BLOCKAGE OF ABCC1/MPR1 EXPRESSION ENHANCED CHEMOTHERAPEUTIC EFFICACY VIA THE MANUPULATION OF NRF2 IN HUMAN ETOPOSIDE-RESISTANT ORAL CANCER CELLS

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Introduction: Etoposide (VP-16), a DNA topoisomerase II poison, is an important chemotherapeutic agent for malignancies such as testicular cancer, small-cell lung cancer, leukemias, lymphomas, Kaposi's sarcoma and head and neck cancer. Two etoposide-resistant cell lines, KB-7D and KB-20a, have been generated from human oral epidermoid carcinoma KB cells for investigating the mechanism of drug resistance in oral malignancies. KB-7D and KB-20a showed approximately 50 and 150 folds more resistant to etoposide, respectively, as compared to the parent KB cells. They also showed cross-resistant to chemotherapeutic agents such as doxorubicin, vincristine, mitoxantrone and methotrexate. It has been demonstrated that multidrug resistance-associated protein 1 (ABCC1/MRP1) is overexpressed in KB-7D cell lines and would be associated with the uptake of anticancer drugs.

Purpose: The aims of this study are to investigate the mechanism of action of etoposide-derived drug resistance and intent to find the molecular target to manipulate therapeutic efficacy of chemo-refratory cancers.

Methods: Expression level of RNA transcripts and proteins was detected using qRT-PCR and Immunoblot assay. Transient transfection of siRNA was performed using Lipofectamin 2000 reagent according to the manufacturer's protocol (Invitrogen). Cell viability was measured using the methylene blue staining.

Results: Silencing the expression of ABCC1/MRP1 by siRNA enhanced the cytotoxicity of etoposide and doxorubicin in KB-7D and KB-20a cells. In addition, pharmacological manipulation of ABCC1/MRP1 expression with a specific inhibitor MK-571 also increased drug sensitivity to etoposide and doxorubicin in these cells. These results suggested that ABCC1/MRP1 was responsible for the efflux of both etoposide and doxorubicin in human oral epidermoid carcinoma cells. To determine transcription factors that regulate the expression of ABCC1/MRP1, the promoter region of ABCC1/MRP1 gene was examined. The nuclear factor-E2-related factor 2 (Nrf2) binding sequence, antioxidant responsive element (ARE), has been found in the ABCC1/MRP1 promoter region, at -470 to -458 upstream from the transcription start site. Nrf2 is a basic-region leucine zipper transcription factor that regulates the expression of key cytoprotective enzymes that counteract oxidative and electrophilic attacks. We demonstrated that the expression level of Nrf2 mRNA and protein in etoposide-resistant cells was 1.4 to 1.8 folds higher than that of in parent cells. Furthermore, expression of Nrf2-mediated downstream genes such as NQO1, AKR1C1, -2 and -3 was also increased in KB-7D and KB-20a cells, as compared to KB cells. Down-regulated Nrf2/ARE dependent proteins by knockdown of Nrf2 or overexpression of Nrf2 sequester, Keap1, could effectively increased chemosensitivity of KB-7D and KB-20a cells against selected anticancer drugs. These results suggested that Nrf2-dependent transcriptional pathway was involved in inducing etoposide-resistance in human cancer cells.

Conclusions: In summary, constitutive activation of Nrf2-dependent ABCC1/MRP1 contributes to the function of chemoresistance in etoposide-derived drug resistant cells. Blockage of ABCC1/MRP1 expression by manipulation of Nrf2 signaling enhances chemotherapeutic efficacy in these cells. Thus, we propose that targeting Nrf2 may be able to reverse chemoresistance in chemo-refractory oral malignancies. In addition, targeting this molecule can be a new strategy for human anti-cancer therapy.