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Establishment of Doxorubicin Resistant Human Osteosarcoma Cell Lines

Zhen Li[1] and Suwei Dong[2*]

[1]Department of Medical Oncology, The Second Affiliated Hospital of Xuzhou Medical University, Xuzhou 221000, People's Republic of China
[2]Department of Orthopaedics, The Second Affiliated Hospital of Xuzhou Medical University, Xuzhou 221000, People's Republic of China

*Corresponding author: Suwei Dong (e-mail: dong.suwei@163.com).

Abstract **Background:** Osteosarcoma (OS) is the most frequent highly malignant primary bone tumour in children and adolescence. The underlying mechanisms of OS progression behind multi-drug resistance s was still not completely clear and needed to uncover the underlying mechanisms of chemoresistance to develop more effective therapeutic strategies. **Objective:** The purpose of this study is to establish drug resistance OS MG-63 and U2OS cells induced by DOX, which will support further investigation into the underlying mechanisms. **Method:** MG63 and U2OS cell lines were treated with repeated exposure to gradually increasing concentrations of doxorubicin. The IC_{50} value of doxorubicin resistance MG63 and U2OS and the parental cells were detected by MTT. **Results:** The IC_{50} value (1065±12.32 ng/ml for MG-63, 1417±15.28 ng/ml for U2OS) of doxorubicin resistance MG63 and U2OS were18.86 and 3.1-fold that for the parental cells ($p < 0.001$, both). **Conclusion:** Our established doxorubicin-resistant cell lines should be a useful tool for identifying new mechanisms of drug resistance and new drug targets in osteosarcoma.

Key Words Osteosarcoma, Doxorubicin, Drug Resistance

INTRODUCTION

Osteosarcoma (OS) is the most frequent highly malignant primary bone tumour in children and adolescence [1]. Although with the great improvement in surgical technique and neoadjuvant chemotherapy, the clinical effectiveness is hampered by the acquisition of multi-drug resistance, which ultimately leads to a low 5-year survival rate of just 16%-20% [2-4]. The underlying mechanism of OS progression behind multi-drug resistance s was still not completely clear. Drug resistance in osteosarcoma involves multiple factors and mechanisms. Current research indicates that osteosarcoma cells can alleviate the cytotoxic effects of drugs through various mechanisms, including enhanced activity of drug efflux pumps, alteration of drug targets, repair of drug-induced DNA damage [5,6]. To uncover the underlying mechanisms of chemoresistance, it is essential to develop more effective therapeutic strategies [7].

Developing drug-resistant cancer cell model is a useful approach to uncover the mechanisms of chemoresistance in cancer cells. Conventional first-line chemotherapy of OS relies on a combination of high-dose methotrexate (HD-MTX), cisplatin (CDDP), ifosfamide (IFO) and doxorubicin (DOX) [8,9]. DOX is an anthracycline inhibitor of DNA topoisomerase II, a traditional chemotherapeutic agent used for a wide variety of tumours [10].

In this study, we established drug resistant OS MG-63 and U2OS cells induced by DOX, which support the further investigate the underlying mechanisms.

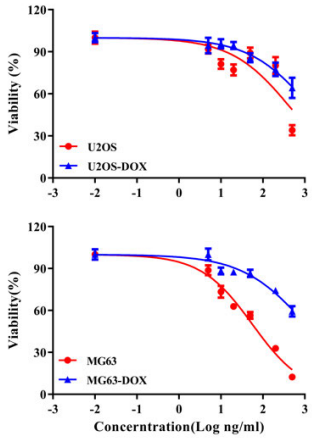
METHODS

Cell Culture

Human osteosarcoma cell line MG63 and U2OS were obtained from National Certified Cell Culture Collection Centre (Shanghai, China). Cell lines were cultured in MEM medium (Gibco, USA) containing 10% FBS (Hyclone, USA). All cells were placed in a 37 °C incubator with 5% CO_2.

Establishment of Drug-Resistant Osteosarcoma Cells

Drug-resistant OS cells were induced as previously described [11]. Briefly, When the MG63 and U2OS cells grew to the logarithmic phase, DOX (A429989, Sangon Biotech, China) was added at a concentration of 0.0035 μM and incubated for 72h. Surviving cells were subsequently passaged and then replaced with fresh medium without



Cell line	IC50 (ng/ml)
MG63	56.47± 1.75
Dox-MG63	1065 ± 12.32
U2OS	456.7 ± 11.35
Dox-U2OS	1417 ± 15.28

Figure 1: Sensitivity of drug-resistant cell lines and parental cell line to doxorubicin. Relative cell viability was determined by MTT assay. The cells were treated with doxorubicin for 24h. The detection of cell viability was determined at the indicated time points by measuring the fluorescence signal using a multimode plate reader. All experiments were conducted in triplicate and data were expressed as the Mean±SD (n = 3)

DOX until the surviving cells recover. Then the second generation of cells were treated with DOX at a concentration of 0.035 μM. The third generation of cells were treated with DOX at a concentration of 0.35 μM. Then repeated the above process for 6 months. Cell sublines at end points were stored in liquid nitrogen for further analyses. The parallel MG63 and U2OS cells did not treated with DOX were considered as negative control.

Cell Viability Assay

Cell viability was determined by thiazolyl blue tetrazolium bromide (MTT) assay, as previously described [12]. Cells were seeded in 96-well plates in triplicate. Following overnight incubation, the cells were treated with different concentrations of DOX for 24h. Then the cells were incubated with 10 μL of freshly prepared MTT reagent. The formazan crystals produced were dissolved in 100 μL of dimethyl sulfoxide, a Microplate Reader (Life Science, Hercules, CA, USA) was used to collect the optical densities at a wavelength of 570 nm. Data gained from three independent experiments. The half-maximal inhibitory concentration (IC50) was estimated as the drug concentration that reduces the cells in the medium by half, calculated using the Bliss method [13]. Subsequently the resistance index was calculated according to the following equation:

$$\text{Resistance Index} = \frac{\text{IC50 of Resistant Cell Line}}{\text{IC50 of Parental Cell Line}}$$

Three independent experiments were set up in this assay.

Statistical Analysis

Statistical analysis was performed with SPSS18.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 8.02. All data are expressed as mean±standard deviation. Comparisons of differences in the quantitative data among groups were performed using Student's t-test. $p < 0.05$ was considered statistically significant.

RESULTS

Establishment of a DOX-resistant OS cell lines

During the past 6 months, OS cells were exposed to progressively higher concentrations of DOX repeatedly, DOX-resistant cell lines were established. The IC50 values of parent cells and DOX-resistant cells to DOX were 56.47±1.75 (95% confidence interval [CI] 48.50-65.76) and 1065±12.32ng/ml (95% confidence interval [CI] 729.1-1556) for MG-63 (18.86-fold), 456.7± 11.35 (95% confidence interval [CI] 233.9-891.5) and 1417 ± 15.28 ng/ml (95% confidence interval [CI] 580.3-3462) for U2OS (3.1-fold) (Figure1). The new cells were thus successfully established as a DOX-resistant OS cell lines: DOX-MG-63 and DOX-U2OS.

DISCUSSION

Neoadjuvant chemotherapy has significantly improved prognosis of OS. Unfortunately, tumour cells become resistant to chemotherapeutics due to various mechanisms, such as inadequate drug concentrations or doses, high degree of plasma protein binding, low level of tissue binding and poor vascular supply [14]. Based on these, using low doses of drugs and pulsed therapy, where cells are given intermittent "repair time" in drug-free medium, resulting in cell models with 2-8 fold resistance compared to the parental cell lines. In the present study, a DOX-resistant cells were established successfully. Cell resistance is determined by several groups of mechanisms [15]. The most universal mechanism that determines chemoresistance to drugs of various classes is increased cellular efflux associated with the drug efflux mediated by the drug efflux pump and the ATP-binding Cassette transporters (ABC transporters) uses ATP to efflux a variety of compounds through the cell membrane, including P-glycoprotein (Pgp) [16]. Most of P-gp is located in cell membrane with a small amount in endoplasmic reticulum and Golgi. P-gp is an energy dependent medicine pump. Drug binds to the nucleotide binding site of the gene. The energy released after hydrolysis pumps the drug out of the cell, resulting in the decline of drug concentration in tumour cell, which will make the killing effect of chemotherapy drugs on tumour cell reduced or complete loss, developing resistance. It protects the cell from foreign substances, while neutralizing the therapeutic potential of a wide range of drugs. Moreover, drug resistant of tumour cells is a complex process that combines multiple genes, multiple factors and multiple steps. A further understanding of drug resistance in osteosarcoma is necessary in order to improve therapy. Present study established DOX-resistant cells would provide a basic material for further genome and proteome investigations to uncover the underlying mechanisms.

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