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Understanding the Role of Serine 26 Phosphorylation Changes in Peroxidemediated Trafficking of System x_c^- to the Cell Membrane

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Understanding the role of serine 26 phosphorylation changes in peroxide-mediated trafficking of system x_c^- to the cell membrane

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Background

- System x_c^- is made of xCT and 4F2hc (4F2)
- xCT is a transporter involved in importing cystine and exporting glutamate and 4F2 is involved in moving xCT to the cell membrane
- The activity of this transporter is important in the protection of cells from oxidative stress, ferroptosis, and in neurotransmitter signaling
- A previous study found that mTORC2 regulates system x_c^- by phosphorylating the serine 26 (S26) of xCT (Gu et al. 2017)
- Phosphorylating S26 inhibits xCT activity by taking it off the cell membrane and onto lysosomes (Mukhopadhyay et al. 2021)
- The Chase lab has found that more xCT is on the cell membrane in response to hydrogen peroxide (H_2O_2), which may be the cell's adaptive response to cell stress

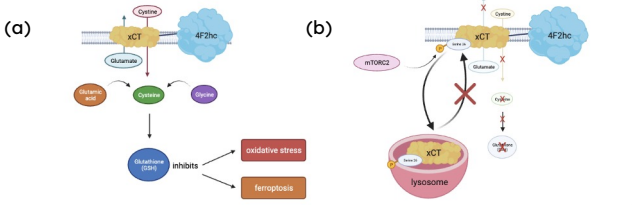


Figure 1. Diagram of mTORC2 regulator activity without (a) and with (b) phosphorylation of serine 26 of the cytosolic N-terminus of xCT.

Purpose

To determine if a change in xCT S26 phosphorylation plays a role in peroxide-mediated movement of system x_c^- to the cell membrane.

Hypothesis

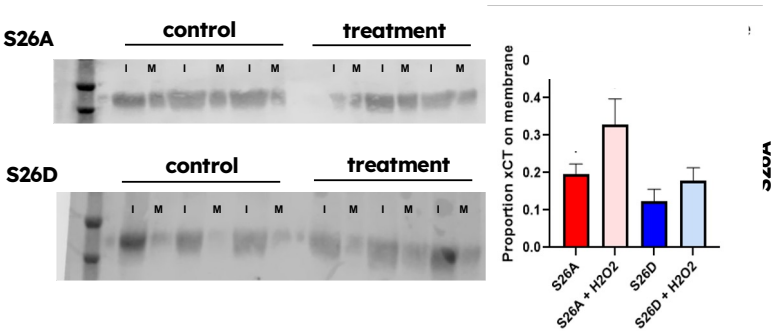
H_2O_2 triggers the loss of phosphorylation of S26, leading to its increased localization of xCT to the plasma membrane.

- To test this hypothesis, we sought to determine if phosphorylation status of S26 affected localization to the membrane by comparing S26A (phosphonull mutant) and S26D (phosphomimetic mutant).
- We also examined the effect of H_2O_2 exposure on localization to the membrane of both mutants.

Methods

- *Immunocytochemistry and confocal microscopy* – used to compare ratios of xCT to 4F2HC on cell membranes of S26A and S26D xCT control and peroxide-treatment groups.
- *Biotinylation assay and Western Blot* – used to determine the relative amount of membrane-bound xCT between S26A and S26D xCT control and peroxide-treatment groups.

Phosphorylation status of S26 affects localization to membrane, but not sensitivity to H_2O_2



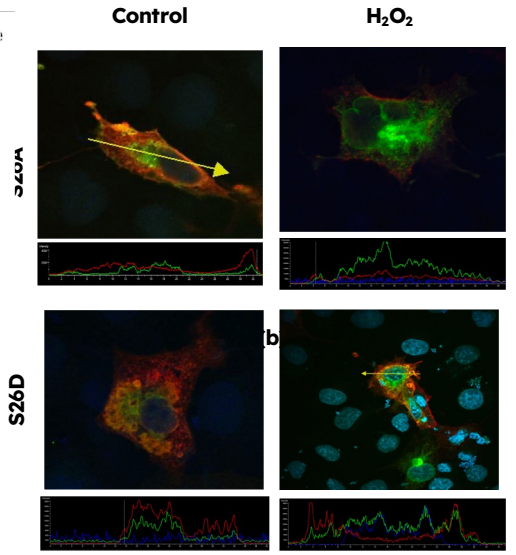


Figure 3. A Biotinylation results: Western blot analysis showing intracellular (I) and membrane (M)-bound xCT in even lanes. **B.** Analysis of biotinylation results including TWO-WAY ANOVA. There is a significant effect of mutant ($F_{1,20}$=6.56, p=0.019) and a significant effect of H_2O_2 ($F_{1,20}$)=4.66 H_2O_2, p=0.043, but no interaction effect H_2O_2(0.3 mM) was added for 20 min **C.** Immunocytochemistry results: xCT (S26A and S26D) and 4F2HC were qualitatively examined for cell membrane expression in the presence and absence of H_2O_2.

Conclusions and Future Research

Biotinylation results

- Our results suggest that S26D exhibits less membrane localization relative to S26A as has been previously shown.
- However, our results suggest that both S26A and S26D may show the ability to the membrane following a 10 min H_2O_2 exposure.

Immunocytochemistry results

- We were unable to complete the quantitative analysis of xCT and 4F2HC association on the plasma membrane because we did not standardize the gain across all cells analyzed.
- Qualitatively, our results support our biotinylation data, demonstrating less S26D localized to the membrane compared to S26A

Interpretation of results

- Our preliminary results suggest that the change in phosphorylation status of S26 is not responsible for the shift in localization of xCT to the plasma membrane.

Future research

1. Repeat biotinylation procedures to increase number of replicates.
2. Repeat immunocytochemistry procedure using standard gain.
3. Use lysosome trackers to determine if S26D xCT is moving to lysosomes
4. Perform repeat of immunoprecipitation procedure to look at overall association between xCT and 4F2 in each mutant

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