



# Down-regulation of Thioredoxin-1 Confers Resistance to Cisplatin and Sensitivity to the ROS

## Generating Agent Elesclomol in Lung Cancer

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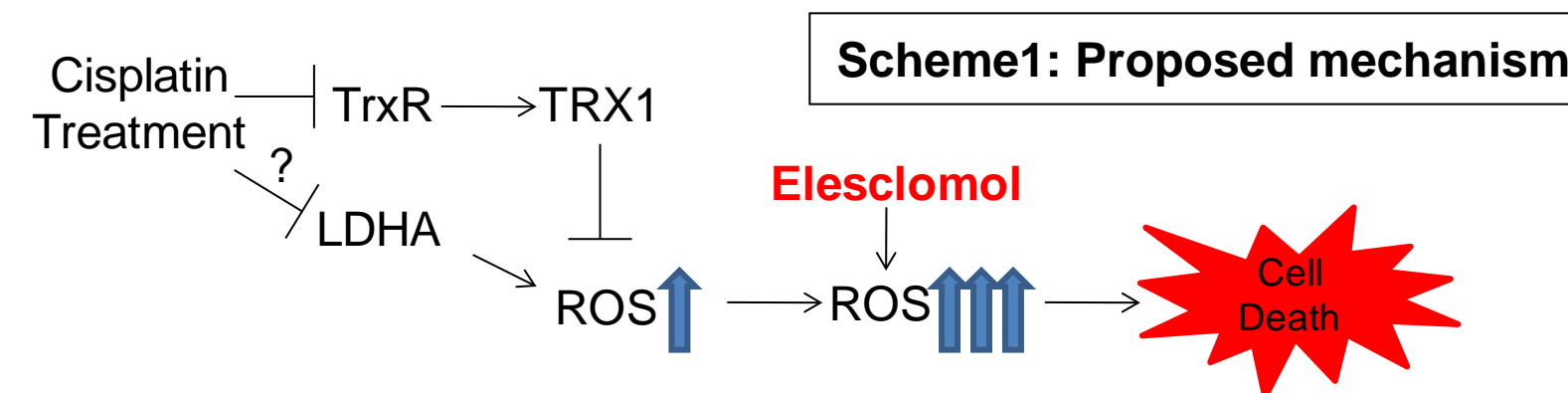


### Introduction

We have previously discovered a unique and important finding that all of our cisplatin resistant (CR) lung cancer cell lines, regardless of their signaling mechanisms, possess high levels of ROS (Reactive Oxygen Species) when compared to their parental cancer cell counterparts as well as normal cells (1). Importantly, these CR cells are sensitive to elesclomol (*N-malonyl-bis (N'-methyl-N'-thiobenzoylhydrazide*, Synta Pharmaceuticals), a new compound which kills cancer cells by generating ROS (2). The question remains why these CR cells possess intrinsically higher levels of ROS.

It is known that one of the pharmacologic actions of cisplatin is the disruption of redox system through inhibition of thioredoxin reductase-1 (TrxR1) (3). TrxR catalyses the NADPH-dependent reduction of the redox protein thioredoxin-1 (TRX1). TRX-1 is an important protein that acts as an antioxidant by facilitating the reduction of other enzymes. Using our CR cell models, we have found that TrxR1 activities as well as TRX-1 levels are significantly decreased. To further verify that TRX-1 is an important contributory factor to the higher ROS levels seen in CR cells, we knocked down TRX-1 protein expression in parental cells using siRNA. These TRX1-knocked down cells generated significantly higher levels of ROS and were resistant to cisplatin as well as hypersensitive to elesclomol treatment. Correspondingly, overexpression of TRX-1 protein in the CR cells using the pCMV6 vector containing full length TRX-1 cDNA, resulted in decreased ROS production but increased sensitivity to cisplatin. These TRX1-overexpressing cells also became more resistant to elesclomol treatment.

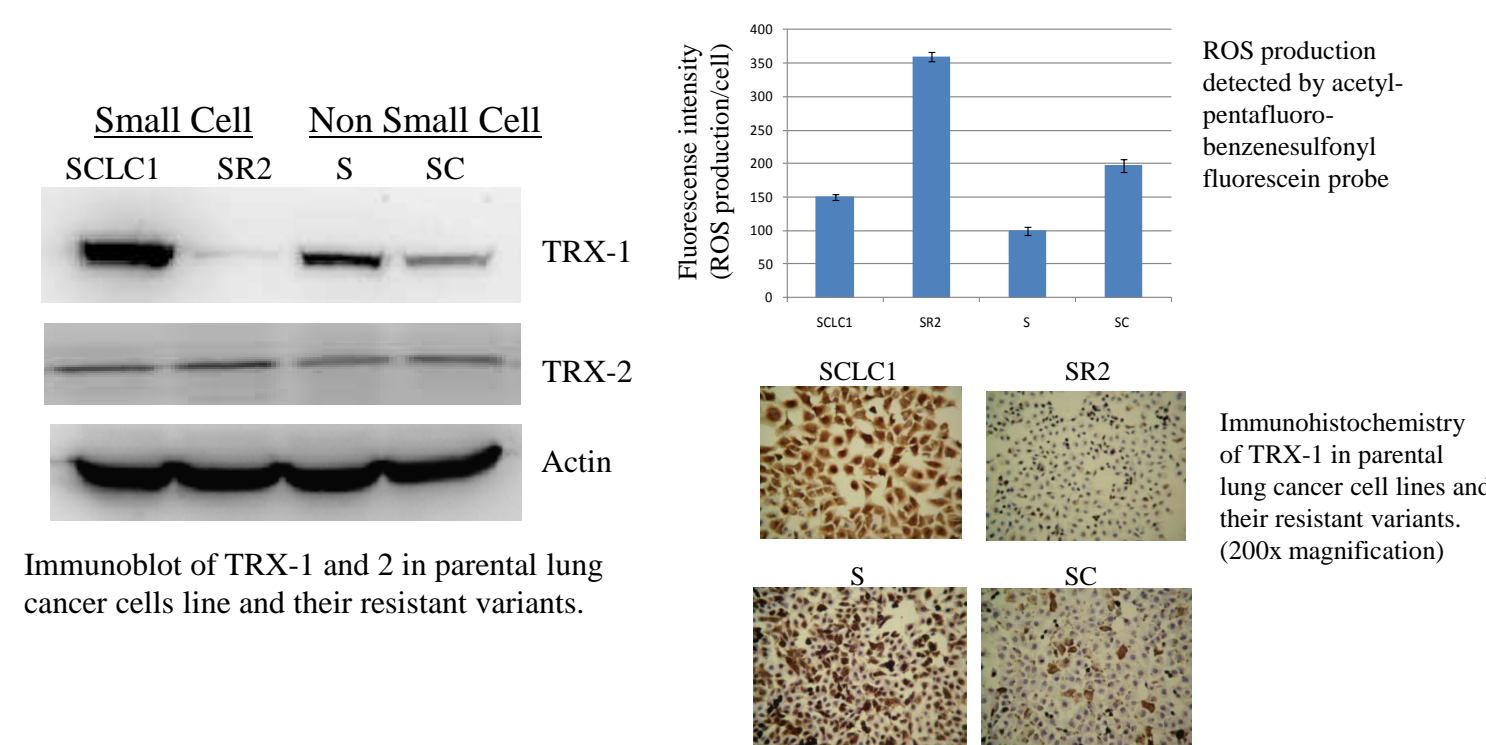
Moreover, we found that all CR cells have 3-5 fold lower levels of lactate dehydrogenase A (LDHA) levels. Interestingly, it has been reported that diminished elesclomol activity is influenced by high LDHA levels (4). Here, we found that TRX1-overexpression cells also exhibit higher LDHA levels and confer resistance to elesclomol. Our findings suggest another novel approach to selectively kill CR lung tumors which intrinsically produce higher ROS and express lower TRX-1 and LDHA levels.



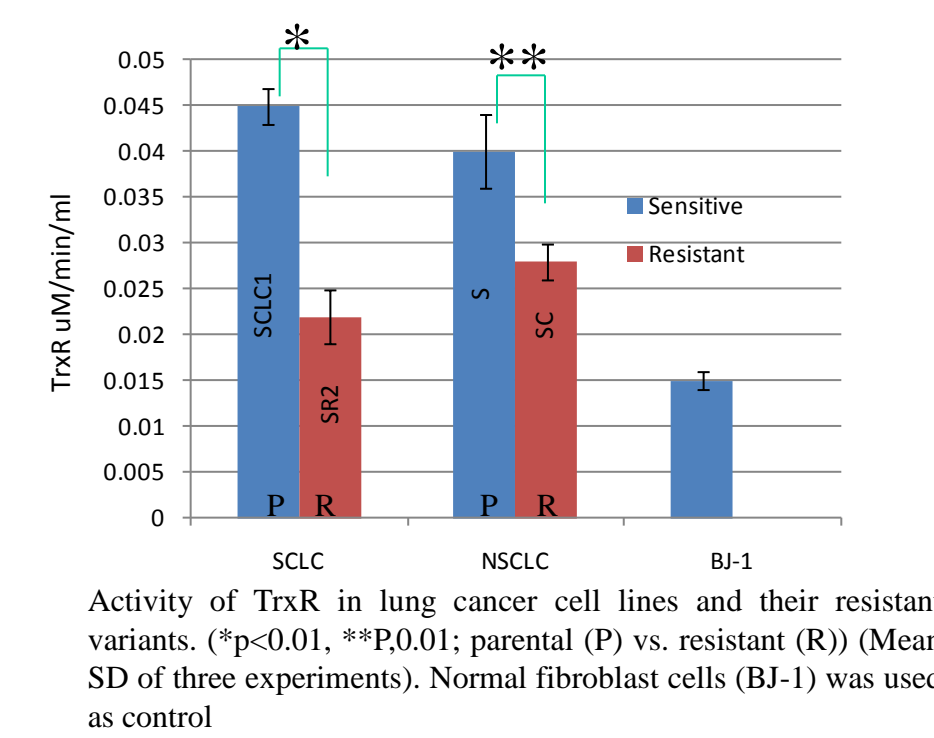
Support by James and Esther King Biomedical Research Program and VA Merit Review Grant

### Results

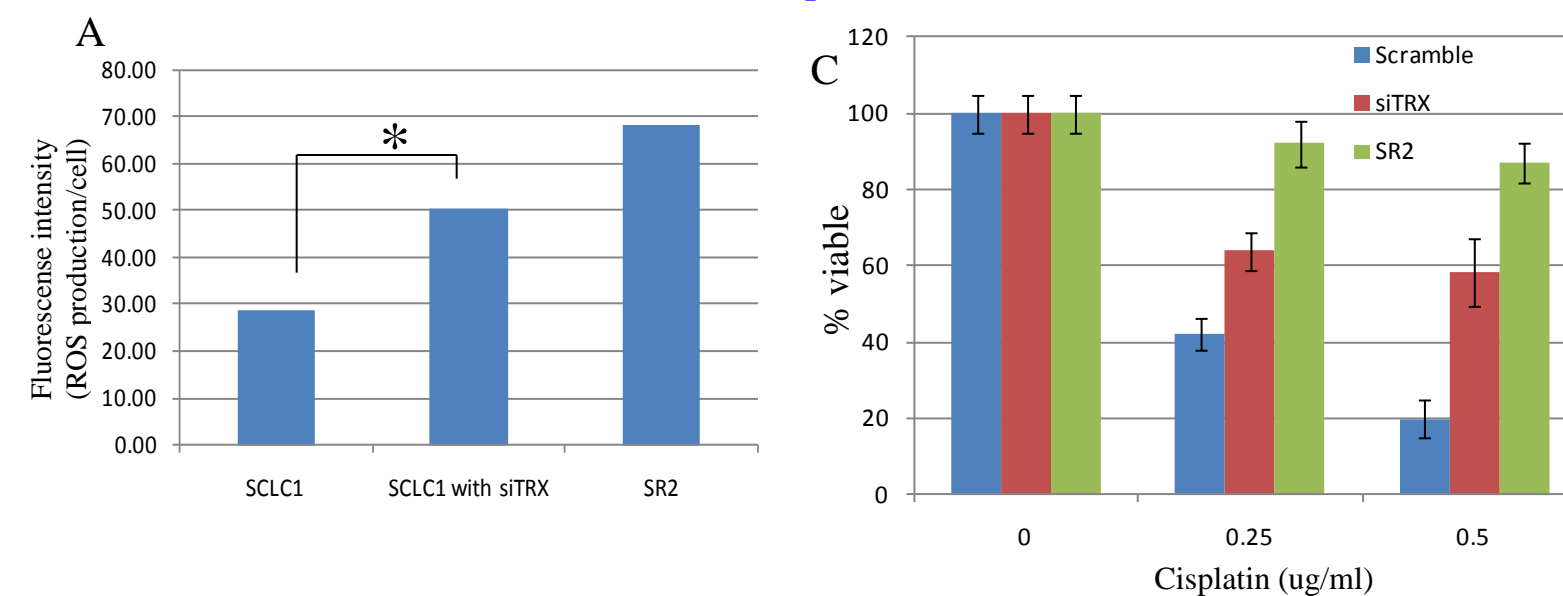
#### Decreased expression of TRX-1 correlates with higher levels of ROS production



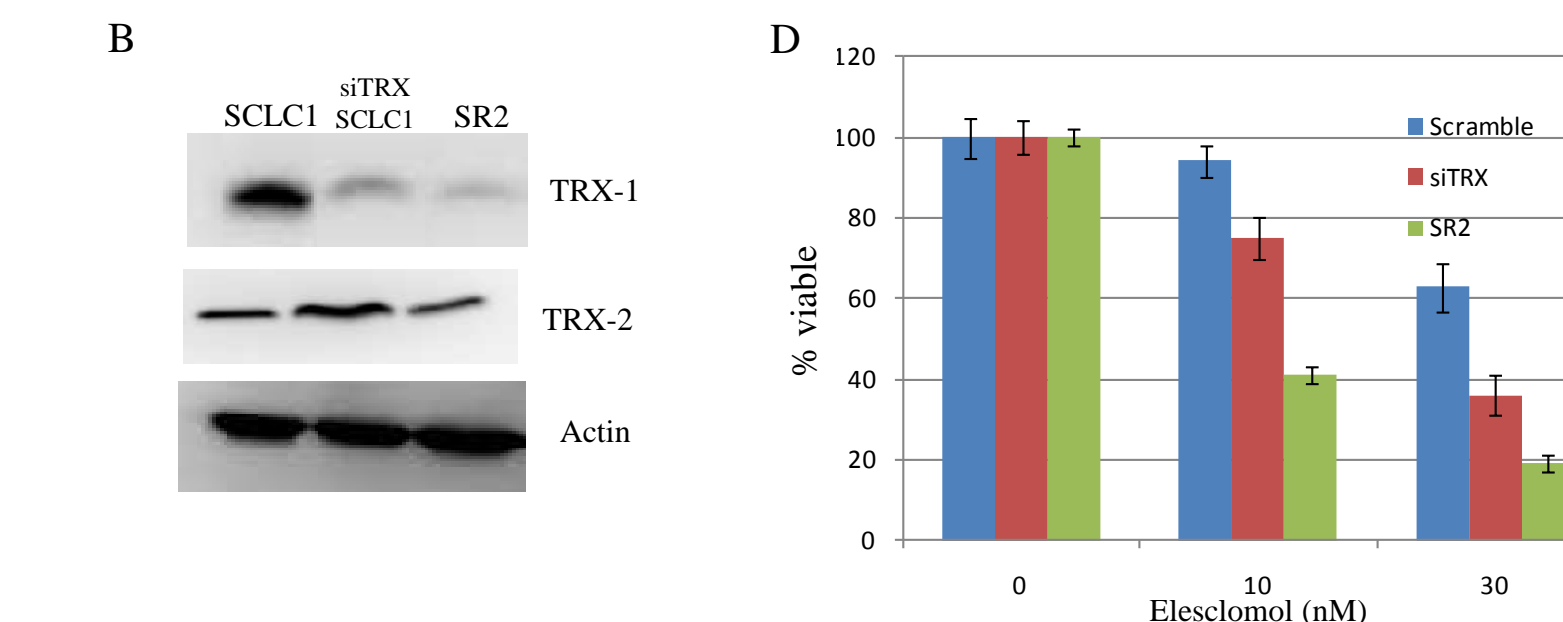
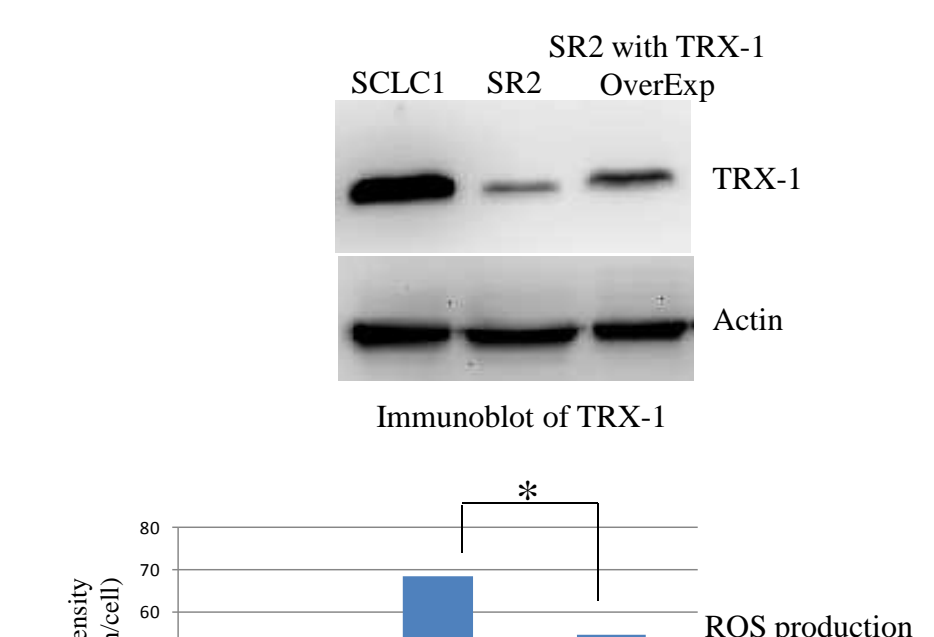
#### Decreased thioredoxin reductase (TrxR) activities are observed in CR lung cancer cell lines.



#### Down-regulation of TRX-1 resulted in increased ROS production, sensitivity to elesclomol, and resistance to cisplatin in SCLC1 cell line



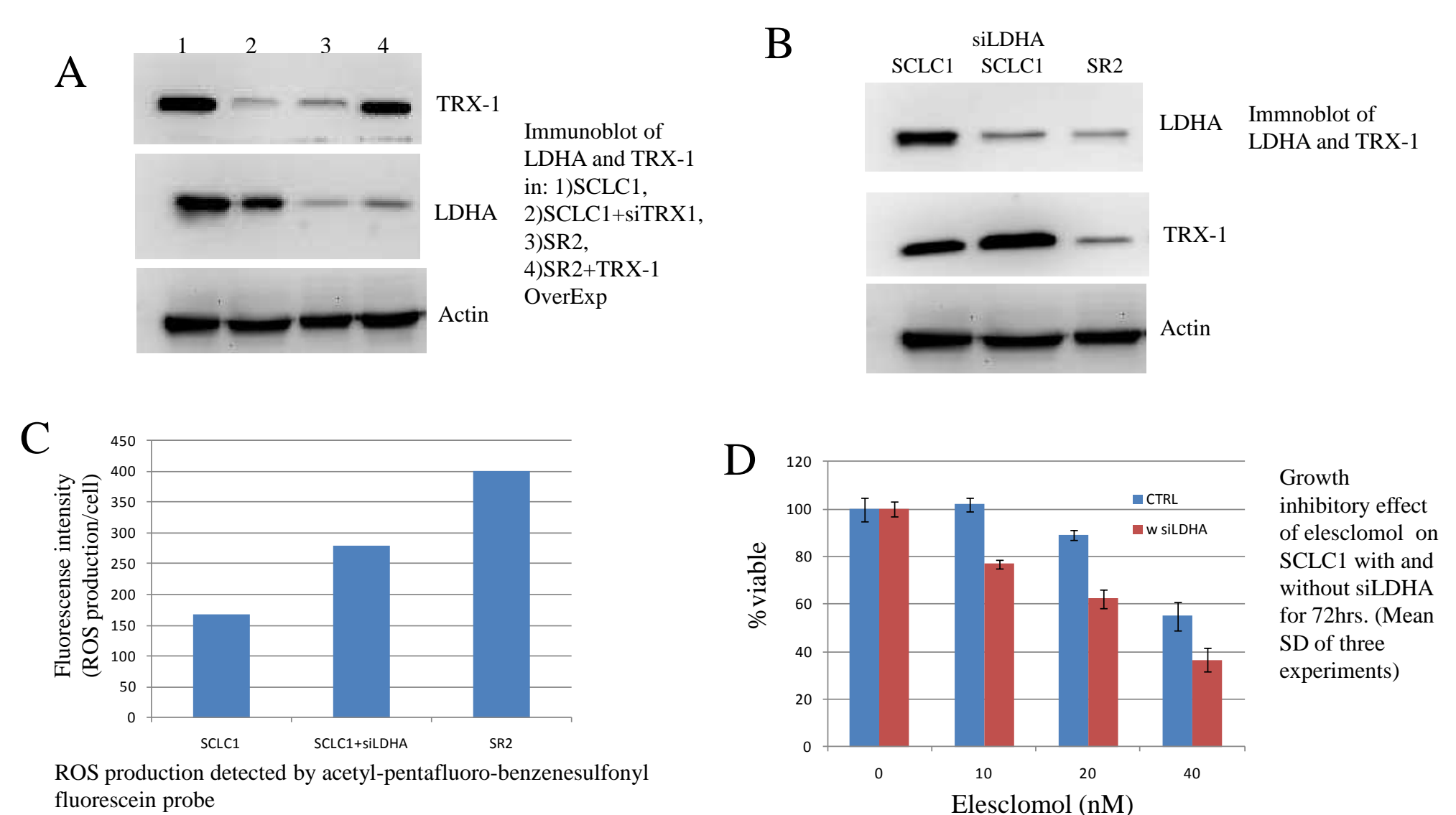
#### Over-expression of TRX-1 in CR cells resulted in decreased ROS production and resistance to elesclomol



(A) siRNA directed against TRX-1 increased the ROS production in parental cell, SCLC1. SR2 was used as positive control. \*p<0.001. (B) Immunoblot of TRX-1 in SCLC1 and SR2. (C) Growth inhibitory effect of cisplatin on SCLC for 72hrs. (D) Growth inhibitory effect of elesclomol on SCLC for 72hrs. (Mean SD of three experiments)

### Results

#### Decreased expression of LDHA correlates with higher levels of ROS production and sensitivity to elesclomol



### Summary

- Cisplatin resistant cell lines have higher basal ROS than their parental cell counterparts.
- Thioredoxin-1 (TRX-1) is a major contributory factor in higher ROS levels found in cisplatin resistant (CR) lung cancer cells.
- Decreased levels of TRX-1 and LDHA lead to increasing ROS.
- Further increase of ROS in these cisplatin resistant cells can push them beyond their tolerance limit which ultimately leads to cell death.

### References

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2:Kirshner JR, He S, Balasubramanyam V, et al. Elesclomol induces cancer cell apoptosis through oxidative stress. *Mol Cancer Ther* 2008;7(8):2319-27  
3:Sasada T, Nakamura H, Ueda S, et al. Possible involvement of thioredoxin reductase as well as thioredoxin in cellular sensitivity to cis-diamminedichloroplatinum (II). *Free Radic Biol Med* 1999;27(5-6):504-14  
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