ABOLISHING MULTIDRUG RESISTANCE IN CULTURED LUNG CANCER CELLS WITH RNA INTERFERENCE.

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ABSTRACT

TITLE: Abolishing Multidrug Resistance in Cultured Lung Cancer Cells with RNA Interference

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The gene, *cox-1,* is over-expressed in cultured GLC4 small cell lung cancer cells concurrent with the development of multi-drug resistance (MDR) as a result of the use of the chemotherapeutic agent used to combat the cancer, doxorubicin. Prevention of MDR has been a tremendous challenge in cancer research and this research is concerned with abolishment of MDR as a cancer survival strategy. RNA-mediated interference technology (RNAi) was employed using siRNA to decrease *cox-1* expression and temporarily restore the susceptibility of the cells to doxorubicin. GLC4 cells are of three types: S (sensitive cells never exposed to doxorubicin); ADR (MDR cells cultured in doxorubicin), and; REV (revertant cells previously cultured in presence of doxorubicin but no longer). REV and ADR cells were transfected with *cox-1* siRNA. After 24 h, $1x10^6$ cells were used for RNA isolation and 1 µg of RNA was used for RT-PCR to assess down-regulation of *cox-1* RNA. RT-PCR results indicated that *cox-1*

RNA was down-regulated to basal levels seen before exposure to doxorubicin. Ct values for GLC4/ADR and *cox-1* down-regulated GLC4/ADR cells were 23 and 34, respectively. The result indicated abundant levels and moderate levels of *cox-1* mRNA in the ADR cells and the transfected ADR cells respectively. The relative expression level of *cox-1* mRNA was 33% higher in the non-transfected GLCR/ADR cells as compared to the transfected GLCR/ADR cells as shown by the curve. Two hundred thousand cells were used for hemacytometer cell counts in the presence of trypan blue to assess cell viability. *cox-1* down-regulation in ADR cells resulted in a significantly higher percentage of non-viable cells (25.4%) as compared to its non-transfected control (20.5%) using a Student's *t*-test (*P <0.05). Similarly, fluorescence microscopy confirmed that apoptosis was significantly increased in the ADR cells treated with doxorubicin and *cox-1* siRNA simultaneously (69.4%) as compared to its non-transfected control (56.7%) (*= P <0.01). A Western blot analysis performed by Fernando Cuadrado indicated that siRNA transfection decreased the expression of COX-1 by 66% in GLC4/ ADR cells as compared to the non-transfected control using densitometry. However, no conclusive results were obtained using flow cytometry as the flow cytometer was incapable of analyzing the mixed cell population (adherent and suspension) which is a characteristic of this cell line, GLC4. Thus, we have clearly demonstrated that MDR cancer cells can be altered temporarily to become susceptible to doxorubicin, a potentially important finding for the treatment of cancer patients.