Article

Study effect and mechanism of andrographolide on breast cancer cell MCF-7 and HCT116

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Abstract: The aim of this study was to investigate the inhibitory effect of andrographolide on breast cancer cell line MCF-7 and HCT116 proliferation and its mechanism. MCF-7 and HCT116 in breast cancer cell were collected in vitro, according to different concentration of andrographolide (AND) dealt were divided into four groups, 15mol/LAND was as low concentration group, 30 mol/L AND was as middle concentration group, 60 mol/L AND was as high concentration group, no AND dealt was as blank control group, cell proliferation was detected by MTS, morphological change of cells were observed by microscope, apoptosis related proteins Caspase-3, Caspase-9 and Bal-2 mRNA express were detected by RT-PCR. The result showed that the cell proliferation inhibition rate of AND dealt groups were significantly increased, and with different concentration and dealt time changed significantly, AND concentration was high, the dealt time was longer, cell proliferation inhibition rate were higher (P<0.05), the microscope showed, MCF-7 and HCT116 cell shrinkage, shedding, assume apoptosis. With AND concentration increasing, Caspase-3, Caspase-9mRNA express gradually increased, Bal-2 mRNA express gradually weakened, comparison between groups with significant difference (P<0.05). Andrographolide on breast cancer MCF-7 and HCT116 cell proliferation has obvious concentration and time dependent inhibition effect, its mechanism may be down-regulating Bal-2 or up-regulating Caspase-3, Caspase-9 protein express.

1. Introduction

Breast cancer is a serious threat to human health of one of the malignant tumor, recent studies have shown that the incidence of breast cancer were significantly elevated trend, and gradually tend to be younger [1-3]. Malignant tumor has more genes and molecules involved in the pathological process of Invasion and metastasis, and a tumor cell degeneration, fall off, cell matrix degradation process such as vascular invasion and lymph node are also involved [2-4]. Andrographolide (AND) is one of the Chinese medicine active ingredients of Andrographolide, its system of angiospermae plant andrographis Andrographispaniculaa (Burm. F.) Nees diterpene lactone class extracts compounds, and has function such as antitumor, antimicrobial infection and protects liver function [5-7]. But research about Andrographolide in breast cancer cell line MCF-7 and HCT116 proliferation inhibition and its mechanism is less.

2. Experiment Materials

MTT, DiOC6, penicillin, streptomycin were for American Sigma products. DMEM medium and fetal bovine serum was from GibcoBRL Company. DMSO is Guangdong Guanghua chemical plants product, Caspase 3, Caspase-9, Bal-2 antibodies bought from the Cell Signaling company, Andrographolide, AND(batch number: 110797-200307, purity > 98%) bought in Guangdong Medicine Institute, dimethyl sulfoxide (DMSO, Guangdong Guanghua chemical plants products). The MTS test kit bought form Promega Company. Enzyme standard instrument (Thermo company, type of MK3), electron microscope (NIKON company, model TE2000) and Trizol bought from Shanghai Sangong biological engineering company. RT-PCR kit purchased from Shanghai China Treasure Biological Technology Company.

3. Methods

3.1. Cell lines and cell culture

The MCF-7 and HCT116 cell line was obtained from the China Type Culture Collection Cells were cultured in Dulbecco's modified Eagle medium (DMEM) (Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillinand 60 μg/ml kanamycin. All the cells were cultured to logarithm growth period, digested and implanted. The cells were cultured in conditioned medium including 0.1% bovine serum albumin, 1% calf serum, 1.0 μmol green copperas.

3.2. Paniculta lactone solution preparation

Using dimethyl sulfoxide (DMSO) dissolve paniculta lactone, with DMEM medium dilution to 15mmol/L (low concentration group), 25 mmol/L (mid concentration group) and 50mmol/ L (High concentration group), using EP tube aseptic packaging after filtering and cryopreserved in-20℃.With DMEM medium diluted to the required concentration.

3.3. Inhibition of cell growth

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Cell growth was measured using the 3-(4,5 dimethylthiazol-2yl)-2,5-diphenyl-tetrazolium bromide(MTT; Sigma, USA) colorimetric dye reduction method. 5000 Cells were trypsinized and seeded into 96 well plates (4×10^3cell/well). After overnight culture, cells were exposed to increasing concentrations of celastrol ranging from 0.008 to 25 μg/ml for another 24 hours. MTT of 20 μl (1 mg/ml) was added to each well for 4 hours at 37℃ to allow MTT to form formazan crystals by reacting with metabolically active cells. Subsequently the formazan crystals were solubilized by 150 μl of DMSO. The absorbance of each well was measured in a microplate reader at 490 nm (A490). For assessment of efficiency of growth inhibition, an IC_{50} value was calculated using the dose-response curve.

Table 1 Cell proliferation inhibition rate comparison between groups.

Groups	12h	24h
50 mmol/L group	55.32±2.31ab	75.21±5.12ab
25 mmol/L group	33.24±3.01a	55.59±4.13a
15 mmol/L group	11.04±2.01	23.56±3.12
Blank control group	0.00±0.00	0.00±0.00
F	5.16	4.99
P	0.001	0.001

Note: compared with 15 mmol/L group, a: P=0.001, and compared with 25 mmol/L group, b: P=0.001.

3.4. RT-PCR method

Cells are regulated commonly and added with 1 ml TRIZOL for schizolysis. After being shaken and stewing for 10 minutes, cells were added with 200ml phenol chloroform and being shaken and centrifuged to give convenience to take supernatant materials. Using isopropanol to settle supernatant materials and 75% ethanol to extract Ribonucleic Acid (RNA). Determining premier was according to real-time quantification. Premier of Caspase-3: Sence (5'-TGATGGTGTCT-GCTGGAAAG-3') and Antisense (5'-GACACGT-GAAAAGTGCCTTG-3'). The premier length is 280 bp. Premier of Caspase-9: Sence (5'-CAGAGATGC GTGG AGAGTCG-3') and Antisense (5'-CAAAGGCGTCGTCAATCACC-3'), Premier of Bal-2: Sence (5'-CCGAT-GTGGTGTTCCAGACA-3') Antisense (5'-TGGC-CTC GTATGT GGCATACT-3'), the premier length is 159 bp.1~5Lg total RNA was added into the reaction pipe. The pipe was heat to 75℃ and lasted for 5 minutes and put on ice quickly. Adding 4L 5×buffer and 2L dNTP and heat the pipe to 95℃ and lasted for 5 minutes, and then 94℃ for 30 seconds, and then 60℃ for 30 seconds, and then 72℃ for 30 seconds, heating circulation for 40 times. The collected pure PCR product was taken as relative quantitative criteria product. IGF-IR and 18sr RNA was taken as a positive model and diluted according to 10-5 and 10-9

and relative copy number was determined. Quantitative reaction was finished and standard cure was achieved. The pipe was heat to 95℃ and lasted for 4 minutes, and then 95℃ for 20 seconds, and then 59℃ for 20 seconds, heating circulation for 40 times. Lastly, the pipe was heated to 94℃ and lasted for 0 second, and then 60℃ for 15 seconds, and then 95℃ for 0 second. Fluorescence signal was collected and analyzed with solubility curve. Every sample includes 3 wells. Standard curve and solubility curve were finished and sample relative quantitative copy number was determined by the data.
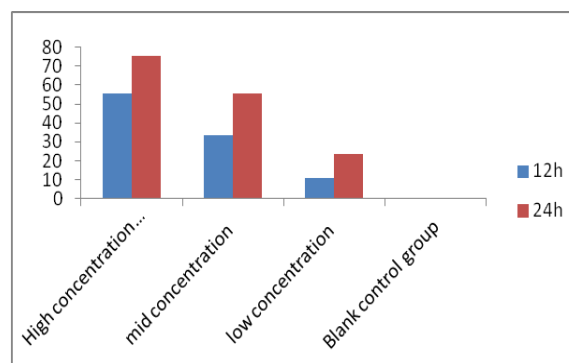

Figure 1. Cell proliferation inhibition rate comparison between groups.

3.5. Electron microscope morphological changes

Logarithmic phase human breast cancer cell line MCF-7 and HCT116 cells were collected, and pancreatic with enzyme digestion of 0.25 orifice, observing cell morphological change with microscope in low concentration group, the concentration, the high concentration group and the blank control group after action 24h.

3.6. Statistical methods

The statistical software used was SPSS 17.0 software. Two groups of measuring data are using T test, the count of data between the two groups are using x^2 test. P <0.05 was considered statistically significant.

4. Results

4.1. Cell proliferation inhibition rate comparison between groups

High concentration cell proliferation inhibition rate was 55.32%, significantly higher than the mid concentration in the group (33.24%), low concentration (11.04%) (P<0.05). Cell proliferation inhibition concentration group was obviously higher than that in low concentration group (P<0.05), and longer duration of drug treatment, cell proliferation inhibition rate increased significantly, different drugs demanding time is statistically significant difference too (P < 0.05), Table 1, Figure 1.

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4.2. Cell morphological change observing with microscope

MCF-7 and HCT116 cell shrinkage, fall off, apoptosis, which is most obvious in high concentration group, Figure 2.
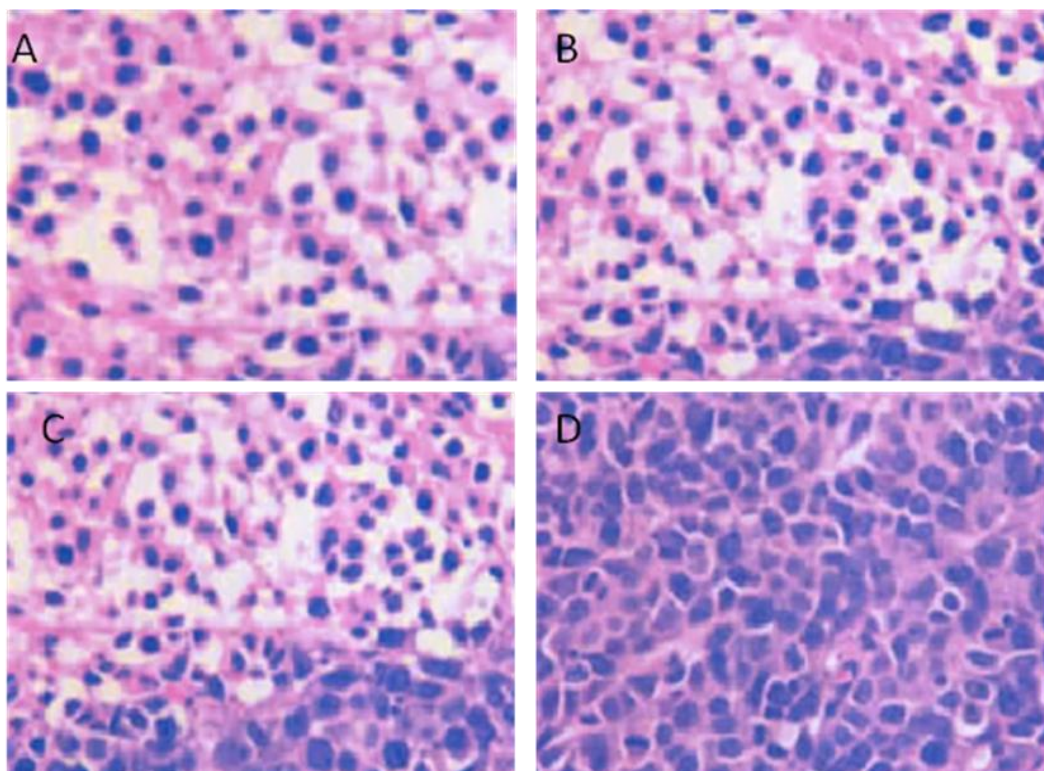

Figure 2. Change of breast MCF - 7, HCT116 cells in electron microscope (×400).
A: high concentration group, focal tumor cell shrinkage, fall off, apoptosis; B: mid concentration group, focal tumor cell shrinkage, fall off, apoptosis; C: low concentration group, focal tumor cell shrinkage, fall off, apoptosis; D: blank control group, tumor cell density

4.3. Caspase 3, Caspase-9, Bal-2 expression between groups

High concentration group of Caspase 3, concentration of Caspase-9 mRNA expression was significantly higher in than mid group and low concentration group and the control group, the above indicators were significantly higher than that of low concentration group and the control group (P<0.05), high concentration group of Bal-2 mRNA expression was lower than that in mid group and low concentration group and the control group, the Bal2 mRNA expression in mid concentration group was obviously lower than that of low concentration and the control group, low concentration group of Bal-2 mRNA expression was significantly lower than the control group, (P< 0.05), Table 2 , Figure 3.

Table 2 Comparison of Caspase 3, Caspase-9, Bal-2 mRNA expression.

Groups	Caspase-3	Caspase-9	Bal-2
50 mmol/L group	1.89±0.11ab	1.75±0.05ab	0.33±0.05ab
25 mmol/L group	1.56±0.08a	1.62±0.04a	0.27±0.04a
15 mmol/L group	1.03±0.05	1.43±0.03	0.22±0.03
Blank control group	0.71±0.09	1.32±0.02	0.15±0.01
F	4.12	3.65	3.98
P	<0.05	<0.05	<0.05

Note: compared with 15 mmol/L group, a: P=0.001, and compared with 25 mmol/L group, b: P=0.001.

5. Discussion

Paniculta lactone induced lung cancer, cervical cancer and prostate cancer cells apoptosis thus play a role of anti-tumor [8]. Paniculta lactone can inhibit malignant tumor cell proliferation, the human breast cancer MCF-7 and HCT116 cell proliferation inhibition increased significantly within 24 h of

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medication, which increased with the increase of drug concentration and processing time, the tumor cell proliferation inhibition is more apparent. Related research confirmed that the 10 mu mol/L, 30 mu mol/L and 60 mu mol/L of Paniculta lactone had proliferation inhibition rate of 10.18%, 31.99% and 57.96% in cervical cancer, respectively. All of them were significantly higher than that of blank control group [9-10]. Therefore, Paniculta lactone has obvious effect in the inhibition of proliferation of malignant tumor cell, and with the increase of concentration, the tumor cell proliferation inhibition increased significantly. Paniculta lactone has obvious inhibitory effect of malignant tumor cell proliferation. On the other hand, Paniculta lactone as an anti-cancer Chinese medicine preparation, will be one of the effective components for injection [11-13]. The pharmacological experiments confirmed that Paniculta lactone have obvious anti-tumor effect by injection in digestive tract malignant tumor, respiratory tract malignant tumor and gynecologic malignant tumors [14-16]. Second, the literature indicates that paniculta lactone being with antitumor function as the chemotherapy drug paclitaxel and cisplatin former by inhibiting tumor cell proliferation [17-19]. Research report also confirmed paniculta lactone play a role of effective suppressor by inhibiting prostate cancer (PC) - 3 cell shrinkage, fall off and apoptosis [20-22]. But in human breast cancer cell line MCF-7 and HCT116, paniculta lactone proliferation inhibition and its mechanism research is less.
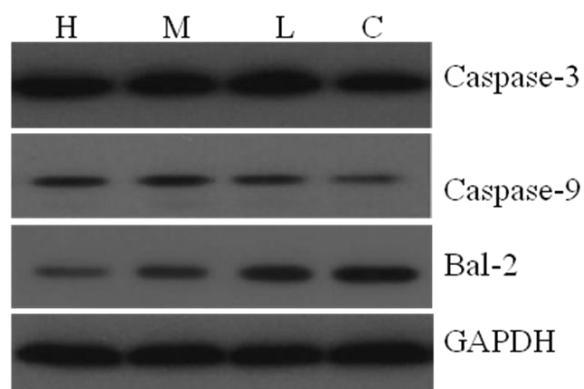

Figure 3. Caspase-3、Caspase-9、Bal-2 mRNA express in MCF-7 、HCT116 cell, H: high concentration group, M: middle concentration group, L: low concentration group, C: control group.

According to the results of our research .Breast cancer MCF - 7, HCT116 cell proliferation inhibition rate increased obviously after AND treatment. It can inhibit tumor cell proliferation [23]. Moreover, with the drug concentration and processing time of change, the higher drug concentration, the longer the processing time, cell proliferation inhibition rate is higher. As a result, AND has obvious inhibiting effect

on tumor cell drug dose and time dependent [24]. With the increase of drug concentration (15, 30, 60mu mol/L), 12 hour for the treatment of tumor cell proliferation inhibition rates were 11.04%, 33.24%, 55.32%, and 24 hour treatment of tumor cell proliferation inhibition rates were 23.56%, 55.59%, 75.21%. Prompted by 60 mu mol/L medication 12 h, tumor cell proliferation inhibition rate is as high as 50% above, after 30 umol/L medication within 24 h, tumor cell proliferation inhibition rate is as high as 50% above. Choosing appropriate concentration and action time helps to inhibit breast cancer MCF-7, HCT116 cell proliferation, improve the curative effect of treatment of traditional Chinese medicine effective component paniculta lactone. In order to further confirm paniculta lactone inhibiting tumor cell proliferation and apoptosis, this study through observe MCF-7 AND HCT116 cell morphological changes with electronic microscope, at the same time as the AND drug concentration increased, cell shrinkage, fall off, apoptosis change, Caspase 3, Caspase -9 mRNA expression gradually strengthen, and the Bal-2 mRNA expression fade out. It showed that paniculta lactone promote Caspase 3, Caspase-9 activation and result in tumor target cells apoptosis, inhibition of cell proliferation by activating Caspase 3, Caspase apoptosis-9. Bal-2 as antiapoptotic proteins, adjust he permeability of the mitochondrial membrane channels to stabilize the mitochondria, inhibite apoptosis related proteins activation. In combination with the results of this study, paniculta lactone play a significant anti-tumor effect, by inhibiting the Bal-2 and the promotion of Caspase 3, Caspase - 9 expression , which the dose, the greater the effect, the longer its antitumor effect.

6. Conclusion

Andrographolide on breast cancer MCF-7 and HCT116 cell proliferation has obvious concentration and time dependent inhibition effect, its mechanism may be down-regulating Bal-2 or up-regulating Caspase-3, Caspase-9 protein express.

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