



Targeting ROS to kill cisplatin resistant cells

Medhi Wangpaichitr*, Chunjing Wu*, Min You**, Marcus T. Kuo***, Vy Dinh*, Lynn G. Feun**, Theodore J. Lampidis**, and Niramol Savaraj*.*

* V.A. Medical Research, V.A. Healthcare System, Miami, FL 33125

**Sylvester Cancer Center, University of Miami School of Medicine, Miami FL 33136

***M.D. Anderson Cancer Center, University of Texas, Houston, TX 77054



Introduction

BACKGROUND: Cisplatin resistance remains a major problem in the treatment of both small cell and non small cell lung cancer. We have previously shown that inhibiting mTOR can restore cisplatin sensitivity; however, not all cisplatin resistant cell lines are sensitive to mTOR inhibitor (1, 2). Our findings have led us to search for an alternative target in these cisplatin resistant cells and we have discovered that cisplatin resistant cells share one common biochemical parameter which is increased reactive oxygen species (ROS). Thus, further increased ROS in these cisplatin resistant cells can push them beyond their tolerance limit which ultimately leads to cell death.

RESULTS: We have found that increased expression of NADPH Oxidase-4 (NOX4) and lower levels of thioredoxin (TRX) correlate with the high levels of ROS seen in cisplatin resistant cell lines. Thus, using elesclomol (Synta Pharmaceuticals), an agent which is known to increase ROS (3), we demonstrated that elesclomol is significantly more cytotoxic toward cisplatin resistant cells with the ID₅₀ of 4-10 fold less than their parental cells counterpart and normal cells. The cytotoxic effect of elesclomol in cisplatin resistant cells is accompanied by further decrease in TRX and glutathione (GSH) antioxidant systems, while opposite results were found in parental and normal cells. This cytotoxic effect; however, can be reversed by an antioxidant, N-acetylcysteine (NAC) which suggested that cytotoxic effect results from increased ROS. Furthermore, we also found that elesclomol also increased intracellular cisplatin and hence enhanced cisplatin sensitivity in resistance cells.

We concluded that elesclomol is highly effective in cisplatin resistant cells which express higher basal levels of ROS and should be considered for treatment of cisplatin resistant tumor.

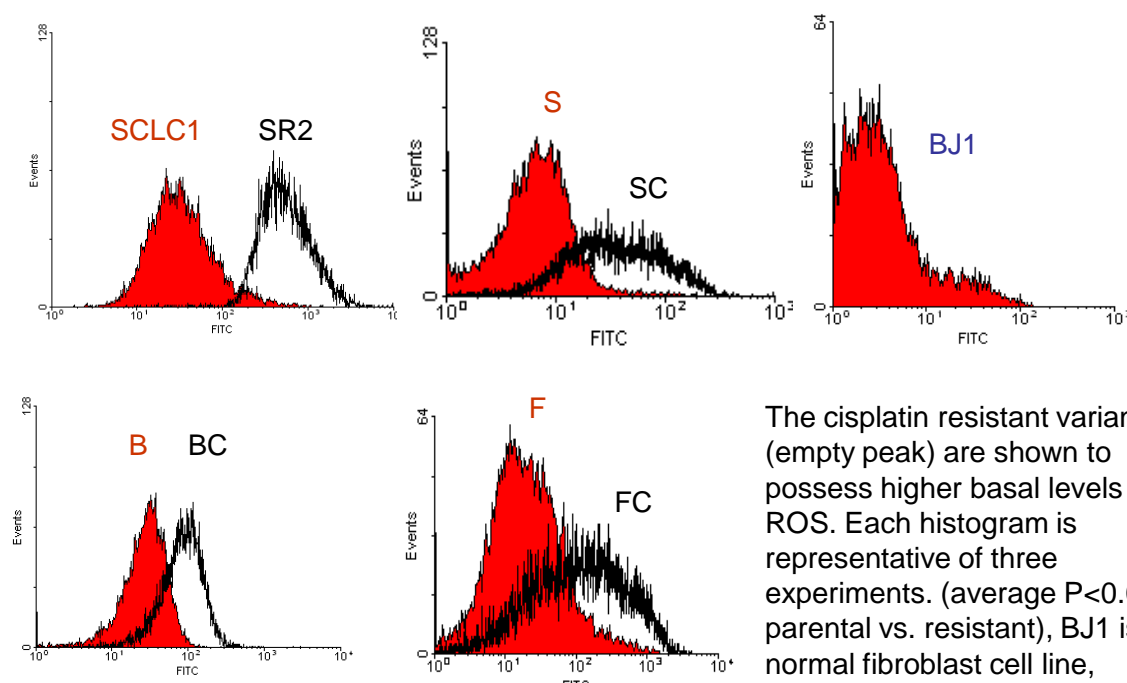
Objectives

- To determine and confirm that cisplatin resistant cell lines possess higher basal level of ROS
- To determine whether elesclomol which increase ROS are more cytotoxic toward the cisplatin resistant cells, and less toxic to parental and normal cells.
- To determine whether combination of elesclomol can enhanced cisplatin sensitivity in resistance cells

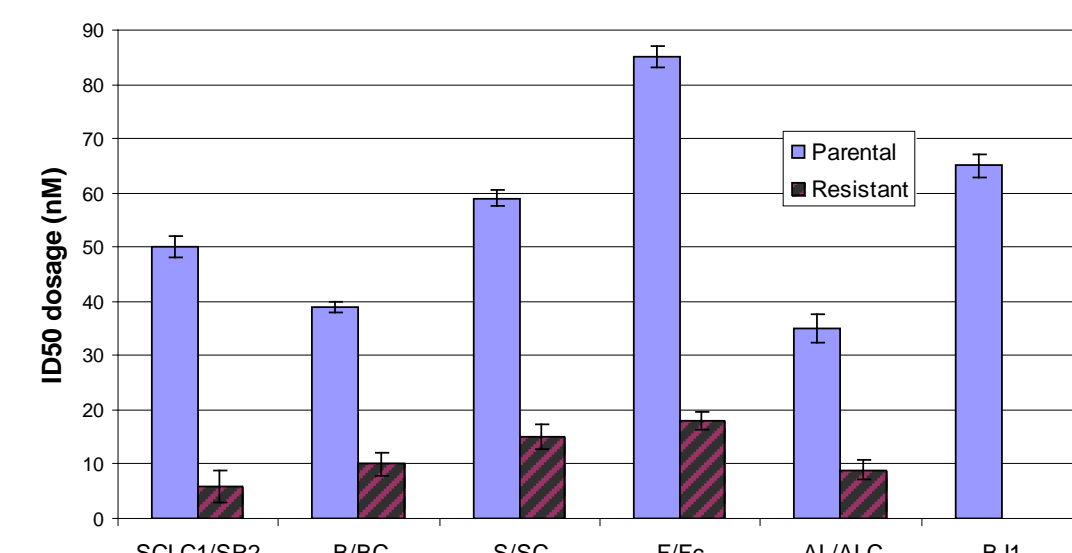
Supported by VA research funding

Results

Flow cytometry analysis of basal level of ROS in various lung cancer cell lines detected by DCF-DA probe.

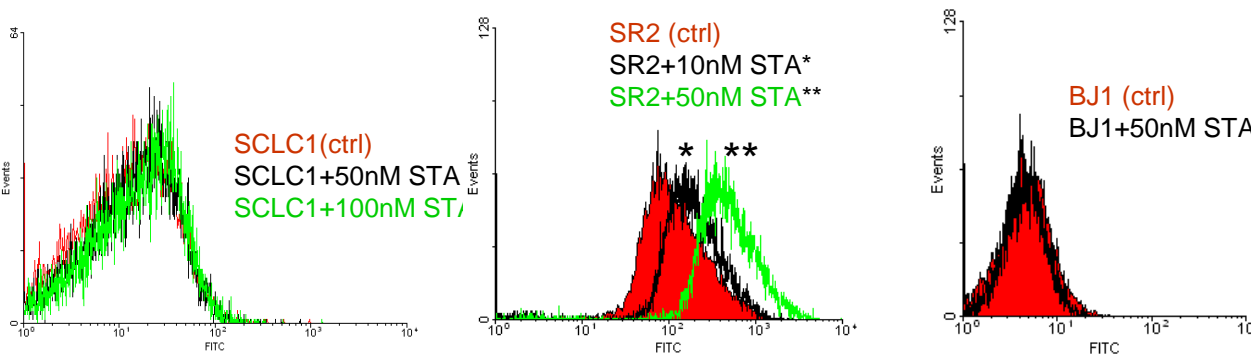


ID₅₀ dosages of lung cancer cell lines treated with elesclomol for 72hrs

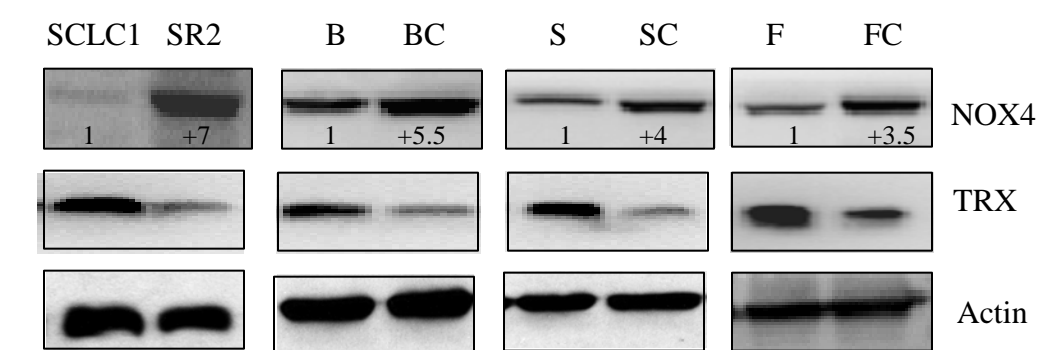


Elesclomol is shown to have striking activity in all of the cisplatin resistant cell lines. (Mean SD of three experiments, average p values < 0.005; parental vs. resistant)

Flow cytometry analysis of ROS in SCLC1, SR2, and BJ1 cell lines when treated with elesclomol.

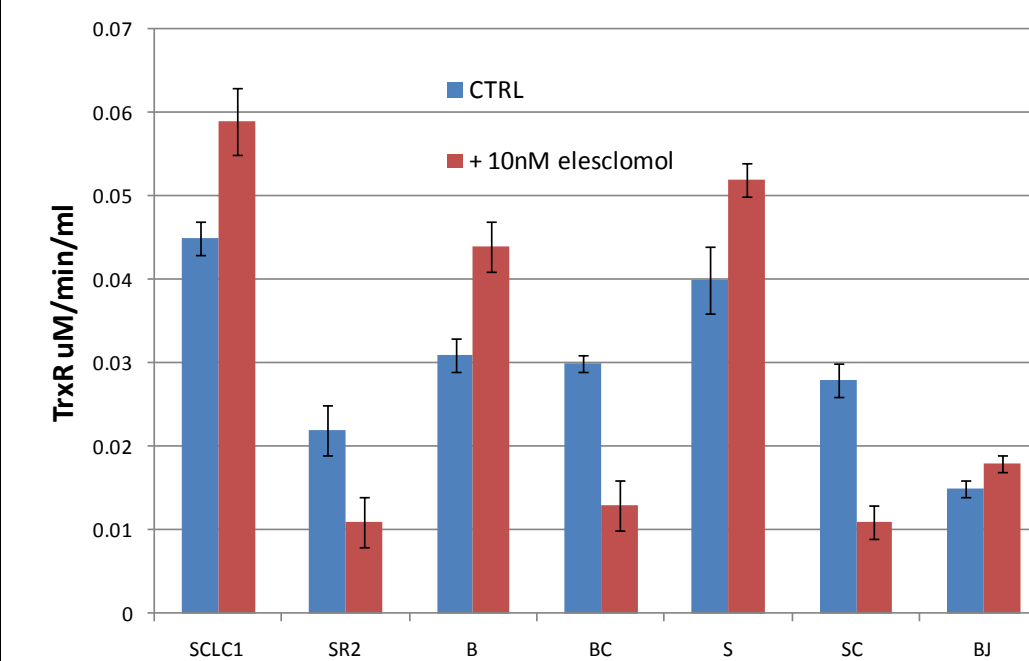


Increased expression in NADPH Oxidase Type 4 (NOX4) and decreased expression in TRX correlates with the high levels of ROS



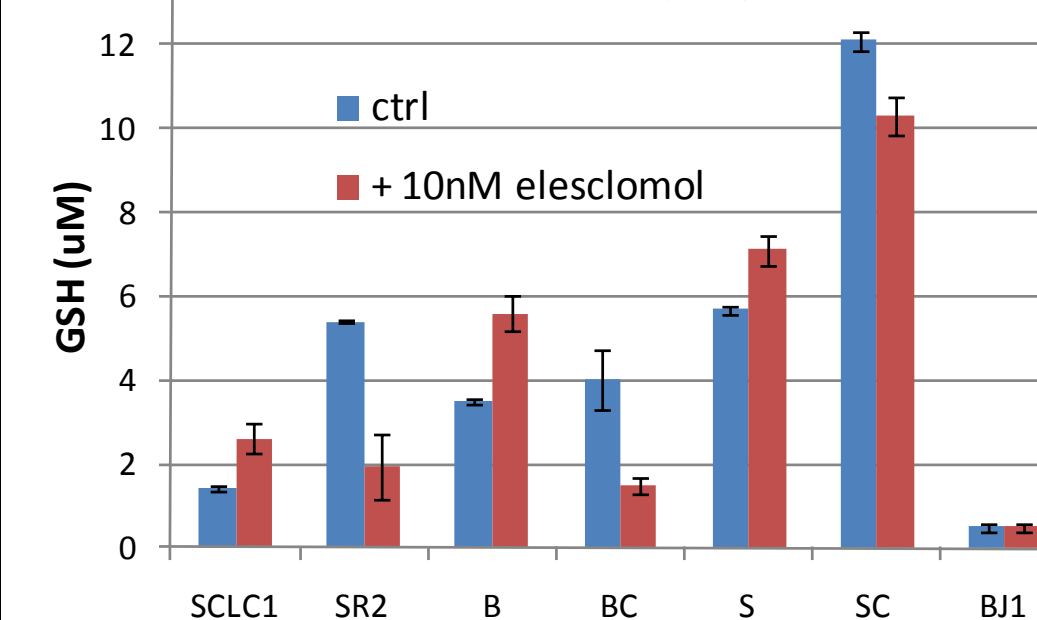
Immunoblot of NOX4 and TRX in parental lung cancer cell line and their resistant variants. The number below each lane depicted the changes from its control which was arbitrarily set at 1. All the band intensity was normalized with actin. Note that all of the cisplatin resistant lines have significant lower levels of TRX expression.

Thioredoxin Reductase Activity (24hr)



TrxR activity are decreased in resistant lines and further decreased when treated with elesclomol (Mean SD of three experiments).

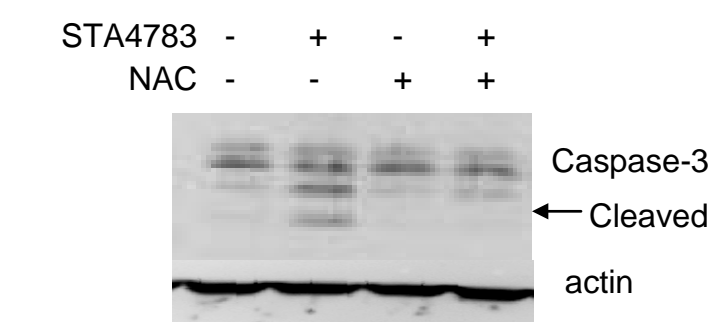
Intracellular GSH (24hr)



After treatment with elesclomol, the levels of GSH are decreased in all of the resistant cell lines (Mean SD of three experiments).

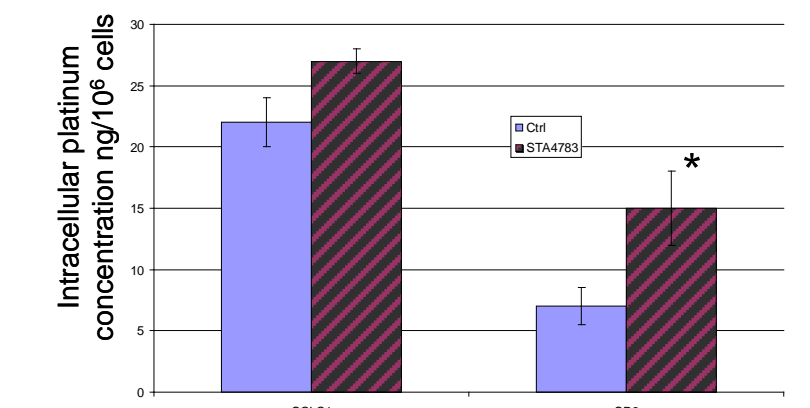
Results

Immunoblot of cleaved caspase-3



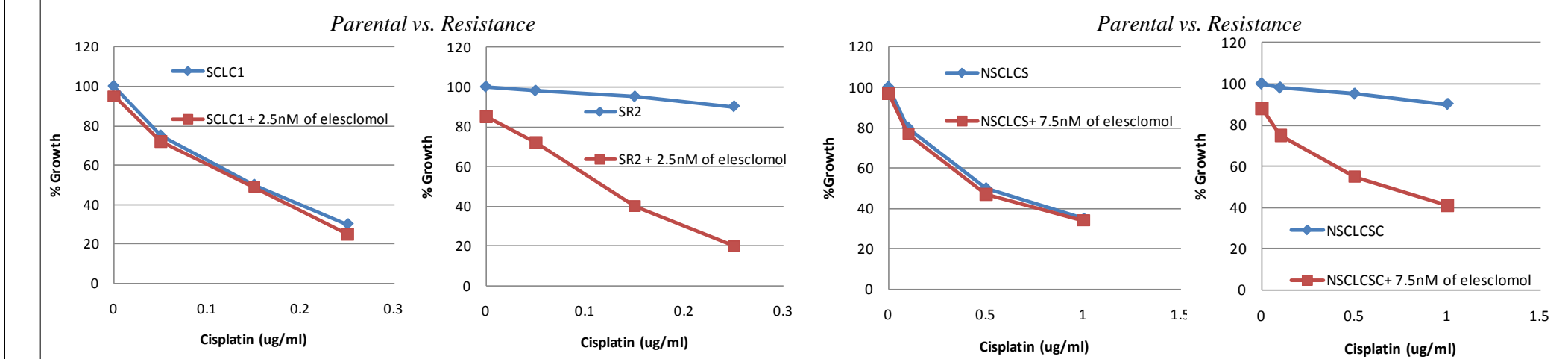
In SR2 cell lines, treatment with 10nM of elesclomol for 48hrs induced caspase 3 cleavage (arrow) which can be reversed with 0.1mM of NAC.

Uptake Assay of Cisplatin



Elesclomol increased the cisplatin uptake in both cell lines, however, the uptake is more apparent in the resistant line. (p<0.05)

Elesclomol stimulates greater growth inhibitory effect in cisplatin resistant cell line when combined with cisplatin.



Summary

- Cisplatin resistant cell lines have higher basal ROS than their parental cell counterparts.
- Further increased ROS in these cisplatin resistant cells can push them beyond their tolerance limit which ultimately leads to cell death.
- Elesclomol induced ROS production in cisplatin resistant lines.
- Elesclomol increased intracellular cisplatin which in turn enhanced cisplatin sensitivity in resistance cells.
- Overall, the results obtained here represent a novel approach to treat cisplatin resistant tumors while sparing normal cells. This concept can also potentially be applied to tumors resistant to other chemotherapeutic agents which are known to generate ROS.

References

1. Wangpaichitr, M., Wu, C., You, M., Kuo, M. T., Feun, L., Lampidis, T., and Savaraj, N. Inhibition of mTOR restores cisplatin sensitivity through down-regulation of growth and anti-apoptotic proteins. *Eur J Pharmacol*, 591: 124-127, 2008.
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3. Kirshner, J. R., He, S., Balasubramanyam, V., Kepros, J., Yang, C. Y., Zhang, M., Du, Z., Barsoum, J., and Bertin, J. Elesclomol induces cancer cell apoptosis through oxidative stress. *Mol Cancer Ther*, 7: 2319-2327, 2008.