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## **Downregulation of TRX-1 confers resistance to cisplatin and sensitivity to the ROS generating agent elesclomol.**

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We have previously discovered a unique and important finding that all of our cisplatin resistant (CR) small cell and non small cell lung cancer cells (5 pairs of cell lines, parental vs. CR cells, and one primary line tested) possess high levels of ROS (Reactive Oxygen Species) compared to their parental cancer cell counterparts and normal cells. Importantly, these CR cells are sensitive to elesclomol (Synta Pharmaceuticals), a new compound which kills cells by generating ROS through mitochondria. (Cancers Ref.). The question remains why these CR cells possess intrinsically high ROS levels. We have found that the common biochemical feature in CR cells is lower expression of the antioxidant protein thioredoxin-1 (TRX1). CR cells have an average of 6 to 8 fold lower TRX-1 as compared to their parental counterparts and normal cells. We have previously reported that expression of other antioxidant systems, such as glutathione (GSH), are higher in CR cells but this does not result in reduced ROS. To further verify that TRX1 is an important contributory factor to the high ROS levels seen in CR cells, we knocked down TRX1 (by 80%) in parental lung cancer cells (SCLC1) using siRNA against TRX1. These SCLC1/TRX1(-) transfectants generated significantly higher ROS ( $P < 0.001$ ) when compared to the control SCLC1+scramble sequence. SCLC1/TRX1(-) cells are resistant to cisplatin treatment with the ID50 of 1.1  $\mu\text{g/ml}$  vs. 0.2  $\mu\text{g/ml}$  in the control transfectants. Moreover, SCLC1/TRX1(-) cells are also hypersensitive to elesclomol treatment (ID50 of 18nM vs. 45nM). Correspondingly, overexpressing TRX1 in the CR cell line SR2, using the pCMV6 vector which contains the full length cDNA of TRX1, resulted in decreased ROS production but increased sensitivity to cisplatin. The ID50 of cisplatin in SR2/TRX1(+) transfectant was 1.2  $\mu\text{g/ml}$  compared to 2.6  $\mu\text{g/ml}$  in SR2 cells. SR2/TRX1(+) cells also became more resistant to elesclomol (ID50 of 35nM vs. 6nM). Thus, our data strongly indicate that TRX1 is a major contributory factor in higher ROS levels found in CR lung cancer cells. Interestingly, it has been reported that diminished elesclomol activity is influenced by high LDHA levels. Our data show that all CR cells have 3 to 5 fold lower levels of LDHA levels when compared with parental cell counterparts. Importantly, SR2/TRX1(+) transfectant also exhibits higher LDHA levels. Thus, it is possible that ROS production seen in CR cells may be in part due to low TRX1 levels resulting in altered cellular metabolism (more oxidative phosphorylation) and lower LDHA. Treatment of these cells with elesclomol results in a greater increase in

ROS levels beyond their tolerance leading to cell death. Lung tumor cells which have higher LDHA most likely have lower basal ROS levels and retain the ability to turn on TRX1 system. Thus, ROS generation by elesclomol is unable to eradicate these tumor cells. Our findings suggest another novel approach to selectively kill cisplatin resistant (CR) small cell and non small cell lung tumors which intrinsically produce higher ROS and express lower TRX1 and LDHA levels.