Mapping and Quantifying Protein O-GlcNAcylation in Human Brain for Studies of Alzheimer's Disease

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Overview

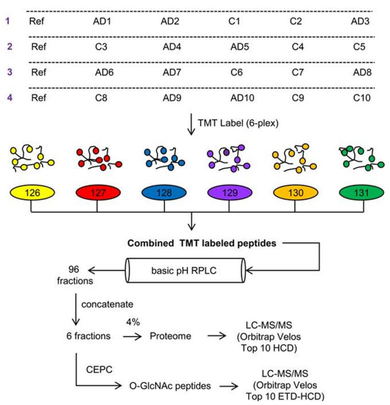
- A proteomic analysis pipeline has been developed for large-scale quantitative profiling of protein O-GlcNAcylation.
- Protein O-GlcNAcylation in post-mortem human brains of Alzheimer's disease (AD) cases and age-matched healthy controls was compared applying this proteomic pipeline.
- 1,850 O-GlcNAc peptides covering 530 proteins were identified; 1,094 O-GlcNAc sites were assigned confidently.
- 128 O-GlcNAc peptides covering 78 proteins (e.g., synaptic proteins, memory-associated proteins) changed significantly in AD brain.

Introduction

- O-GlcNAcylation, in which Ser/Thr residues are modified with a single β-N-acetylglucosamine, is a widespread dynamic protein modification.
- The link between O-GlcNAc and AD is emerging as an important topic[1].
- Issues such as the extremely low abundance and labile nature of O-GlcNAcylation pose great analytical challenges on direct analysis of O-GlcNAc peptides by LC-MS.
- We developed a proteomic pipeline consisting of isobaric tandem mass tag (TMT) labeling, high-pH RPLC fractionation, and chemoenzymatic photocleavage (CEPC) enrichment[2] for comprehensive LC-MS/MS analysis of protein O-GlcNAcylation.
- Protein O-GlcNAcylation in post-mortem brains with or without AD (10 brain samples in each group) was characterized and compared using this proteomic pipeline.

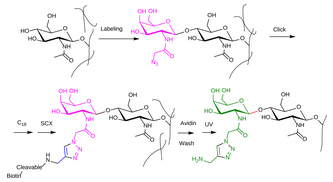
Methods

TMT-6 Labeling Scheme and Workflow



- Ref: a pooled sample with equal contribution from each individual sample
- Proteome and O-GlcNAc samples were split after TMT-6 labeling and fractionation
- Alternating HCD and ETD were used for quantification (TMT reporter ions) and O-GlcNAc site determination, respectively

Chemical Principle of CEPC Enrichment



Results

Mapping of O-GlcNAc in Human Brain

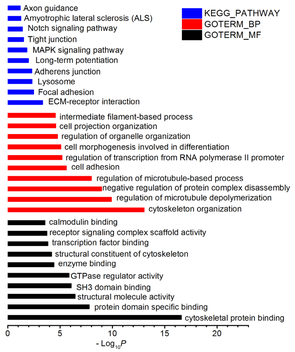


Figure 1. Gene ontology (DAVID) analysis of all the identified O-GlcNAc proteins.

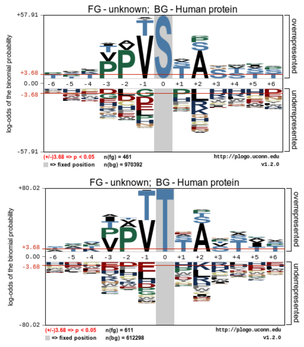


Figure 2. Motif analysis of O-GlcNAc sites with pLogo. Red horizontal lines: thresholds of p = 0.05. Upper panel, the S motif; lower panel, the T motif. FG, foreground; BG, background.

Change of O-GlcNAc in AD

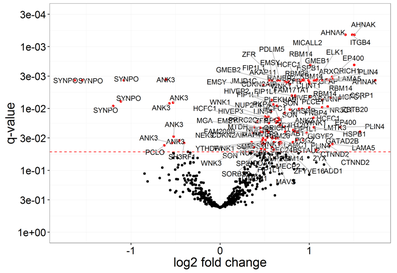


Figure 3. Quantification of the O-GlcNAc peptides in the human brain: Volcano plot of the O-GlcNAc peptides with >50% occurrence in all samples. The proteins (gene name) that carry the O-GlcNAc peptide(s) with q < 0.05 (red dots) are labeled.

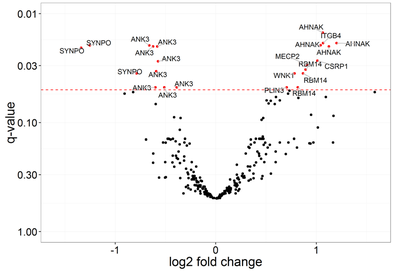


Figure 4. The changes in O-GlcNAc stoichiometry of human brain proteins as shown in volcano plot. The proteins (gene name) that carry the O-GlcNAc peptide(s) with q < 0.05 (red dots) are labeled.

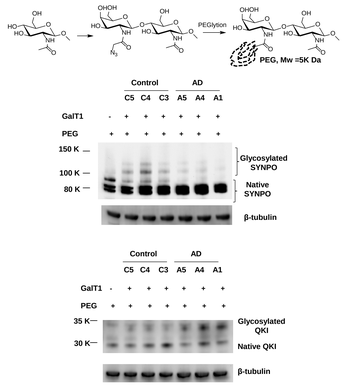


Figure 5. Verification of O-GlcNAc quantification with Western blotting. Upper panel: principle of PEG labeling of O-GlcNAcylated proteins; middle and lower panels: Western blots of brain homogenates, probed with antibody against synaptopodin (SYNPO) or Protein quaking (QKI), respectively. β-tubulin was used for loading control.

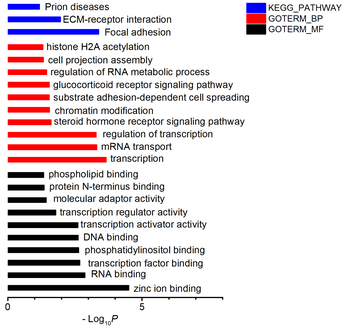


Figure 6. Gene ontology (DAVID) analysis of proteins carrying significantly changed O-GlcNAc in AD.

Conclusions

- The present study is the most comprehensive quantitative proteomic study of brain O-GlcNAcylation to date, leading to confident assignment of over one thousand O-GlcNAc sites from post-mortem human brains.
- The use of 6-plex TMT labeling with the pooled common reference strategy allowed for reliable large-scale quantification of O-GlcNAcylation in AD.
- The altered O-GlcNAcylated proteins in AD belong to several structural and functional categories, including synaptic proteins, cytoskeleton proteins, and memory-associated proteins.
- Identification of altered O-GlcNAc proteins such as ankyrin-3, SYNPO and QKI may open new avenues for investigating the molecular mechanisms of sporadic AD.

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References

1. Liu F, Iqbal K, Grundke-Iqbal I, Hart GW, & Gong C-X (2004) O-GlcNAcylation regulates phosphorylation of tau: a mechanism involved in Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* 101(29): 10804-10809.
2. Alfaro JF, *et al.* (2012) Tandem mass spectrometry identifies many mouse brain O-GlcNAcylated proteins including EGF domain-specific O-GlcNAc transferase targets. *Proc. Natl. Acad. Sci. U.S.A.* 109(19): 7280-7285.

Career Opportunities: For potential openings in the Integrative Omics Group at PNNL please visit http://omics.pnl.gov/careers

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