Loss of N-glycanase 1 alters translational and transcriptional regulation

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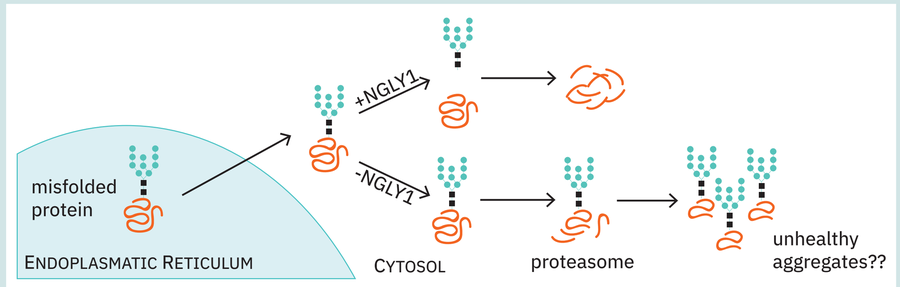
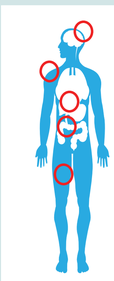
INTRODUCTION

N-Glycanase 1 (NGLY1) deficiency is a rare, complex and devastating neuromuscular disease that affects fewer than 55 identified patients globally. The NGLY1 protein is a cytosolic deglycosylase involved in the degradation of misfolded proteins translocated from the endoplasmic reticulum (ER). Whole genome and whole exome sequencing found autosomal-recessive mutations common to multiple patients with similar phenotypes, identifying NGLY1 as causative for the deficiency. The most common mutations result in premature stop codons and are predicted to lead to mRNA degradation via nonsense-mediated decay. Patients are usually young and display a variety of phenotypes including developmental delay, movement disorder, and a lack or decreased ability to produce tears. NGLY1 deficient cells have been reported to exhibit phenotypes including decreased deglycosylation activity, cloudy cytoplasm, and an increased sensitivity to proteasome inhibitors. Here, we show that the loss of NGLY1 causes substantial changes in the RNA and protein landscape of K562 cells. Loss of NGLY1 results in down regulation of proteasomal subunits, consistent with its activity in processing of the transcription factor NFE2L1.





Disease symptoms:
Global developmental delay
Lack of language development
Hyperkinetic Movements
Alacrima (lack of tears)
Swallowing difficulties
Seizures
Neuropathy
Constipation

1. Test the hypothesis: Are protein aggregates detectable in NGLY1 deficient cells?

2. Discover pathway perturbations linked to NGLY1 deficiency.

3. Screen compounds for those that rescue loss of NGLY1 or return patient cells to full functionality and those that exacerbate NGLY1 phenotypes.

WORKFLOW

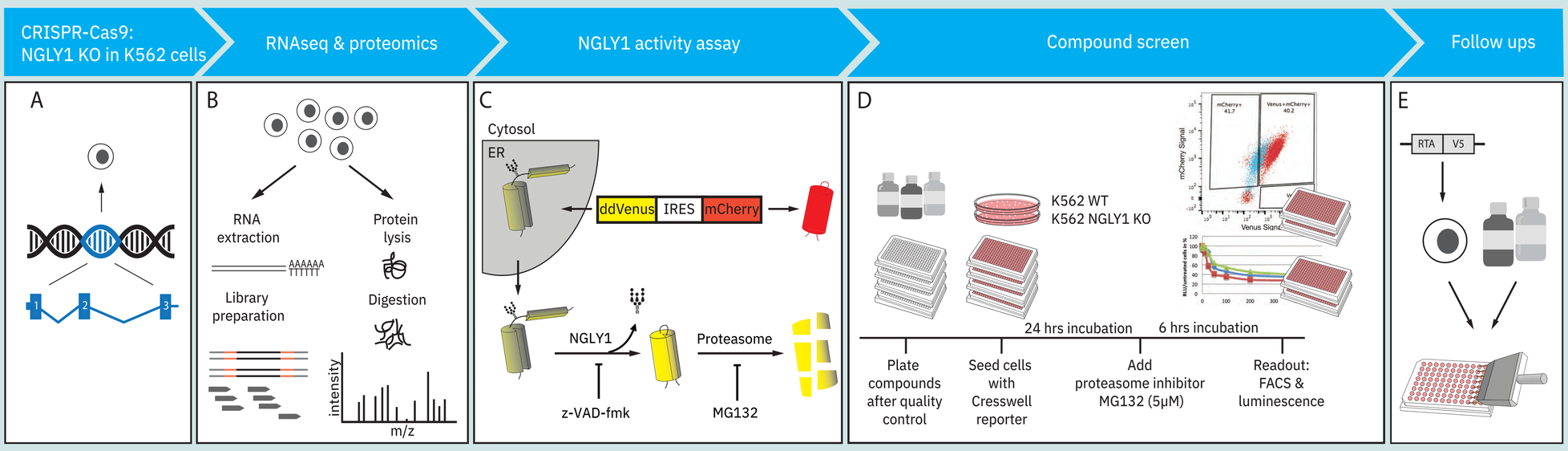
A: Generation of NGLY1 KO in K562 cells via CRISPR-Cas9 and validation of sensitivity to proteasome inhibition.

B: Analysis of RNA and protein of NGLY1 deficient K562 cells

C: Adaptation of an existing deglycosylation depended reporter assay for a compound screen

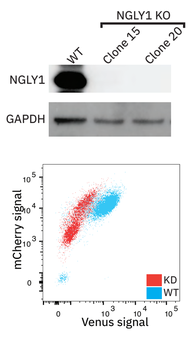
D: Scheme of the compound screen

E: Target validation

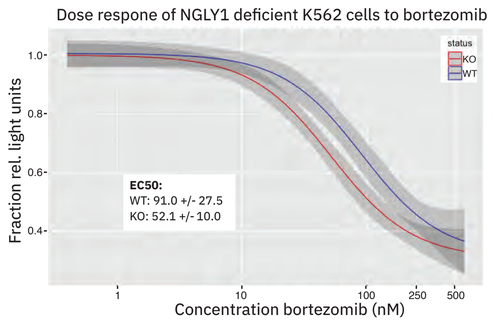


RESULTS

NGLY1 knockout (KO) in K562 cells was verified by Western blot and stable cell lines including a dual fluoresence deglycosylation reporter for FACS were created. We exposed our K562 cell lines to increasing concentrations of bortezomib (a proteasome inhibitor) and observed a ~2-fold higher sensitivity of NGLY1 KO K562 cells to treatment than controls (Albanell and Adams 2002).



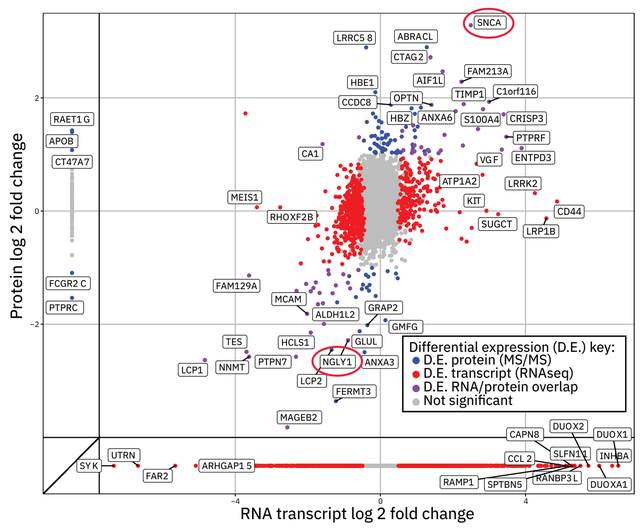
Dose respone of NGLY1 deficient K562 cells to bortezomib



We harvested whole cell mRNA and protein fractions and performed RNA-seq and LC-MS/MS analyses to determine transcriptional and translational processes that are influenced by NGLY1.

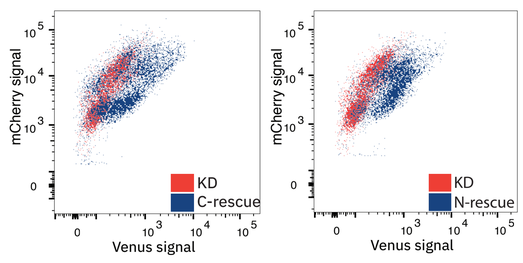
59 genes showed significantly increased/decreased signal on protein and transcript levels (points in purple). This set of genes contains NGLY1. SNCA, the gene coding for α-synuclein, one of the major proteins involved in aggregate formation in Parkinson's disease (Cookson 2010), was the most upregulated gene in both our RNA and protein datasets.

RNA vs protein data set in NGLY1 deficient K562 cells

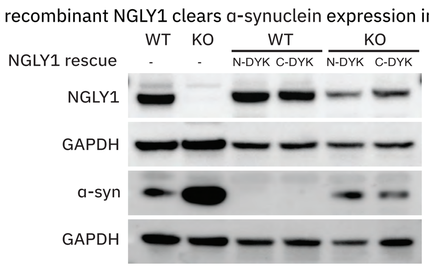


We tested whether restoration of NGLY1 expression in these cells would reset SNCA expression. For this we transfected K562 cells with DYK-tagged NGLY1-protein and tested activity using the ddVenus FACS assay. Both constructs rescue NGLY1 activity. Accordingly, rescue of NGLY1 expression with a C- or N-terminally DYK-tagged recombinant protein decreased α-synuclein expression back to levels similar to that of K562 WT cells.

Fluorescent reporter: DYK tagged NGLY1 rescues deglycosylation in NGLY1 KO K562 cells

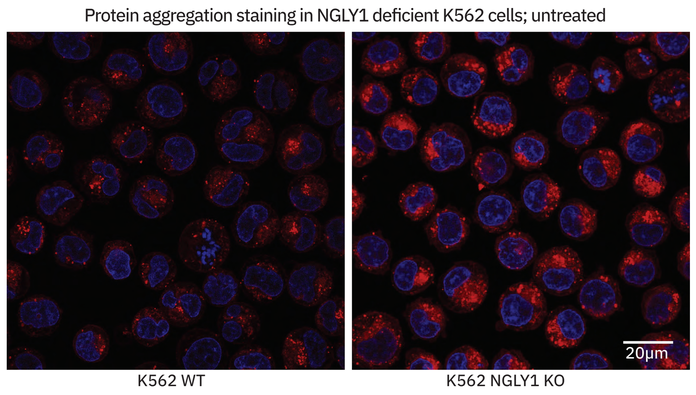


Western Blot: recombinant NGLY1 clears α-synuclein expression in NGLY1 KO K562 cells



Having linked expression of an aggregation-prone protein to NGLY1, we then tested for protein aggregation in the NGLY1-deficient K562 cells using the Proteostat protein aggregation dye (Enzo BioSciences). We found that there was a trend toward increased staining of protein aggregation in K562 NGLY1 deficient cells. However, it remains to be determined if aggregates contain α-synuclein.

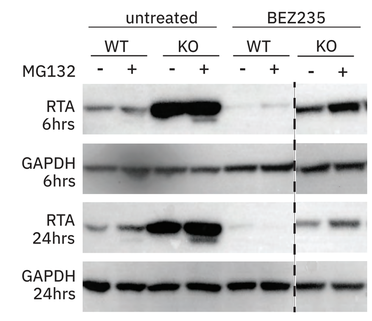
Protein aggregation staining in NGLY1 deficient K562 cells; untreated



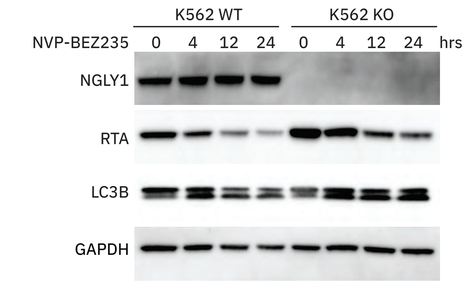
K562 WT K562 NGLY1 KO

To identify a compound that rescues the NGLY1 deficiency phenotype, we used our transcriptome data and the CMap database to determine potential compounds. However, a screen of 48 candidate molecules in NGLY1 KO reporter cells did not reveal any hits. Nevertheless, we identified several inhibitors of NGLY1 function in the WT reporter cells.

To characterize the mechanism by which NVP-BEZ235 inhibits NGLY1, we created a stable cell line expressing the ricin-toxin A chain (RTA), a known target of NGLY1 whose protein levels accumulate in the absence of NGLY1.



However, treatment with NVP-BEZ235 caused a decrease in RTA signal over time. Hypothesizing that this is due to increased autphagy, we assayed the modification of LC3 to LC3-II, an indicator of autophagic flux (Maira et al. 2008), in a timecourse experiment.



We see a consistent decrease in the level of RTA signal and simultaneously an increase in the proportion of LC3-II to LC3 upon treatment with NVP-BEZ235. These data suggest that autophagy may clear aberrant substrates during NGLY1 deficiency.

CONCLUSION

1. NGLY1 deficiency results in the accumulation of aggregates.

2. NGLY1 dependent protein accumulation can be decreased through the activation of autophagy.

3. It is possible that modulation of autophagy is a therapeutic option for NGLY1 deficient patients.

NEXT STEPS

1. Determine the subcellular localization of the aggregates.

2. Determine if a specific sub type of autophagy is effected by NGLY1.

3. Test other autophagy modifying compounds to determine their influence on NGLY1 deficient systems.

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