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Does Phosphorylation on Serine 26 of System xc^- Lead to Changes in Cell Surface Expression?

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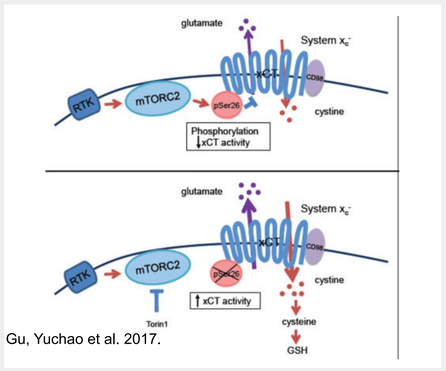
Background: Phosphorylation of serine 26 of system x_c^-

- System x_c^- catalyzes the cellular uptake of cystine and the release of glutamate via membrane antiporter xCT (Bridges et al. 2012).
- The activity of this transporter is important in the protection of cells from oxidative stress.
- A previous study has demonstrated that mTORC2 regulates system x_c^- by phosphorylation of serine 26 (S26) on the cytosolic N-terminus of xCT (Gu et al. 2017).
- Phosphorylation of S26 was found to inhibit xCT activity.



Gu, Yuchao et al. 2017.

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

Research Question

- This experiment aims to determine the specific mechanism by which phosphorylation of serine 26 inhibits system x_c^- by studying modified S26A, S26D, and wild type S26 (WT).
- S26A contains a modified serine that is unable to be phosphorylated, and S26D contains a modified serine that chemically mimics the permanently phosphorylated state.

Hypothesis:

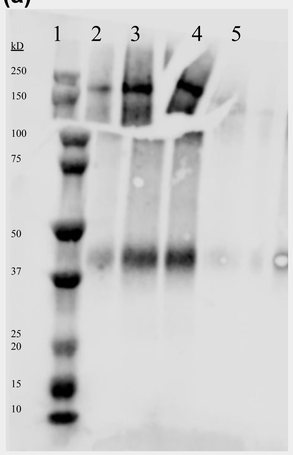
The phosphorylation of serine 26 of xCT inhibits system x_c^- activity by taking xCT off of the cell membrane and back into the cell interior. It may also modulate the association of xCT with a high molecular weight complex.

Methods

- *Immunocytochemistry and confocal microscope image analysis* used to compare ratios of xCT to 4F2HC on cell membranes of WT xCT and S26A xCT.
- *Immunoprecipitation assay and Western Blot* was used to examine myc-xCT molecular weight in WT myc-xCT, S26A myc-xCT, and S26D myc-xCT.

Results: Western Blot

(a)



Key
Lane 1: Standard
Lane 2: none*
Lane 3: WT
Lane 4: S26A
Lane 5: S26D

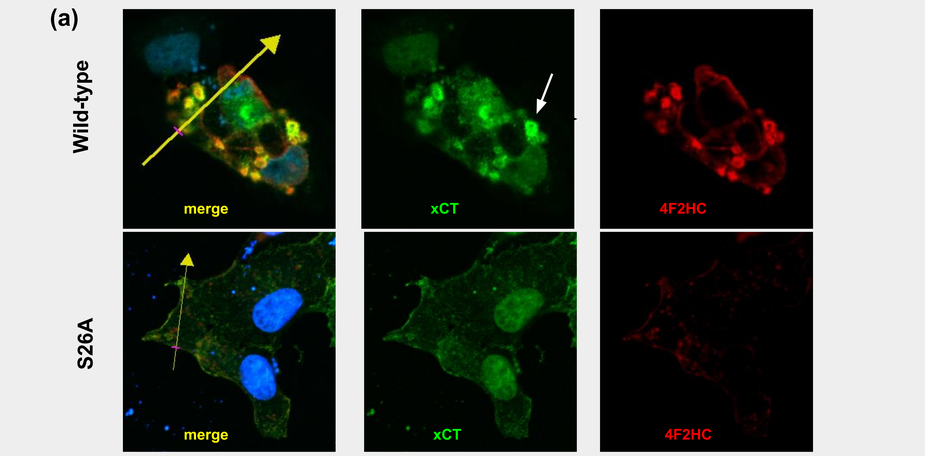
*some WT xCT contaminated Lane 2

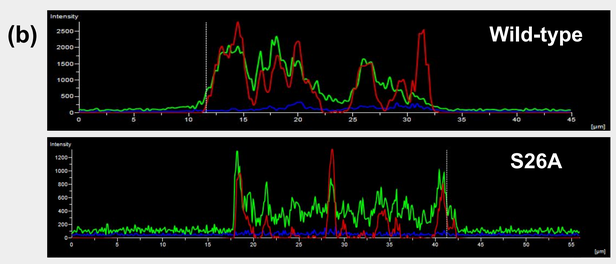
(b)

myc-xCT Type	high:low MW ratio
WT	3.83
S26A	2.63

Figure 2.
(a) Western Blot of WT myc-xCT and variant S26A myc-xCT show bands at different molecular weights, one at 150 kD and one between 37 and 50 kD. S26D myc-xCT was unable to be analyzed due to unusually low expression of the protein.
(b) Ratios of high:low molecular weight xCT for WT and S26A myc-xCT.

Results: Colocalization of xCT and 4F2HC on cell membrane





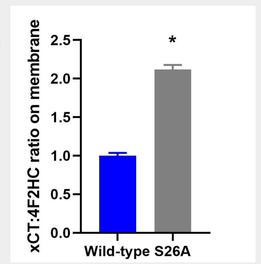


Figure 3.
(a) Immunocytochemistry shows that more xCT is concentrated on the membrane than the interior of the cell in cells with S26A xCT, while more xCT is concentrated in the cytosol of the cell in cells with WT xCT. Some cells exhibited large vesicles containing xCT which may be autophagosomes (white arrow).
(b) Intensity plots quantify xCT and 4F2HC immunocytochemistry signals across cells.
(c) Mean ratio of xCT to 4F2HC on cell membranes for each cell type. Cells transfected with 26A xCT have a mean ratio (μ=2.13) twice as large as cells with WT xCT (μ=1.00) ($t=3.660$, $df=41$, $p < 0.001$).

Conclusions

- myc-xCT WT type shows higher proportion of high-molecular weight xCT than myc-xCT S26A
- The mean ratio of xCT to 4F2HC on the cell membrane is approximately twice as large in S26A-transfected cells compared to WT-transfected cells.
- A higher proportion of xCT appears on the membrane of S26A-transfected cells than WT-transfected cells.
- Since this mutant is unable to be phosphorylated, these data suggest that perhaps a significant fraction of xCT is phosphorylated at S26 under basal conditions, limiting xCT expression on the membrane.

Future Research

- Repeat of the Western Blot procedure and immunocytochemistry is required to determine if results can be replicated.
- If so, this would suggest that the higher molecular weight bands are less active forms of xCT
- Because S26D xCT did not show strong bands in Western Blot we need to repeat this experiment.
- If low expression of S26D xCT persists, this could indicate that phosphorylation of s26 may lead to reduction in transporter expression.
- Complete cell surface biotinylation assay to measure xCT cell surface expression.

Acknowledgements

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