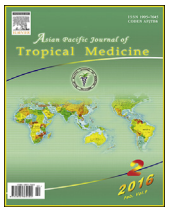
HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm



Original research http://dx.doi.org/10.1016/j.apjtm.2016.01.010

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

Yun Zhang[1], Qing-Zhan Liu[2], Su-Ping Xing[1*], Jin-Ling Zhang[1]

[1]*Oncology Department No. 2, Linyi People's Hospital, Linyi City, Shandong Province, 276000, China*

[2]*Radiotherapy Department, Linyi People's Hospital, Linyi City, Shandong Province, 276000, China*

ARTICLE INFO

Article history:
Received 15 Nov 2015
Received in revised form 20 Dec 2015
Accepted 30 Dec 2015
Available online 11 Jan 2016

Keywords:
Breast cancer
Recombinant human endostatin
Ginsenoside Rg3
Autophagy

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

*Corresponding author: Su-Ping Xing, Bachelor, Oncology Department No. 2, Linyi People's Hospital, No. 27, East Section of Jiefang Road, Lanshan District, Linyi City, Shandong Province, 276000, China.
Tel: +86 13563988539
E-mail: fenglin_w@126.com

Peer review under responsibility of Hainan Medical College.
Foundation project: It is supported by Linyi City Science and Technology Development Plan in 2014 (No. 201413010).

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]$P < 0.05$.