Preliminary research on the effect of Taxus chinensis var. mairei aqueous extract on malignant biological behavior of ovarian cancer cell line SKOV-3

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ARTICLE INFO

Objective: To study the effect of Taxus chinensis var. mairei aqueous extract on malignant biological behavior of ovarian cancer cell line SKOV-3. Methods: Ovarian cancer cell line SKOV-3 were cultured and divided into the control group (processed with DMEM medium without fetal bovine serum) and the treatment group (processed with 600 µ g/L, 800 µ g/L and 1000 µ g/L Taxus chinensis var. mairei aqueous extract). Then cell viability, migration ability, ATP generation and mRNA contents of related genes were detected. Results: (1) Cell viability and migration ability: Taxus chinensis var. mairei aqueous extract could decrease the MTS value and cell migration rate on dose-dependent manner; (2) ATP generation: Taxus chinensis var. mairei aqueous extract could decrease ATP generation on dose-dependent manner; (3) Gene mRNA contents: Taxus chinensis var. mairei aqueous extract could decrease expressions of IGF-β 1, CD44v, Survivin, MIF, HIF-1 and VEGF on dose-dependent manner. Conclusion: Taxus chinensis var. mairei aqueous extract can inhibit viability, migration ability and ATP generation of ovarian cancer cell; it can also reduce expressions of proliferation and migration related genes as well as angiogenesis related genes.

Ovarian cancer is a common malignant tumor of female reproductive system. For patients with advanced ovarian cancer, chemotherapy regimen based on platinum drugs is mostly suggested, which can effectively kill cancer cells and prolong patients' survival time. Nonetheless, there are still some patients who are insensitive to chemotherapeutic drugs and thus chemotherapy fails; there are also some patients who cannot tolerate the adverse reactions of chemotherapeutic drugs and thus chemotherapy is interrupted[1]. This has become a major cause of ovarian cancer progression and metastasis [2]. In recent years, clinical scholars are dedicated to explore more effective and less toxic drugs to treat ovarian cancer. Taxus chinensis var. mairei is a kind of natural plant containing a variety of antitumor ingredients. Its aqueous extract has antitumor effect. In the following research, effect of Taxus chinensis var. mairei aqueous extract on malignant biological behavior of ovarian cancer cell line SKOV-3 was analyzed.

1. Materials and methods

1.1 Materials

Ovarian cancer cell line SKOV-3 were purchased from the Institute of Cell Library; cell culture medium and serum were purchased from Gibco Company; cell viability assay kit was purchased from Promega Corporation; ATP assay kit was purchased from Pik Company; fluorescence quantitative PCR detection kit was purchased from TAKARA company; Taxus chinensis var. mairei aqueous extract was provided by the Department of Pharmacy.

1.2 Methods

1.2.1 Cell culturing and processing methods

After recovery, cells were cultured in DMEM medium that
contained 5% fetal bovine serum. When growing to 70-80%, the cells were generated and processed, processing method being as follows: the control group was processed with DMEM medium without fetal bovine serum and the treatment group was processed with 600 μg/L, 800 μg/L and 1000 μg/L Taxus chinensis var. mairei aqueous extract.

1.2.2 Cell viability detection
After cells were processed for 24h in 96-well plates, MTS assay from Promega Corporation was added, 20 μL per well; then cells were continued to be incubated for 3h; after solution color changed, absorbance at 490 nm of microplate was detected to reflect cell viability. For pair wise comparison, cell viability of the control group was set to 100 to calculate that of the treatment group.

1.2.3 Cell migration detection
Cell migration process was detected by wound-healing test. Before the drug treatment, a scratch was done in the middle of the bottom of cell plate using 200 μL pipetting head and the scratch pattern under the microscope was recorded; 24h after the drug treatment, the scratch pattern under the microscope was recorded again to calculate cell migration rate before and after the drug treatment.

1.2.4 ATP detection
After the cells were processed for 24h, ATP assay kit produced by Pik Company was used to detect cell ATP content and corresponding total protein content at the same time to calculate ATP content produced by unit number of protein.

1.2.5 Gene mRNA contents detection
Lysate was added to cells. Total RNA was extracted, RT synthesized into cDNA. Fluorescence quantitative PCR was used to amplify IGT-β1, CD44v, Survivin, MIF, HIF-1, VEGF as well as β-actin. Target gene mRNA contents were calculated. For pair wise comparison, target gene mRNA content of the control group was set to 100 to calculate that of the treatment group.

1.2.6 Statistical methods
Data were input and analyzed by SPSS18.0 software, measurement data for variance analysis and pair wise comparison for LSD-t test. Differences were considered to be statistically significant at a level of P< 0.05.

2. Results

2.1 Cell proliferation and migration ability
MTS was used to detect cell viability and the results showed that Taxus chinensis var. mairei aqueous extract could decrease the MTS value and inhibit cell viability on dose-dependent manner; scratch test was done to detect migration ability and the results showed that Taxus chinensis var. mairei aqueous extract could decrease cell migration rate and inhibit cell migration on dose-dependent manner. See Figure 1 for details.

2.2 Expressions of proliferation and adhesion genes
Real-time PCR was used to detect cell mRNA contents of proliferation and adhesion genes. Pair wise comparison after variance analysis and LSD test showed that Taxus chinensis var. mairei aqueous extract could decrease mRNA contents of IGT-β1, CD44v and survivin on dose-dependent manner. See Figure 2 for details.

2.3 Cell energy metabolism
Fluorescence method was used to detect cell ATP content. Pair wise comparison after variance analysis and LSD test showed that Taxus chinensis var. mairei aqueous extract could decrease the ATP value and inhibit cell energy metabolism on dose-dependant manner. See Figure 3 for details.
Treating ovarian cancer has always been a hot spot of clinical study. For patients who cannot receive surgical excision, chemotherapy is mostly needed. However, the effect of chemotherapy is not precise. There are situations that patients are insensitive to chemotherapy or cannot tolerate the adverse reactions. In recent years, the exploration for more effective and less toxic target drugs to treat ovarian cancer has been a hot spot for study. Taxus chinensis var. mairei is Taxaceae taxus. Its branches and leaves contain multiple substances with antitumor activity such as paclitaxel, paclitaxel flavonoids, 10-deacetylation baccatin, etc. Its aqueous extract can inhibit processes of tumor proliferation and migration, etc. Now, studies of Chinese scholars SHU Qi-jin [3] and XU Ying-fei [4] show that Taxus chinensis var. mairei aqueous extract can induce lung cancer cell line apoptosis. But using the drug to treat ovarian cancer has not been reported yet. In the research, ovarian cancer cell line proliferation and migration behaviors were detected at first. MTS is the most common method to detect cell viability, which can reflect the number of living cells, then reflecting proliferation ability; scratch test, also called wound-healing assay, can reflect cell migration ability through migration rate. Analysis results of the research showed that Taxus chinensis var. mairei aqueous extract could decrease the MTS value and inhibit cell migration rate on dose-dependent manner, which indicated that Taxus chinensis var. mairei aqueous extract had the effect of inhibiting ovarian cancer cell proliferation and migration.

Tumor cell proliferation and migration are two types of malignant biological behaviors that are mediated by multiple molecules. Survivin is a newly discovered apoptosis-inhibiting protein, which can directly inhibit function of apoptosis-executing molecules such as caspase-3 and caspase-7 [5]. Excessive expression of the molecule can enhance cell proliferation ability and inhibiting its expression can induce cell apoptosis. Adhesion molecules are important molecules that participate in cell migration process [6]. CD44 belongs to cell transmembrane adhesion proteins. Exons have standard form and variation form. According to differences of exons, CD44 can be divided into two types: CD44s and CD44v [7]. The former one is mainly expressed in normal tissues and cells, which helps to maintain normal cell function; the latter one is mainly expressed in cancer cells, which can mediate adhesion between cancer cells as well as adhesion between cancer cells and normal cells. Its molecular basis causing distant metastasis of malignant tumor [8]. IGT-1 plays a key role in processes of adhesion between cells as well as adhesion between cells and extracellular matrix, which plays the adhesion effect through combining with corresponding ICAM-1 and VCAM-1. Its important molecule causing cancer cell metastasis and infiltration [9–10]. The research detected contents of proliferation and adhesion related genes and found out that Taxus chinensis var. mairei aqueous extract could decrease mRNA contents of IGT-1, CD44v and survivin on dose-dependent manner, which indicated that Taxus chinensis var. mairei aqueous extract processing could inhibit expressions of proliferation and adhesion related genes.

Malignant behaviors like proliferation and migration, etc are all active and energy-consuming biological processes that need energy provided by oxidative phosphorylation generation of ATP as well as oxygen and nutrients provided by local angiogenesis. ATP is a factor that directly provides energy for all kinds of active behaviors in the body. Malignant tumor needs a large number of ATP generated by oxidative phosphorylation to perform proliferation and migration function. Inhibiting ATP existence will surely be able to inhibit malignant biological behaviors of tumors [11]. Oxidative phosphorylation process needs oxygen and glucose. Tumor tissue needs to establish an independent blood supply.
system in order to get enough nutrients from the body. Therefore, a sufficient number of angiogenesis formed in the local lesion are the basis of oxidative phosphorylation[12]. Tumor angiogenesis process is regulated by a variety of molecules. VEGF is the most explicit angiogenesis-promoting factor known for now, which can directly induce endothelial cells to form vascular structure[13]; MIF is a type of cytokine produced by active T cells, which can inhibit specific immune dissolution response of tumor cells and enhance angiogenesis formation[14]; HIF-1 is a type of nuclear transcription factor, which can maintain strong proliferation ability of malignant tumor cells in the anoxic environment[15]. The research detected ATP generation and contents of angiogenesis related genes and found that Taxus chinensis var. mairei aqueous extract processing could reduce ATP generation and inhibit MIF, HIF-1 and VEGF expressions.

References


