

Mechanisms of Docetaxel Resistance in Breast Cancer Cells

Qiang Jing, Zhiliang Cong, Liang Guo*

The Fourth People's Hospital of Jinan City, Taishan Medical College, Shandong, China, 250031

*Corresponding author

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Abstract: Breast cancer is a common malignant tumor in women. The incidence of breast cancer in China is increasing year by year. Drug resistance in cancer cells is an important reason for the failure of breast cancer treatment. Drug-resistant cell lines are important models for studying the occurrence and reversal of MDR in tumors. This paper established a docetaxel-resistant human breast cancer cell line in vitro by low dose incremental induction, and studied its biological and drug resistance mechanism. It was pointed out that overexpression of MDR1 gene and protein was one of the main mechanisms of acquired drug resistance.

1. Introduction

Breast cancer is the most common malignant tumor in women. In China, the incidence of breast cancer is increasing year by year, and the age of onset is also younger, which has seriously threatened women's health. At present, the treatment of breast cancer is based on the staging and physical condition of the tumors, using surgery, radiotherapy, chemotherapy, endocrine therapy, biological targeting therapy and traditional Chinese medicine adjuvant treatment and other means of comprehensive treatment. The adjuvant chemotherapy and neoadjuvant chemotherapy are important means in the treatment of breast cancer. Drug resistance often occurs in the course of chemotherapy for breast cancer, which is a difficult point in clinical treatment. Therefore, it is of great significance to study the molecular mechanism of chemoresistance in breast cancer for choosing drug regimen, improving therapeutic effect and improving prognosis of patients.

2. Clinical application and drug resistance of docetaxel

Docetaxel is a semi-synthetic second-generation taxol drug extracted from the branches and leaves of *Taxus chinensis*, which is a semi-synthetic taxol drug. Docetaxel has stronger anticancer activity than paclitaxel. Its intracellular concentration is three times higher than that of paclitaxel, and its intracellular retention time is longer. It is an important reason for its high anticancer activity, and there is no cross-resistance with paclitaxel. Studies have shown that docetaxel is the most effective single drug in the treatment of metastatic breast cancer than doxorubicin. Because of its unique mechanism of action and good tolerance, it has been widely used in combination with anthracycline, platinum and gemcitabine as a first-line drug in breast cancer chemotherapy. Nevertheless, drug resistance often occurs in the treatment of breast cancer with docetaxel, which seriously affects the efficacy of docetaxel.

In the clinical treatment of patients with advanced metastatic breast cancer, docetaxel-based combination chemotherapy has been widely used and achieved high efficiency. Regardless of the number and location of metastatic organs, HER-2 negative or positive, and different pathological types of breast cancer, docetaxel-based combination therapy has better therapeutic effect. Docetaxel combined with other drugs still has a certain therapeutic effect on the failure of anthracycline therapy. But the resistance of breast cancer to docetaxel is the main reason that restricts the therapeutic effect of docetaxel in clinic. Therefore, once the mechanism of docetaxel resistance is clarified, it will be of great significance for clinical rational drug use, even reversal of drug resistance, and increase the efficiency of breast cancer treatment.

3. Effect of FoxM1b on docetaxel resistance

The anticancer activity of docetaxel was stronger than that of paclitaxel, and there was no cross-resistance with paclitaxel. It binds to specific sites of cell beta-tubulin, promotes tubulin aggregation and inhibits its depolymerization, which results in cell cycle being blocked in G2/M phase, and induces apoptotic substance release to induce cell apoptosis. Although docetaxel is the first-line drug in breast cancer chemotherapy, drug resistance is still an important factor affecting the therapeutic effect of breast cancer. Doc cells were established by stimulating breast cancer cells with docetaxel for several months. Doc cells showed significant resistance to docetaxel and showed significant differences in multiple concentrations, as shown in Figure 1.

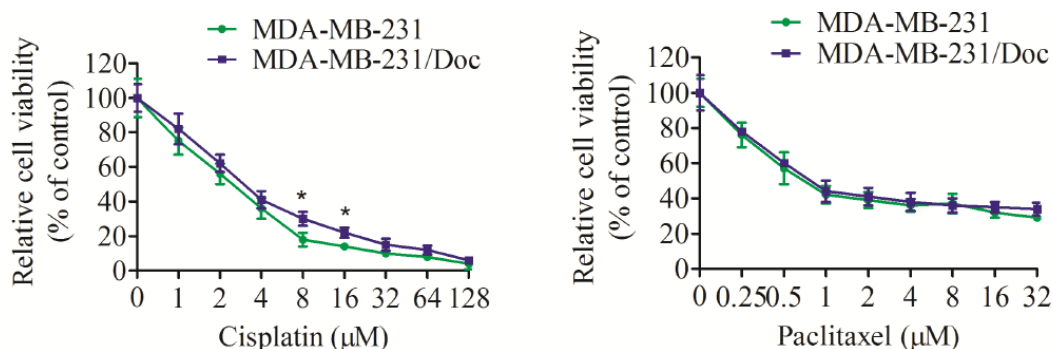


Fig1. Comparisons of drug resistance of docetaxel-resistant cells

Docetaxel-resistant cells were screened out by long-term stimulation of human breast cancer cells with docetaxel. Morphological changes of the two cells in different concentrations of docetaxel were observed under a microscope. The difference of drug resistance between the two cells was verified by MTT test (as shown in Figure 1), which further proved the reliability of the drug-resistant cells screened. The expression of FoxM1 in breast cancer was detected by immunohistochemical staining. FoxM1 was found to be positive in most breast cancer tissues. In order to further clarify the expression and function of splicing isomers of FoxM1 in breast cancer, three splicing isomers of FoxM1a, B and C were detected by reverse transcription PCR. The results showed that FoxM1a, B and C were amplified, indicating that FoxM1 B played a role in resistance to docetaxel.

Because FoxM1a, B and C genes have little difference in sequence, it is impossible to detect the difference of protein expression in breast cancer cells. Six breast cancer cells, including MDA-MB-231 and MDA-MB-231/Doc, were used for Western blotting. The results showed that FoxM1 protein was expressed in all six breast cancer cells to varying degrees. Compared with the parental cell line MDA-MB-231, the expression of FoxM1 in MDA-MB-231/Doc cells was significantly increased, which further indicated that FoxM1 was associated with docetaxel resistance in breast cancer.

4. Mechanisms of docetaxel resistance in breast cancer cells

Docetaxel (Doc) is a new type of antineoplastic drug based on paclitaxel, which is a natural antineoplastic drug. It has good solubility and better efficacy. Clinical practice shows that Doc-based combination chemotherapy is the most promising treatment for breast cancer, especially for lymph node-positive patients. But the problem of drug resistance cannot be ignored. Drug-resistant cell lines are important models for studying the occurrence and reversal of MDR in tumors. Docetaxel-resistant human breast cancer strains were induced by low dose incremental method in vitro, and their biological and drug resistance characteristics were studied.

4.1 Inducing drug resistant strains of tumor cells in vitro results

The methods of inducing drug-resistant strains of cancer cells in vitro include low-dose stepwise addition method and high-dose short-term stimulation method, and the combination of the two

methods. In contrast, although the low dose incremental method is cumbersome and time-consuming, it has less risk, easier to grasp and higher success rate. In this study, human breast cancer cell line MCF-7/Doc resistant to docetaxel was induced by low dose incremental method. Compared with parental cells, the growth rate of MCF-7/Doc was significantly slowed down, blocked in G0/G1 phase, and the population doubling time was prolonged. This indicated that MCF-7/S was in a slow cycle or even resting state after drug resistance was induced by Doc in the model, and its sensitivity to chemotherapeutic drugs was reduced, thus possessing the characteristics of MDR.

Doc-induced MCF-7/Doc resistant strains are not only resistant to Doc, but also cross-resistant to other chemotherapeutic drugs such as methotrexate, hydroxycamptothecin, paclitaxel, epirubicin and gemcitabine, suggesting that common pathways may be involved in the formation of MCF-7/Doc resistance, such as the high expression of P-gp. It has been reported that the expression of P-gp is mainly related to drug resistance of alkaloids, anthracyclines and podophylls, but not cross-resistance with alkylators and platinum drugs. The results also support that MCF-7/Doc drug-resistant strains have no obvious resistance to carboplatin and cisplatin, which provides important information for drug selection after clinical Doc resistance.

4.2 P-gp plays a key role in Doc acquired drug resistance

MDR is a difficult problem that needs to be solved urgently in clinical chemotherapy. The mechanism of drug resistance is still unclear. Overexpression of P-gp is currently recognized as the biological basis of MDR, and is also considered as a classical or basic marker of drug-resistant cells. P-gp is a transmembrane glycoprotein encoded by MDR1 gene related to drug resistance in the human MDR gene family. It not only acts as a drug efflux pump to protect cells from toxic substances, but also participates in cell proliferation, differentiation and apoptosis, and mediates the production of MDR. By RT-q PCR and Western Blot, it was found that MCF-7/Doc overexpressed MDR1/P-gp, while MCF-7/S did not. It was confirmed that P-gp was induced and played a key role in the acquired drug resistance of Doc. With MDR1/P-gp as the target, 3 micromol/L MS-209 (a quinoline reversal agent) can completely reverse the resistance of multidrug resistant solid tumor transplantation model and cell model to Doc, which can be seen that P-gp is closely related to Doc resistance.

4.3 Gene expression patterns of different drug-resistant cell lines are different

Compared with MCF-7/S, some genes such as MDR1, ECM-related genes, cytokines and growth factor signaling pathway genes, reactive oxygen species (ROS) metabolism and epithelial mesenchymal transformation (EMT) related genes are different. The difference in the expression of MCF-7/120 nmol/L Doc was more obvious than that in MCF-7/30 nmol/L Doc, which indicated that the acquisition of MDR phenotype by MCF-7 was a gradual and dose-dependent process, i.e. the difference in the expression of related genes increased with the increase of drug resistance. It was found that the process of EMT was related to the resistance of MCF-7 to Doc. At the same time, the expression of zinc finger transcription factor slug and vimentin was up-regulated while the expression of E-cadherin was down-regulated.

5. Conclusions

Chemotherapy is an irreplaceable and important method for the treatment of breast cancer. However, multidrug resistance often leads to treatment failure and recurrence and metastasis of tumors in later stage, which seriously threatens the survival of patients. Docetaxel is a new type of antineoplastic drug based on natural antineoplastic drug paclitaxel, which has good solubility and better efficacy after structural modification. Doc drug-resistant cell model of human breast cancer was established in this paper. Doc drug-resistant strains were induced by low concentration of Doc and gradually added. Their biological characteristics were evaluated by MTT drug sensitivity test and flow cytometry. FoxM1 protein was expressed in breast cancer cells to a certain extent. The expression of FoxM1 protein may be related to docetaxel in breast cancer. Doc cells have typical

multidrug resistance. Overexpression of MDR1 gene and protein is one of the main mechanisms of acquired drug resistance.

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