Increased topoisomerase II alpha level correlates with doxorubicin resistance in triple negative breast cancer cell lines

Lili Bao, Krzysztof Moroz and Srikanta Dash. Department of Pathology and Laboratory Medicine, Tulane University Health Sciences Center, New Orleans, LA-70112

Introduction: Chemotherapy remains the only treatment option available for patients with triple negative breast cancer. However, a considerable proportion of women develop resistance and recurrent cancer at distant metastatic sites after adjuvant chemotherapy. The mechanisms of chemoresistance are not well understood. Aim: To understand the molecular mechanisms of doxorubicin resistance in vitro as well as in vivo mouse model using triple negative highly metastatic murine as well as human breast cancer cell lines. Methodology: Doxorubicin resistant highly metastatic triple negative human breast adenocarcinoma cell line (MDA-MB-231) and murine breast adenocarcinoma (4T1) cell lines were generated after long-term culture in a growth medium supplemented with doxorubicin. The chemoresistant tumor lines were characterized by their ability to escape from the cytotoxic action of doxorubicin by MTT assay, cell proliferation and cell cycle analysis. To understand the mechanisms of resistance, intracellular drug uptake and distribution was examined by a fluorescence microscopy. Furthermore, topoisomerase 2-alpha expression level was determined between sensitive and resistant cell line by Western blot analysis to find out whether it is a determinant for the doxorubicin resistance. Results: The results of MTT assay showed increased dose dependent cellular cytotoxicity of doxorubicin in sensitive cell lines (S-MDA-MB-231, S-4T1) as compared to resistant (R-MDA-MB-231, R-4T1) tumor cell line. Cell cycle analysis revealed that doxorubicin treatment of sensitive cell lines (S-MDA-MB-231, S-4T1) resulted in G2/M growth arrest. However, Doxorubicin mediated cell cycle arrest was totally prevented in resistant tumor cell lines (R-MDA-MB-231, R-4T1). Cellular uptake studies revealed there is reduced doxorubicin trafficking and entry into the nucleus in the resistant cell line that leads to decreased drug induced DNA damage. Most of doxorubicin localized in the cytoplasm of the resistant tumor cell lines compared to sensitive cells where drugs were localized predominantly in the nucleus. Doxorubicin inhibited topoisomerase 2-alpha in the sensitive cells in a time dependent manner but not in the resistant cell line. Increase level of topoisomerase 2 alpha was detected in doxorubicin resistant tumor cell line derived from human as well as mouse breast adenocarcinomas. Conclusions: We developed doxorubicin sensitive and resistant breast cancer cell lines of human and mouse origin. Doxorubicin inhibits topoisomerase-2 alpha levels in the sensitive line of human and mouse origin but not in the resistant cell lines. The topoisomerase levels were high in the doxorubicin resistant tumor cell line. The mechanisms of doxorubicin resistance appear to be similar between the mouse and human breast tumor cell line.