“Overcoming Multidrug Resistance in Breast Cancer: Targeting MRP Proteins for Enhanced Therapeutic Efficacy”

PURPOSE: Chemotherapy remains one of the major treatment options for metastatic breast cancer. Resistance to chemotherapeutic agents is a major reason for cancer treatment failure. We plan to utilize nanotechnology to overcome chemoresistance mechanisms. It’s been reported that down-regulating the nuclear expression of MDR proteins (P-gp, MRP, BCRP etc.) by siRNA could increase the delivery of cancer curing drugs (i.e. doxorubicin) to drug resistant breast cancer cells. However, unless those siRNA-encapsulated nanoparticles are targeted specifically to cancer cells, they will have hardly any impact; rather they will create notorious undesired side effects to normal tissues. We plan to overcome these problems by developing a targeted nanocarrier delivery system for siRNA into breast cancer cells. Our hypothesis is that conjugating nanoparticles with a cancer cell specific aptamer should enhance the knockdown of multidrug resistant genes, which will increase the delivery of Dox (i.e. doxorubicin) into breast cancer cells leading to enhanced cellular toxicity and antitumor effect as compared to unconjugated nanoparticles. This study is intended to know whether silencing the expression of P-gp or MRP-1 by aptamer-labeled siRNA nanoparticles could enhance the delivery of doxorubicin into breast cancer cells in culture. METHODS: For targeted delivery, Aptamer-A6 has been used which can bind to Her-2 receptors on breast cancer cells. The particles were prepared by high pressure homogenization (HPH) using different amount of DOTAP, cholesterol, PLGA or PLGA-PEG and Mal-PEG. After siRNA encapsulation, the particles were incubated with aptamer-A6 for surface labeling. The liposomal particles were characterized for their size, surface charge and cytotoxicity. The delivery of P-gp siRNA or MRP-1 siRNA into 4T1-R cells has been assessed by immunofluorescence, PCR and FACS analysis. The doxorubicin accumulation into the cells has also been observed before and after the knockdown of MDR proteins by immunofluorescence and FACS analysis. RESULTS: This study has shown that the uptake of Dox by Dox-resistant 4T1-R is significantly less than Dox-sensitive 4T1-S which is partly attributed to the higher expression of drug-efflux pump (i.e. ABC transporter proteins P-gp, MRP-1, BCRP etc.) on the surface of the resistant cells. The targeted knockdown of P-gp or MRP-1 has been enhanced when the particles carrying P-gp siRNA or MRP-1 siRNA, respectively were labeled with aptamer. Concurrently, the uptake of Dox into the Dox-resistant 4T1-R breast cancer cells has increased significantly when the MDR proteins were knockdown by appropriate siRNA-encapsulated aptamer-labeled nanoparticles. CONCLUSIONS: This preliminary study concludes that aptamer functionalization of the nanoparticles could enhance the knockdown of MDR proteins which increases the delivery of doxorubicin into the breast cancer cells. ACKNOWLEDGEMENT: This work is funded in part by the Louisiana Cancer Research Consortium, NIMHD grant number TL4GM118968, NIGMS grant number UL1GM118967 and R25GM060926, CUR from Xavier University of Louisiana, LBRN and NSF.