SLC7A11 modulates sensitivity to the first-in-class mitochondrial peroxiredoxin 3 inhibitor thiostrepton (RSO-021) via a ferroptosis independent pathway

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Background

Mitochondrial peroxiredoxin 3 (PRX3) has been identified as an actionable cancer vulnerability that is currently being investigated in the first-in-human phase 1/2 clinical trial, MITOPE (NCT05278975). RSO-021 (Thiostrepton or TS) is a redox-active drug that inhibits PRX3's peroxidase activity via covalent adduction of its active site peroxidatic and resolving cysteine residues, forming an irreversible crosslink across the protein dimer (Figure 1). Covalent inhibition of PRX3 results in tumor cell death due to a diminished ability to remove high levels of mitochondrial reactive oxygen species (ROS) in cancer cells. To better understand the mechanism underpinning sensitivity and resistance, human mesothelioma cell lines were subjected to a drug screen comprising chemotherapeutic agents, metabolic inhibitors, ROS-modulating agents, and ferroptosis-inducing compounds, to identify synergistic interactions. Erastin, an SLC7A11 inhibitor exhibited the greatest synergy when combined with TS, potentiated PRX3 covalent crosslinking and cellular ROS levels. This interaction was potentiated by cystine depletion and could be phenocopied by siRNA knockdown of SLC7A11. SLC7A11 was upregulated in cells generated to be tolerant to TS and resistance could be overcome with the erastin. TS-tolerant cell lines have significantly slowed proliferation. Ferroptosis inhibitors did not inhibit TS or TS in combination with erastin. Lastly, SLC7A11 expression was determined to correlate with cytotoxic responses in a cohort of TS-treated mesothelioma surgical explants. In summary, SLC7A11 upregulation confers tolerance to TS through a mechanism involving a cysteine-dependent modulation of the redox state, that can be overcome by the addition of an SLC7A11 inhibitor.

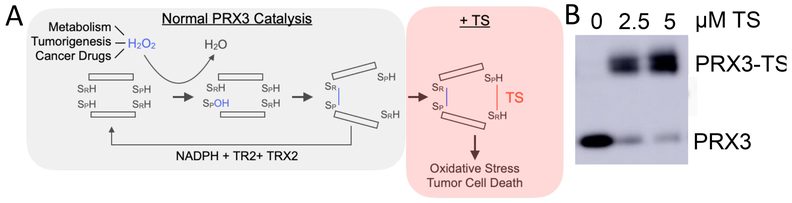

Figure 1: Thiostrepton (TS) is a covalent inhibitor of mitochondrial Peroxiredoxin 3 (PRX3). The proposed MOA of TS. PRX3 is the primary mitochondrial peroxidase required for H_2O_2 clearance from the mitochondria induced by metabolic, tumorigenic, and drug treatment inputs. During the metabolism of H_2O_2, PRX3 forms an intramolecular disulfide bond that orients the second active site for TS-dependent covalent crosslinking, inactivating the protein leading to increased oxidative stress and tumor cell death. **B)** TS-dependent modifications are visualized by reducing SDS-PAGE and western blotting where a dose-dependent irreversible increase in modified PRX3 (PRX3-TS) is present.

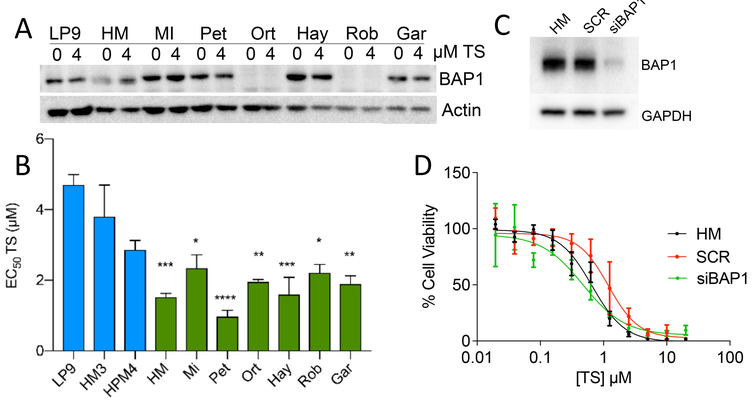

Figure 2: TS activity is independent of BAP1 expression. A) BAP1 protein expression in immortalized mesothelial (LP9), pleural Meso (HM, MI), and peritoneal Meso (Pet-Gar) cell lines. TS treatment at 4 μM does not alter BAP1 protein expression. **B)** EC_{50} values for TS in normal and MM cell lines (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, n = 4 biological replicates) **C)** Protein western blots of BAP1 and GAPDH in HM scramble control (SCR) and BAP1 siRNA expressing (siBAP1) cells. **D)** Cell viability curves of cells treated with TS 48 hours (n = 3).

Results

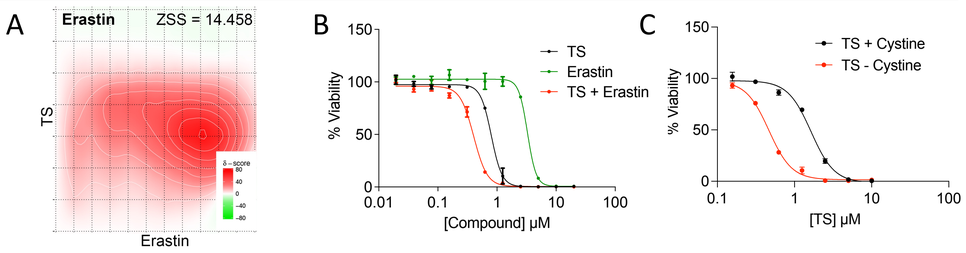

Figure 3. TS and erastin have synergistic activity in Meso cells. A) Synergy distribution plot and Zip Synergy Score (ZSS) for Erastin tested in combination with TS. ZSS > 10 indicates synergy between the two compounds. **B)** Sensitivity of HM cells to TS, Erastin, or TS + erastin (N = 2). **C)** Cell viability curves of HM cells treated with TS in media +/- cystine supplementation (N = 2).

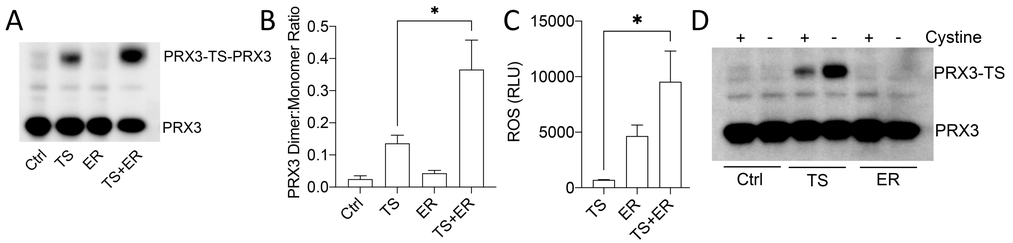

Figure 4. Erastin potentiates PRX3 crosslinking and ROS generation by TS. A) Western blot from HM cells treated with TS (1 μM), erastin (ER, 2.5 μM) or TS + ER for 24 hours. Samples were run under reducing conditions by SDS-PAGE. **B)** Quantification of PRX3 dimer (TS-crosslink) to PRX3 monomer (unmodified PRX3) from protein western blots as in A (n = 2 biological replicates). **C)** ROS levels in cells treated with TS, ER, or TS+ER for 24 hours (n = 2 biological replicates, 3 technical replicates). **D)** Protein western blot of cells cultured in media +/- cystine and treated with TS or ER. Note; PRX3 crosslinking by TS is increased in cells cultured in cystine free (-) media.

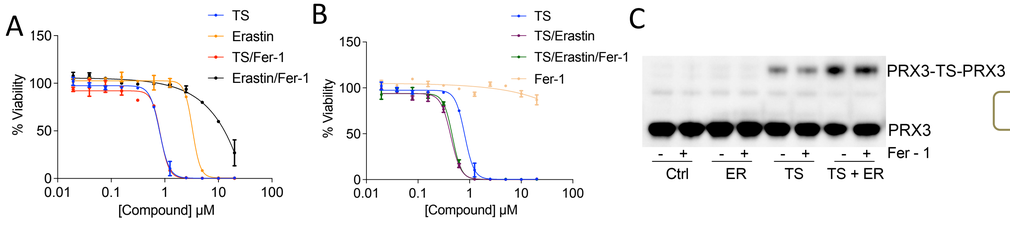

Figure 5. TS synergy with erastin is independent of ferrostatin sensitive MOA. A) Cell viability curves of cells treated with indicated compounds for 48 hours. Note ferrostatin (Fer-1) blocks ER cytotoxicity (orange vs. black line) but not TS cytotoxicity (Red vs. blue lines) **B)** Cell viability curves of cells treated with indicated compounds for 48 hours. Note Fer-1 does not block TS/ER cytotoxic synergy (green vs. purple lines). **C)** Protein western blot of PRX3 crosslinking by TS, ER, and TS + ER in cells treated +/- Fer-1. Fer-1 does not alter PRX3 crosslinking by TS or TS + ER. Similar results obtained with the ferroptosis inhibitor liproxstatin-1.

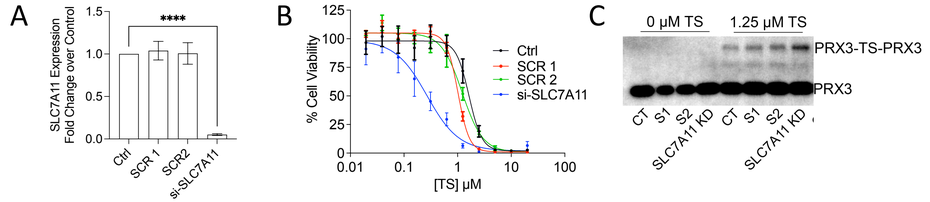

Figure 6. Knockdown of SLC7A11 potentiates TS activity. A) SLC7A11 expression measured by RT-qPCR in HM cells at 48 hrs post transfection with indicated siRNA (Ctrl nontransfected, scramble 1 (SCR 1), scramble 2 (SCR 2) and SLC7A11 siRNA) n = 3 biological replicates. **B)** Cell viability assay of HM cell line with indicated permutations treated with TS, n = 3 biological replicates. **C)** Western blot of PRX3 in the HM cell lines with indicated permutations. PRX3 crosslinking is increased in the SLC7A11 KD samples.

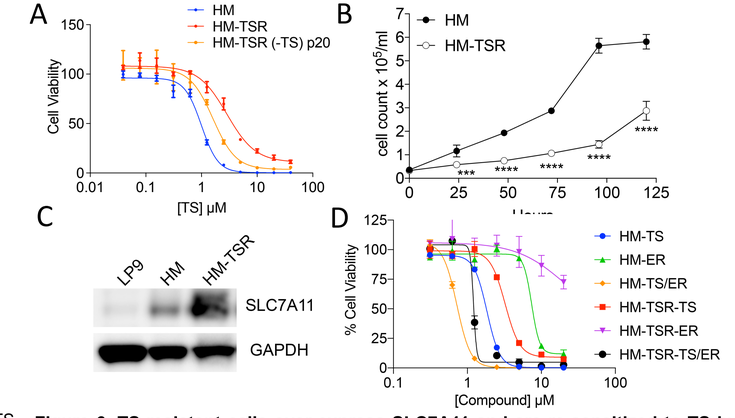

Figure 6. TS-resistant cells over-express SLC7A11 and are re-sensitized to TS by Erastin (ER). HM cells were dosed with TS by repetitive drug treatment with a short 24-48hr recovery period. TS concentration was gradually increased by 1.5-fold until a concentration was reached that no longer provided viable cells. Development of a drug-tolerant cell line with tolerance at 5 μM after 8 months. **A)** Cell viability assay of HM, HM-TSR (TS-resistant) and HM-TSR cells removed from TS pressure for 20 passages. Note; partial resistance of HM-TSR cells is reversible when pressure is removed. **B)** Cell growth analysis of HM and HM-TSR cells over 125 hours (n = 4). **C)** Western blot of SLC7A11 levels in LP9, HM and HM-TSR cells. **D)** Cell viability assay of HM and HM-TSR cells treated with TS, ER or TS + ER for 48 hours. Note the shift in sensitivity of the red line (HM-TSR-TS) to the black line (HM-TSR-TS/ER).

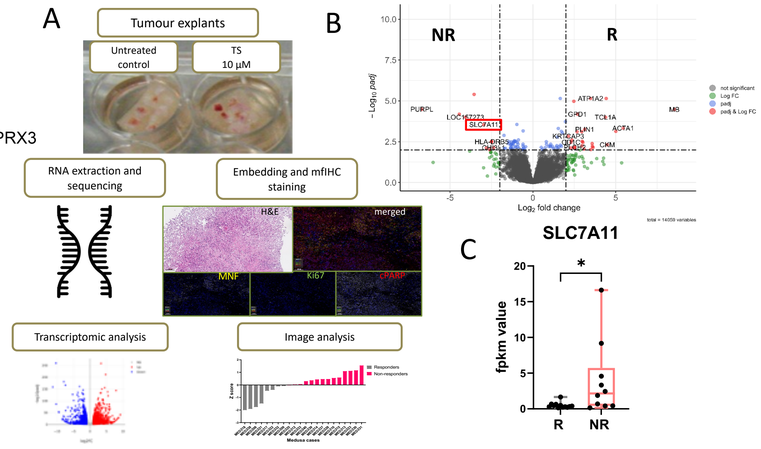

Figure 7. SLC7A11 is enriched in patient derived explants (PDEs) that are non responders to TS. A) Schematic of experiment with PDEs. Assignment to responders (R) or non – responders (NR) was based on cPARP staining results in treated explants in comparison to untreated controls. **B)** DESeq2 analysis of R and NR group showed SLC7A11 upregulation in NR. **C)** This phenomena was confirmed in Mann- Whitney test conducted on normalized transcript level values (fpkm) for R and NR groups.

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