Investigation of the Role of GLYT1 and ATF4 in Cell Proliferation

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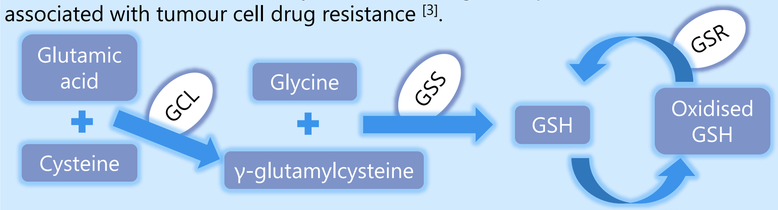
Introduction/Aims

Glycine protects cells against various toxic challenges, possibly by contributing to glutathione (GSH) synthesis. GSH is formed in two ATP-requiring steps by the enzymes glutamate cysteine ligase (GCL) and glutathione synthetase (GSS). As an antioxidant that reduces reactive oxygen species, GSH allows cell proliferation in a hostile environment. Glutathione reductase (GSR) allows the reformation of GSH. Elevated GSH levels, GCL activity and also GCLC gene expression have been associated with tumour cell drug resistance [3].



Glycine transport is crucial for rapid growth of some cancers. Studies [1] found that rapidly proliferating (dividing) cancer cells had a significantly greater glycine requirement than slow proliferating cancer cells and also non-cancerous fast proliferating cells; suggesting the manipulation of glycine availability to be a possible therapeutic target for highly invasive cancers. GLYT1 is a key transporter for glycine influx into cells. The transcription factor ATF4, upregulated in cancer [2], regulates GLYT1 as well as genes involved in GSH biosynthesis.
This project aims to verify if reducing the expression of GLYT1 or ATF4 via gene knockdown influences cancer cell proliferation rate and if the downregulation has an effect on the expression of glutathione synthesis genes.

Methods

Cancer cell lines (A498, A459, HT29) cultured

GLYT1 and ATF4 knockdown
by 72 hour transfection with negative control, GLYT1 and ATF4 siRNA

Verification of effect on cell proliferation
using cell viability assay (CellTiter-Blue, Promega, UK)

Verification of effect on gene expression

RNA Extraction
from cells cultured in 12-well plate with RNA purity and concentration quantified via spectrophotometry

Reverse transcription
to obtain cDNA which was then diluted 1 in 5 for qPCR

Real-time qPCR
using Lightcycler 480 (Roche) to analyse gene expression and verify knockdown efficiency. Three housekeeping genes (TOP1, ATP5B, GAPDH) were used to normalise data and a standard curve of cloned target PCR products was made to infer arbitrary units of relative mRNA expression

Results: Effect on Cell Proliferation

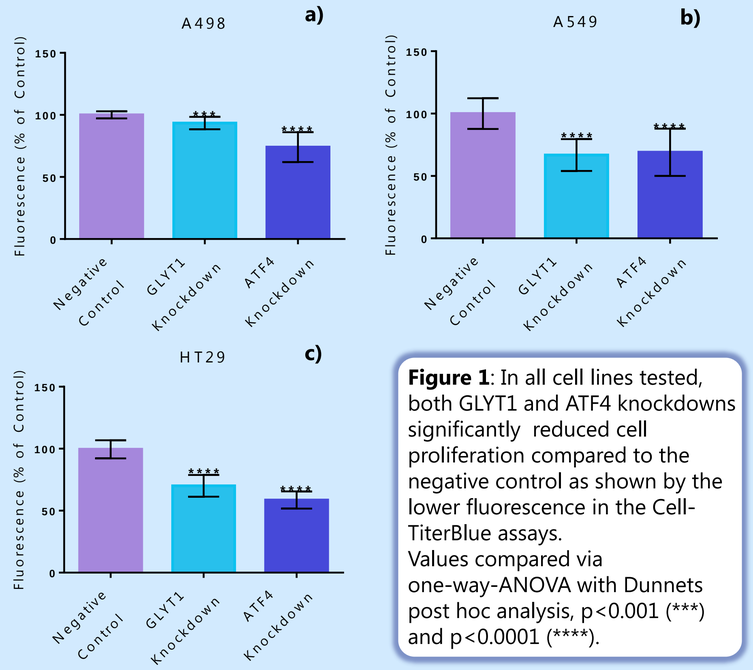


Figure 1: In all cell lines tested, both GLYT1 and ATF4 knockdowns significantly reduced cell proliferation compared to the negative control as shown by the lower fluorescence in the Cell-TiterBlue assays.
Values compared via one-way-ANOVA with Dunnets post hoc analysis, $p<0.001$ (***) and $p<0.0001$ (****).

Results: Effect on Gene Expression

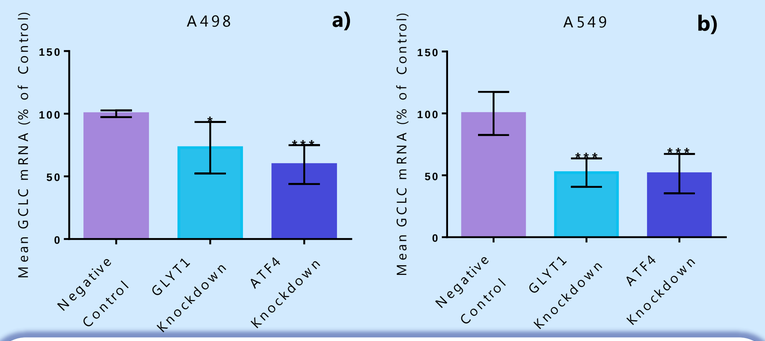


Figure 2: Real-time qPCR analysis showed that both GLYT1 and ATF4 knockdown reduced the abundance of mRNA encoding GCLC in A498 and A549 cells. HT29 cells showed no significant change in GCLC mRNA with either knockdown. Neither GLYT1 nor ATF4 knockdown had any effect on expression of either GCLM, GSS or GSR mRNAs ($p>0.05$).
Values compared via one-way-ANOVA with Dunnets post hoc analysis, $p<0.1$ (*) and $p<0.001$ (***).

Discussion/Conclusions

- A greater reduction in cell growth was observed in the rapidly proliferating cell lines (A459 and HT29) than the slowly proliferating cells (A498). This indicates rapidly proliferating cells are more dependent on GLYT1 and glycine availability; supporting the manipulation of glycine availability as a new chemotherapeutic approach
- While the greater reduction in GCLC expression in A549 cells correlates with the reduction seen in cell proliferation, there was no significant change in GCLC expression observed in HT29 cells. This suggests that cell proliferation and the expression of GSH synthesis genes are independent of one another
- GCL is responsible for catalysing the rate limiting step in GSH synthesis which could explain why GCLC expression was the only GSH synthesis gene that was significantly altered in the knockdown experiments
- From the data, it can be concluded that GLYT1 and ATF4 knockdown affect cancer cell growth however the exact mechanisms of this could be due to many complex pathways, including regulation of GSH synthesis and glycine availability, rather than a direct impact of the knockdowns
- Further research is required to confirm the mechanism behind the effect and, if any, of GSH synthesis

References

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3. Traverso, N., Ricciarelli, R., Nitti, M., Marengo, B., Furfaro, A.L., Pronzato, M.A., Marinari, U.M., Domenicotti, C., 2013 'Role of Glutathione in Cancer Progression and Chemoresistance' *Oxidative Medicine and Cellular Longevity*, 2013: 972913.

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