Anti-tumor Effect of Paclitaxel Enhanced by Psoralen at the Cellular Level

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Abstract　[**Objectives**] To explore the effect of psoralen combined with paclitaxel on the apoptosis of MCF-7 cells. [**Methods**] The effects of different concentrations of psoralen, paclitaxel, or the combination of psoralen and paclitaxel on cell viability were detected using CCK-8 assay kit. Cell cycle distribution and apoptosis after 24 h of psoralen (0.16, 0.32, 0.64 mmol/L), paclitaxel (0.1 μmol/L), combined action of psoralen (0.32 mmol/L) and paclitaxel (0.1 μmol/L) were detected using flow cytometry. [**Results**] Lower concentration of psoralen (0.04 – 0.32 mmol/L) showed no significant inhibitory effect on cells. After combined with paclitaxel, the inhibitory effect on MCF-7 cell proliferation was significantly higher than that of the group treated alone. Compared with the paclitaxel group, the cell apoptosis rate in the drug combination group was significantly increased. Different low concentrations of psoralen can block the cell cycle of MCF-7 at G_0/G_1 phase, while paclitaxel can block the cell cycle at G_2/M phase. After combined action, the number of cells blocked at G_2/M phase decreased. [**Conclusions**] Overall, the combined effect of psoralen and paclitaxel can enhance anti-tumor ability by inhibiting cell proliferation, inducing apoptosis, and blocking cell cycle.

Key words　Psoralen, Paclitaxel, Breast cancer, Apoptosis

1　Introduction

Female breast cancer has surpassed lung cancer induced by radiation as the most common cancer. It is estimated that there are 2.3 million new cases (11.7%) worldwide every year, including 416 000 new cases of breast cancer in China. The mortality rate of breast cancer (6.8%) is second only to that of lung cancer (18%). The number of women suffering from breast cancer continues to increase, which not only seriously threatens women's health and quality of life, but also brings a heavy burden to the global health system[1-2]. Chemotherapy is an important means to treat breast cancer at present, and it can effectively reduce the tumor cell load in patients. Taxol compounds are currently internationally recognized as new anti-tumor drugs, which can block the mitosis of tumor cells, induce apoptosis, and have been proven to be effective in breast cancer[3]. However, some studies have shown that high expression of drug efflux proteins or breast cancer stem cells can lead to paclitaxel resistance[4-5]. Psoralen (PSO) is a furacoumarin compound, and can exert anti-tumor effects by increasing endoplasmic reticulum (ER) stress-dependent cell apoptosis, blocking cell cycle, inhibiting epithelial mesenchymal transition, regulating exosomes secretion, reversing multidrug resistance, and other mechanisms[6-8]. This paper aimed to explore the effect of psoralen combined with paclitaxel on apoptosis of breast cancer cells *in vitro*, so as to provide a theoretical basis for the treatment of breast cancer.

2　Materials and methods

2.1　Cell line　The human breast cancer MCF-7 cells used in this experiment came from the research group of teacher Fanshi Taihe of Guangxi International Zhuang Medicine Hospital. They were cultured in DMEM medium containing 10% fetal bovine serum at 37 ℃ and 5% CO_2.

2.2　Reagents and instruments　Fetal bovine serum (Zhejiang Tianhang Biotechnology Co., Ltd.); DMEM culture solution, PBS buffer (USA Gibco Company); pancreatin, penicillin-streptomycin (dual antibody, containing 100 U/mL penicillin and 100 μg/mL streptomycin, Beijing Solaybio Technology Co., Ltd.); cell-level DMSO (USA Sigma Company), CCK-8 test kit (Biosharp Company); psoralen, paclitaxel (Guangzhou Aichun Pharmaceutical Technology Co., Ltd.); cell apoptosis and cycle detection kit (Shanghai Biyuntian Biotechnology Co., Ltd.); SYNERGY H1M full-wavelength multifunctional enzyme-linked immunosorbent assay (USA BioTek); IncuCyte S3 dynamic functional analysis system for living cells (Germany Sartorius); FACS Calibur flow cytometry (USA BD Company).

2.3　Cell grouping and drug administration　MCF-7 cells were cultured in DMEM medium containing 10% fetal bovine serum, and cells in logarithmic growth phase were divided into control group (without any drug stimulation), paclitaxel group, psoralen group, and psoralen combined with paclitaxel group.

2.4　Detection of cell proliferation activity　Cell viability was detected using the CCK-8 method. MCF-7 cells were inoculated

onto 96-well plates with 1×10^4 (100 μL) cells per well and incubated overnight. After the cells adhered to the wall, the old culture medium was removed. According to the protocol of Section **2.3**, they were divided into groups and administered. Each group had 3 replica wells, and it continued to culture for 24 h. The culture medium was discarded, and 100 μL of culture medium containing 10% (*V/V*) CCK-8 was added to each well. After incubated in dark for 1 h, the *OD* value was measured using an enzyme-linked immunosorbent assay at a wavelength of 450 nm. The inhibition rate was calculated based on the *OD* value, and survival rate (%) = [(*OD* value in experimental group − *OD* value in blank group)/(*OD* value in control group − *OD* value in blank group)] ×100%.

2.5 Detection of cell apoptosis The apoptosis rate was detected using the Annexin V-FITC/PI test kit. MCF-7 cells were inoculated onto a 6-well plate with 1×10^5 (2 mL) cells per well and cultured for 24 h. After the cells adhered to the wall, the old culture medium was removed. According to the protocol of Section **2.3**, they were divided into groups and administered. Each group had 3 replica wells, and it continued to culture for 24 h. Cells were digested and collected with trypsin without EDTA, and washed twice with pre cooled PBS (1 000 g, centrifuged for 5 min). 1×10^5 cells were collected, and 0.5 mL of Binding Buffer was added to suspend cells. After adding 5 μL of Annexin V-FITC, 10 μL of PI was added to mix well. At room temperature, it reacted for 15 min by avoiding light, and observation and detection of flow cytometry were performed within 1 h.

2.6 Detection of cell cycle Cell Cycle and Apoptosis Analysis Kit test kit was used to detect cell cycle. MCF-7 cells were inoculated onto a 6-well plate with 1×10^5 cells per well and cultured for 24 h. After the cells adhered to the wall, the old culture medium was removed. According to the protocol of Section **2.3**, they

were divided into groups and administered. Each group had 3 replica wells, and it continued to culture for 24 h. Cell culture medium was collected, and cells were digested and collected by trypsin. Old cell culture medium was added, and cells were collected (1 000 g, centrifuged for 5 min). Cells were washed once with pre cooled PBS, and 1×10^5 cells were collected. Pre cooled 70% ethanol was added, and it was fixed at 4 ℃ for 24 h. Cells were washed with pre cooled PBS and collected, and 0.5 mL of propidium iodide staining solution was added. It took a dark and warm bath at 37 ℃ for 30 min, and observation and detection of cells were performed using flow cytometry within 24 h.

2.7 Statistical methods Statistical analysis was conducted using Graphpad Prism 9.0, and the experimental results were expressed as mean ± standard deviation. Unpaired two tailed *t* tests was used to analyze the statistical differences between the two groups of data, and one-way ANOVA was used to analyze the statistical differences between multiple groups of data. $P < 0.05$ indicated a statistically significant difference.

3 Results

3.1 Inhibition of MCF-7 cell proliferation The CCK-8 detection results showed that the inhibitory effect of psoralen (PSO) on cells was dose-dependent, with an IC_{50} of 0.777 8 mmol/L. Lower concentrations of psoralen (0.04 − 0.32 mmol/L) showed no significant inhibitory effect on cells (cell survival rate ≥90%). After the administration of paclitaxel (PTX), the survival rate of cells also decreased with increasing concentration. The inhibitory effect of different concentrations of PTX combined with PSO (no significant toxic concentration of 0.32 mmol/L) on MCF-7 cells showed an overall upward trend with the increase of PTX concentration, indicating that psoralen may increase the sensitivity of MCF-7 cells to paclitaxel (Fig. 1).
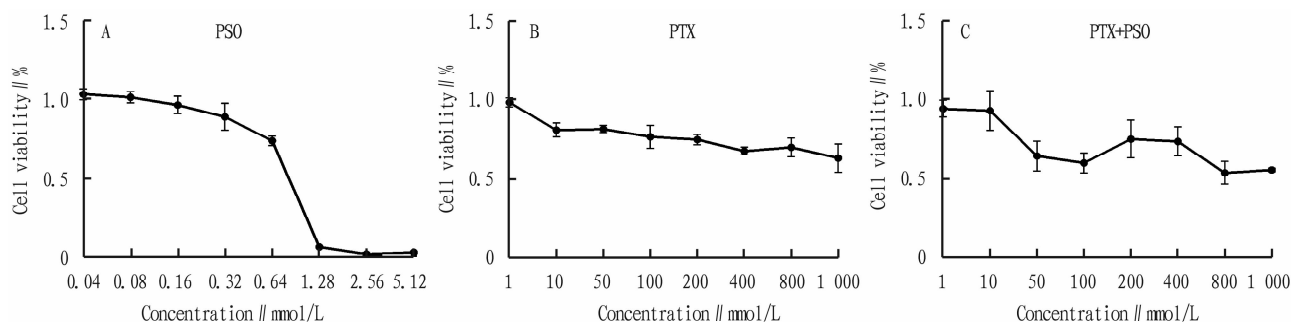

Note: A, B and C are the cell survival rates of psoralen, paclitaxel, and the combination of paclitaxel and psoralen, respectively.
Fig. 1 Effects of psoralen and paclitaxel on the viability of MCF-7 cells ($n = 3$)

3.2 Effects on apoptosis of MCF-7 cells By conducting cell proliferation experiments, the concentration of the combined action of psoralen and paclitaxel was determined, and the apoptosis of cells after psoralen, paclitaxel, and the combined action of psoralen and paclitaxel was detected by flow cytometry. The results showed that compared with the control group (9.36% ±0.49%), the apoptosis

rates of each treatment group were significantly increased, with statistical differences ($P < 0.001$). Compared with the paclitaxel group (36.87% ±0.55%), the combined action of paclitaxel (0.1 μmol/L) and psoralen (0.32 mmol/L) significantly increased the apoptosis rate (45.90% ±0.30%) ($P < 0.001$), indicating that psoralen may help to enhance the sensitivity of breast cancer cells to paclitaxel (Fig. 2).
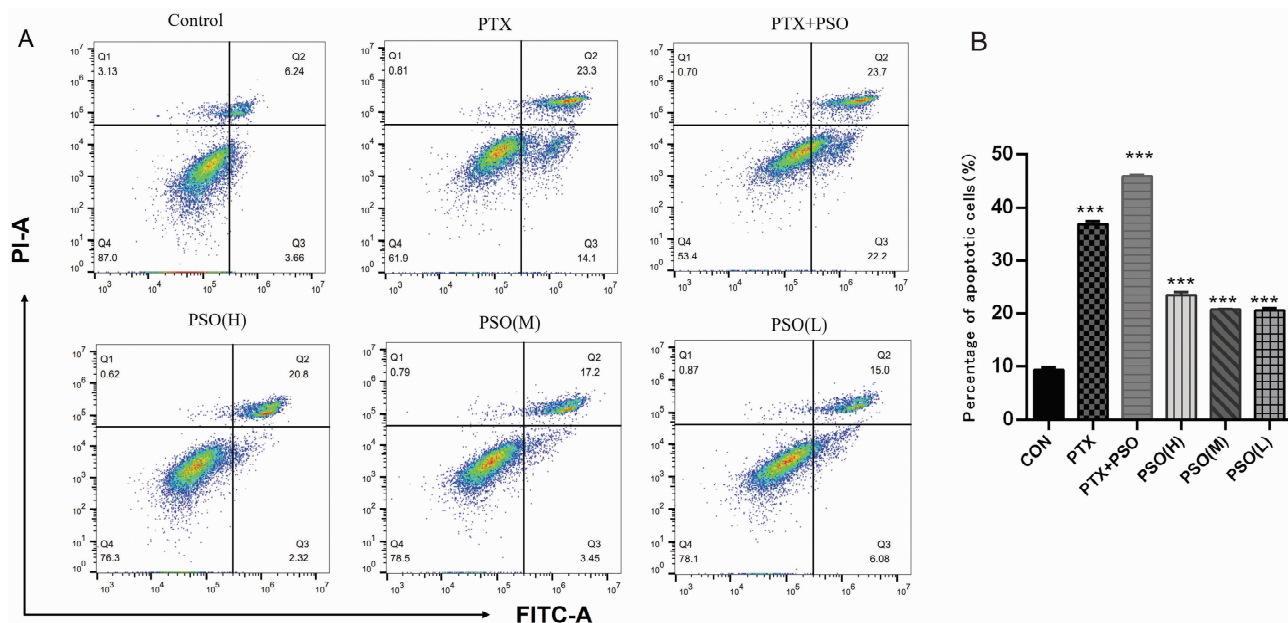
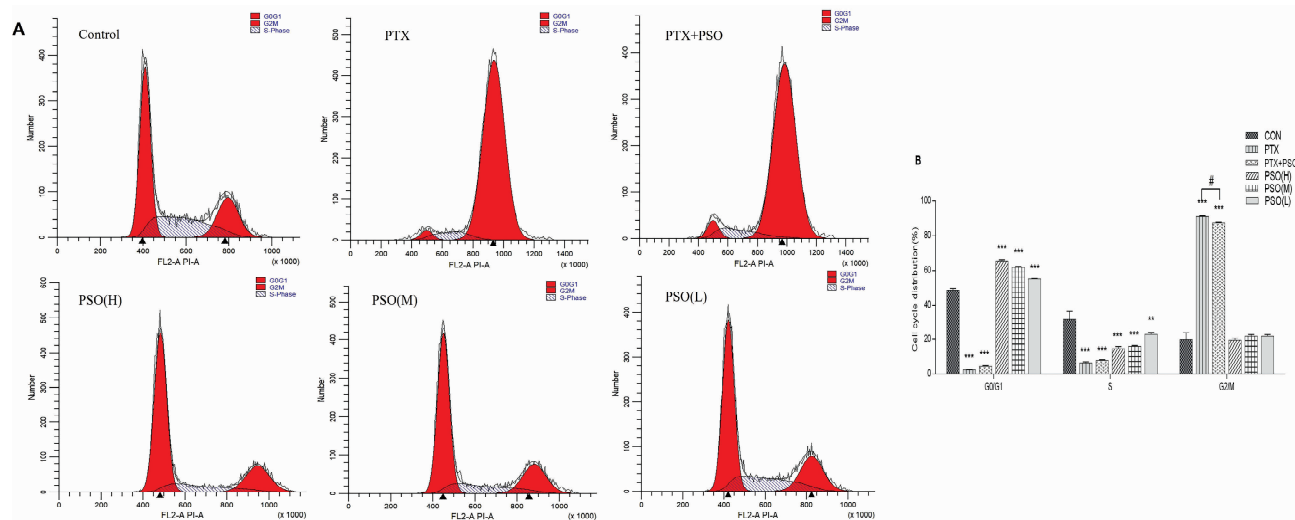Note：A. Apoptosis detection of flow cytometry；B. Statistics of cell apoptosis rate. Compared with control group，***shows $P < 0.001$；compared with paclitaxel，### shows $P < 0.001$.

Fig. 2   Effects of psoralen and paclitaxel on apoptosis of MCF-7 cells ($n = 3$)

3.3   Effects on MCF-7 cell cycle   After confirming that the combination of two drugs can promote cell apoptosis, further changes in cell cycle were detected by flow cytometry. The results indicated that paclitaxel can block the cell cycle in the G_2/M phase. Different concentrations of psoralen can block the cell cycle of MCF-7 in G_0/G_1 phase and inhibit cells from entering S phase, with significant differences compared with the control group

($P < 0.001$). After the combination of medication, the cell cycle was still blocked in the G_2/M phase, but the proportion of cells decreased ($P < 0.05$). The combined effect of psoralen and paclitaxel showed a synergistic effect on the cell cycle, and the proportion of cells in the G_2/M phase was less than that in the paclitaxel group, but more than that in the psoralen group (Fig. 3).

Note：A. Cycle detection of flow cytometry；B. Distribution statistics of cell cycle. Compared with the control group，***shows $P < 0.001$，**shows $P < 0.01$；compared with paclitaxel group，# shows $P < 0.05$.

Fig. 3   Effects of psoralen and paclitaxel on the cell cycle of MCF-7 ($n = 3$)

4   Discussion

At present, chemotherapy is still the cornerstone of comprehensive treatment for breast cancer. Paclitaxel is the first-line treatment

drug for breast cancer, but the resistance of breast cancer to paclitaxel treatment is a major obstacle in clinical application, and also one of the main reasons for death caused by chemotherapy failure.

Factors leading to paclitaxel resistance include mutations of ABC transporter, microRNA (miRNA) or some genes, dryness of breast cancer cells, etc[9].

Cell proliferation and apoptosis are important events in the occurrence and development of tumors. Paclitaxel can promote the aggregation of intracellular microtubule proteins, inhibit depolymerization, induce cell cycle arrest, and inhibit mitosis, thereby promoting cell death[10-11]. The research results indicated that paclitaxel can block the cell cycle of MCF-7 in the G_2/M phase. Research reports that psoralen and its derivatives show good antitumor activity on breast cancer, can block cell cycle in G_0/G_1 phase, and reduce the proportion of cells in S phase. Moreover, change is more significant with the increase of psoralen concentration[12-13]. The results of this study are consistent with the report. Paclitaxel and psoralen may have a certain degree of complementarity due to different action sites, thereby enhancing cell apoptosis. The results showed that psoralen combined with paclitaxel could enhance the anti-tumor ability by inhibiting cell proliferation, inducing apoptosis, and blocking cell cycle when acting on breast cancer cell line MCF-7.

The research on the molecular mechanism of psoralen combined with paclitaxel inducing apoptosis of breast cancer cell MCF-7 is not in-depth, and further research is needed. In addition, psoralen is a kind of furacoumarin, which has extensive pharmacological activities and is related to a variety of anti breast cancer mechanisms. It can play an anti-tumor role by antagonizing metabolic pathways, proteases and cell cycle processes, and even interfering with the cross link between receptor and growth factor mitotic signals[14]. The synergistic effect of psoralen targeting multiple pathways and promoting cancer treatment is worth further exploration.

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