Multidrug resistant (MDR) cancer is the major clinical problem of chemotherapy of cancer patient. It is well-known that the p-glycoprotein (Pgp) expressed on the cell membrane and changed the anticancer drugs’ permeability of cancers cell, thereby reducing the cytotoxicity of anticancer drugs. In our previous study, the drug resistant cell line Ds-5-C5 of human uterine sarcoma was treated with p glycoprotein inhibitor, we found that Ds-5-C5 cell did not completely block the drug resistant ability. Furthermore, the protein kinase C delta (PKC δ) signaling pathway and related gene have significant altered in DNA methylation pattern of Ds-5-C5 cell at microarray and MetaCore pathway analysis. These data lead us to hypothesize that PKC δ signaling transduction pathway may play an important role in drug resistance in Ds-5-C5 cell line. We used western blot and quantitative real-time RT-PCR to verify the genes expression level of PKC δ signaling transduction pathway. Our results showed that PKC δ protein expression level was up-regulated in Ds-5-C5 cell line compared with parental cell line. The mRNA expression level of PKC δ, AP-1 DNA (-2.0 μg) was prepared from each cell line, and PKC δ inhibitor (Rottlerin) was co-transfection. After 48 hours transfection, the cell viability of Ds-5-C5 cell line were assessed by MTT assay. Protein expression level of both cell line were assessed by western blot. 

Materials and Methods

Cell culture. The two drug-resistant cell line Ds-5-C5 (ATCC CRL-1777) was established from the human sarcoma cell line MES-SA (ATCC CRL-1776) in the presence of increasing doxorubicin concentrations. All cell lines were grown in McCoy’s 5A medium supplemented with 10% fetal bovine serum (FBS).

Rottlerin treatment. Each cell line was treated with Rottlerin (Sigma-Aldrich, USA) at 0.5 μM, combining with doxorubicin at indicated concentration for 48 hr. Cell viability of both cell line were assessed by MTT assay. Protein expression level of both cell line were assessed by western blot. 

Downstream Genes related to AP-1 signaling transduction pathway were upregulated in the Ds-5-C5 cell line. The mRNA expression of (A) HMOX1, (B) HSPA6 and (C) EGR1 in MES-SA and Ds-5-C5 cell. ** P value <0.01. Values are expressed as the mean ± S.D. (n=3).

The Role of Protein Kinase C delta in Multidrug resistant Cancer cell line

Introduction

Protein kinase C (PKC) is a family of serine- and threonine-specific protein kinases that play central roles in signal transduction pathways regulating proliferation, apoptosis, and malignant transformation of a variety of cell types. Among the various PKC isotypes, study indicated that PKC δ expression in breast cancer cell lines had been proved to correlate with metastatic potential. Recent study found that over-expression of PKC δ lead to anchorage-independent growth of poorly metastatic cells. Furthermore, it also found that those cells confer an enhanced survival against doxorubicin-related drug through NFκB pathway and increased expression of a wide variety of antiapoptotic genes. Moreover, we noticed that Ds-5-C5 cell still have drug resistant ability even after co-treated with MDR1 inhibitor and doxorubicin in our previous study. In addition, we also noticed that only PKC δ isoform was up-regulated in the Ds-5-C5 cell line in DNA expression microarray analysis. Therefore, we hypothesize that PKC δ signaling transduction pathway may play an important role in drug resistance in Ds-5-C5 cell line. 

Western blot. Total protein extracts (50 μg) from each cell line were analyzed by Western blotting with antibodies of PRKCD and phospho-PRKCD (Thr 505) (Cell Signaling, USA), MDR1(Santa Cruz Bio. USA) and beta-actin (CHEMICON, USA). The beta-actin was used as the internal control for normal gene expression.

Specific Aim

To investigate the role of protein kinase C delta in multiple drug resistance cell line.