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 (N-α-acetylglucosamine) is a widespread dynamic protein modification.

• The link between O-GlcNAc and AD is emerging as an important topic¹.

• 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-MS².

• We developed a proteomic pipeline consisting of isobaric tandem mass tag (TMT) labeling, high-pH RPLC fractionation, and chemenzymatic photocleavage (CEPC) enrichment³ 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.

• Identification of altered O-GlcNAc proteins such as synaptophysin and protein quaking distinguishes many mouse brain O-GlcNAcylated proteins including EGF domain-specific O-GlcNAc transferase (EDEM) and PI3K/AKT, which are also increased in Alzheimer’s disease.

Methods

TMT-6 Labeling Scheme and Workflow

Chemical Principle of CEPC Enrichment

Results

Change of O-GlcNAc in AD

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

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

Figure 3. Quantification of the O-GlcNAc peptides in the human brain: Volcano plot of the O-GlcNAc peptides with >90% 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 blotting of brain homogenate, probed with antibodies against synaptopodin (SYNPO) or Protein quaking (QKI), respectively. [3H]PEG was used for loading control.

Figure 6. Game 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 synaptophysin and protein quaking may open new avenues for investigating the molecular mechanisms of sporadic AD.

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