,c]	16.36 ± 1.82[a,b,c]	26.43 ± 2.89[a,b,c]	34.24 ± 4.12[a,b,c]
F	17.877	24.961	33.812	21.305	20.184
P	<0.05	<0.05	<0.05	<0.05	<0.05

Compared with group A, [a]$P < 0.05$; compared with group B, [b]$P < 0.05$; compared with group C, [c]$P < 0.05$.

4. Discussion

Recombinant human endostatin (Endostar) is endostatin that is independently developed by Chinese scientists and it has anti-angiogenesis effect [3]. The drug uses *Escherichia coli* as expression vector to obtain the desired proteins, the expression efficiency is high, physicochemical properties are stable, and the drug itself has lower toxicity and less adverse reaction [4]. Study has confirmed that application of the drug can inhibit the progression and pathological process of breast cancer, and reduce the expression of pro-angiogenesis molecules and pro-invasion molecules [5]. In the research, after breast cancer tumor-bearing mouse models were established, recombinant human endostatin was used for intervention, and then dynamic changes of tumor volume were measured. Analysis results showed that increasing trend of breast cancer tumor tissue volume of group B was significantly weaker than that of group A, and 7 d, 14 d and 21 d after intervention, breast cancer tumor tissue volume of group B was significantly lower than that of group A. It indicated that recombinant human endostatin could inhibit breast cancer tumor growth in tumor-bearing mice.

Ginsenoside Rg3 is a kind of antitumor composition extracted from extracting solution of ginseng root, and it has inhibiting effect on biological behaviors of a variety of malignant tumor cells [6]. Studies have confirmed that antitumor activity of ginsenoside Rg3 is manifested as inhibiting cancer cell proliferation, invasion and angiogenesis, and molecules that are inhibited include cyclin, vascular endothelial growth factor, matrix metalloproteinase and so on [7,8]. In the research, after breast cancer tumor-bearing mouse models were established, ginsenoside Rg3 was used for intervention, and then dynamic changes of tumor volume were measured. Analysis results showed that increasing trend of breast cancer tumor tissue volume of group C was significantly weaker than that of group A, and 7 d, 14 d and 21 d after intervention, breast cancer tumor tissue volume of group C was significantly lower than that of group A. It indicated that ginsenoside Rg3 could inhibit the breast cancer tumor growth in tumor-bearing mice.

In the development process of breast cancer, malignant biological behaviors of cancer cells are regulated by a variety of molecules, and combined application of drugs with different mechanisms can block different links of malignant biological behaviors, thereby more effectively inhibiting the development of tumor. Ginsenoside Rg3 and recombinant human endostatin can act on different links of malignant biological behaviors of breast cancer cells, and combined application of the two drugs is expected to be able to more effectively inhibit the development of breast cancer. In the research, tumor tissue volume of four groups was compared, and first of all, breast cancer tumor tissue volume of group D was significantly lower than that of group A, which indicated that ginsenoside Rg3 combined with recombinant human endostatin could inhibit breast cancer growth;

second, breast cancer tumor tissue volume of group D was significantly lower than that of groups B and C, which indicated that ginsenoside Rg3 combined with recombinant human endostatin had stronger inhibiting effect on breast cancer growth than single drug.

Local angiogenesis and cell infiltration are the basis of the occurrence and development of tumor, and formation of new blood vessels in tumor tissue as well as infiltration of cancer cells to vascular structure can provide blood supply to the metabolism of tumor cells, meanwhile reduce resistance of vascular system and increase blood flow and blood supply. Tumor angiogenesis is a complex process involving a variety of cytokines, including the links of pro-angiogenesis factor enhancement, cell adhesion and invasion to endothelial basement membrane and formation of vascular structure. VEGF is the currently known cytokine with the strongest pro-angiogenesis effect, and it can specifically act on endothelial cells and promote the formation of vascular structure [9,10]; MMPs are molecules directly related to the degradation of extracellular matrix and basement membrane components, and they can promote cancer cell invasion to vascular system as well as reduce vascular resistance and increase tumor blood supply [11,12]. In the research, after tumor-bearing mice received combined treatment of recombinant human endostatin and ginsenoside Rg3, expression levels of above molecules in tumor tissue were analyzed, and results showed that expression levels of *VEGFA*, *VEGFB* and *VEGFC* as well as *MMP2* and *MMP9* in tumor tissue of groups B, C and D were lower than those of group A and expression levels of above molecules of group D were lower than those of groups B and C. It indicated that both recombinant human endostatin and ginsenoside Rg3 could inhibit the expression of *VEGFs* and *MMPs*, combined application of the two drugs had synergistic effect and the inhibiting effect was more significant.

Study in recent years believes that cell autophagy is related to the occurrence and development of malignant tumors. Autophagy means that cells use lysosomes to degrade damaged organelles and macromolecular substances, and it can cause cell death. The process is another type of programmed cell death different from apoptosis and necrosis, also known as 'Type II cell death'. Activating autophagy process can induce cell apoptosis, and therefore, autophagy has also become a new target for treatment of malignant tumors [13]. LC3 and P62 are autophagy marker molecules, and in the process of autophagy, LC3-I transforms to LC3-II and ubiquitin protein p62 is degraded [14]. In the research, analysis of the expression levels of autophagy marker molecules showed that *LC3-II/LC3-I* in tumor tissue of groups B, C and D was higher than that of group A and mRNA contents of *p62* were lower than that of group A; *LC3-II/LC3-I* in tumor tissue of group D was higher than that of groups B and C and mRNA content of *p62* was lower than those of groups B and C. It indicated that both recombinant human endostatin and ginsenoside Rg3 could enhance cell autophagy and the enhancement effect of combined use of the two drugs was more significant. mTOR/PI3K/Akt and JNK/Beclin-1 are currently known two signaling pathways that regulate autophagy process, and enhancement of their function can inhibit cell autophagy and create an enabling environment for cell proliferation. In the research, analysis of the function of two signaling pathways in tumor tissue showed that both recombinant human endostatin and ginsenoside Rg3 could inhibit the function of *mTOR/PI3K/Akt* and *JNK/Beclin-1* signaling pathways and thus enhance cell autophagy process, and the inhibiting effect of combined use of the two drugs was stronger.

Based on above discussion, it can be concluded that Endostar combined with ginsenoside Rg3 has stronger inhibiting effect on breast cancer tumor growth in tumor-bearing mice than single drug, and it can inhibit angiogenesis and cell invasion, and enhance cell autophagy.

Conflict of interest statement

We declare that we have no conflict of interest.

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