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Inhibition of N-linked Protein Deglycosylation Stimulates Autophagy

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Glycosylation is the process by which sugars are added to biological molecules such as proteins. It regulates a myriad of biochemical processes, such as protein folding, adhesion, targeting and recognition events. Conditions resulting from in-born defects of glycosylation manifest with wide-ranging pathologies impacting nearly every organ system and result in severe multi-system diseases. These disorders are challenging to diagnose and difficult to map onto the clinical presentations, as a single defect in a sugar processing pathway may result in complex and varied downstream effects [1].

Protein folding and degradation

In a healthy cell, N-glycanase removes N-linked glycans from misfolded proteins prior to proteasomal degradation (figure 1).

NGUY1 disorder is a rare congenital disease caused by mutations in the gene that encodes N-glycanase resulting in:

- Loss/reduction of N-glycanase activity
- Build up of misfolded proteins which aggregate in the cell [1,2].

If N-glycanase is inhibited, autophagy is stimulated to compensate for the accumulation of misfolded proteins. N-glycanase inhibition was studied using z-VAD-fmk, an irreversible inhibitor of N-glycanase. As z-VAD-fmk is also a caspase inhibitor, Q-VD-Oph was employed as a control as it has a similar caspase inhibition profile but does not inhibit of N-glycanase.

N-glycanase inhibition causes transient ER stress

Inhibition of N-glycanase using z-VAD-fmk increases protein aggregation and expression of Grp78 (figure 2). Cellular ThioflavinT (ThT) labelling provides a convenient assay for ER stress and protein misfolding [3] (figure 2). Grp78 is upregulated during ER stress and the unfolded protein response (figure 3).

Autophagy deficient cells cannot recover from cellular stress caused by N-glycanase inhibition

Cells lacking ATG13 are unable to form autophagic vesicles (figure 6). Autophagy-deficient ATG13⁻/⁻ MEFs incubated with 50 µM z-VAD-fmk for 72 h showed a significant reduction in cell viability (figure 7a).

Wild type MEFs showed no reduction in cell viability (figure 7b). ATG13⁻/⁻ MEFs showed no reduction in cell viability when treated with Q-VD-Oph, indicating cell toxicity is due to N-glycanase inhibition and not caspase inhibition (figure 7c).

N-glycanase inhibition does not disrupt ER or actin cytoskeletal structure

Figure 8 (a) Confocal images of HEK293 cells treated with 50 µM z-VAD-fmk and transfected with GFP-ER (b) Fluorescent images of HEK293 cells treated with 50 µM z-VAD-fmk. Actin stained with Phalloidin 488 and nucleus with Hoechst. Scale bars = 10 µm, n = 1.

Summary

- Peptide N-glycanase inhibition causes simultaneous ER stress and the accumulation of autophagic vesicles.
- Autophagy enables cells to degrade misfolded proteins and reduce ER stress.
- Cells die if they unable to trigger autophagy during N-glycanase inhibition.

Future work

- CRISPR generated knockouts and clinical mutations of N-glycanase to study long-term inhibition on global/aggregate glycosylation.
- How aggregates are targeted to autophagy network.
- Analysis of proteosomal degradation machinery included in aggregates.

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References


Statistics

Error bars indicate SEM. * indicates P < 0.05. Data analysed using ANOVA and relevant post-hoc test.