Elucidating the role of O-GlcNAc glycosylation in neurobiology and neurodegeneration

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Elizabeth Hwang Jensen

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ABSTRACT

O-GlcNAc glycosylation is a dynamic, inducible post-translational modification (PTM) essential for neuronal homeostasis and found on proteins associated with neurodegenerative diseases such as α-synuclein, amyloid precursor protein, and tau. Intracellularly, O-GlcNAc modification is cycled by two enzymes in mammalian cells: O-GlcNAc transferase (OGT) appends O-GlcNAc to serine or threonine residues and O-GlcNAcase (OGA) removes O-GlcNAc. OGT modifies over 1000 different proteins, but the lack of a well-defined consensus sequence or substrate structural constraints has hampered efforts to predict sites a priori. Furthermore, the identification of O-GlcNAc modification sites has been obstructed by the difficulty of enriching and detecting O-GlcNAc using traditional biochemical methods. Here, we established and employed biological and chemical tools to illuminate the role of O-GlcNAc in neuronal function.

In Chapter 2, we sought to determine the role of O-GlcNAc in learning, memory, and neurodegeneration. Deletion of the OGT gene causes early postnatal lethality in mice, complicating efforts to study O-GlcNAc glycosylation in mature neuronal function and dysfunction. We demonstrated that the loss of OGT in the forebrain of adult mice (OGT cKO) leads to progressive neurodegeneration, including neuronal death, neuroinflammation, hyperphosphorylated tau, amyloidogenic Aβ-peptides, and memory deficits. In the hippocampus, we showed that OGT ablation lead to the upregulation of neuroinflammatory genes and the downregulation of cholesterol biosynthetic genes. Additionally, a gene network analysis (WGCNA), qPCR, and immunohistochemistry (IHC) revealed that loss of O-GlcNAc perturbed cell cycle progression in the hippocampal neurons. In the hippocampus, we identified increased neuroinflammatory gene transcription
in OGT cKO mice and both tau neurofibrillary tangle (NFT)-forming and amyloid-forming Alzheimer’s disease (AD) mouse models. However, only OGT cKO and NFT-forming mice displayed decreased synaptic gene expression, suggesting that NFT formation and OGT cKO compromise hippocampal synaptic transcription. These studies indicate that O-GlcNAcylation regulates pathways vital for the maintenance of neuronal health and suggest that dysfunctional O-GlcNAc signaling may be an important contributor to neurodegenerative diseases.

In order to understand the critical O-GlcNAc-mediated neuronal functions that underlie OGT cKO dysfunction, we next developed and utilized novel biological and chemical tools in order to identify key OGT interactors and substrates in the brain in Chapter 3. Due to the lack of a well-defined OGT substrate sequence and structural constraints, OGT is believed to obtain its substrate specificity through its interactome where specific interactors target OGT to specific substrates. In order to identify these interactors, we used CRISPR/Cas9 to generate a novel mouse with a minimally tagged OGT in order to identify the endogenous OGT brain interactome using tandem affinity purification and MS methods. The preliminary OGT brain interactome consisted of previously identified OGT interactors and substrates as well as novel interactors. The identified OGT interactors were enriched for ribosomal and cytoskeletal proteins in addition to axonal, dendritic, and neuronal cell body proteins, implicating OGT as a pivotal mediator of neuronal structure and function.

In addition to the OGT interactome, we sought to uncover OGT’s substrates or the O-GlcNAcome. We developed an improved approach to quantitatively label and enrich O-GlcNAcylated proteins for site identification. Chemoenzymatic labeling followed by Cu(I)-
catalyzed azide-alkyne cycloaddition (CuAAC) installed a new MS-compatible linker designed for facile purification and release of O-GlcNAcylated proteins for downstream MS analysis. We validated the approach by identifying several established O-GlcNAc sites on the proteins α-crystallin and OGT as well as discovering new, previously unreported sites on both proteins. Notably, these novel sites on OGT lie in key functional domains of OGT, underscoring how this site identification method can reveal important biological insights into protein activity and regulation.

Finally, in Chapters 4 and 5, we focus on the post-translational modification (PTM) code on a specific transcription factor (TF), CREB (cAMP response element binding protein). CREB regulates memory formation through its transcriptional control of neuronal metabolism, activity, differentiation, development, and survival. CREB phosphorylation at serine 133 has been previously shown to enhance CREB-mediated transcription while CREB glycosylation at serine 40 has been shown to decrease CREB-mediated transcription. However, the exact gene networks modulated by and potential interplay between CREB glycosylation and phosphorylation have not been explored. Through differential expression analysis with glycosylation-deficient (S40A) and phosphorylation-deficient (S133A) CREB mutants, we showed that CREB O-GlcNAcylation is important for neuronal activity and excitability while phosphorylation at serine 133 regulated the expression of genes involved in neuronal differentiation. Using WGCNA, we demonstrated that CREB O-GlcNAcylation at serine 40 and phosphorylation at serine 133 mediate mutually exclusive gene networks. The glycosylation-deficient mutant enhanced neuronal activity- and excitotoxicity-related gene networks while the phosphorylation-deficient mutant perturbed neuronal differentiation and amino and fatty acid metabolism-related
gene networks. Our work sheds light on the regulation of CREB through PTMs to modulate neuronal function and delineate the roles of O-GlcNAcylation and phosphorylation in modulating neuronal excitability and neuronal development and metabolism respectively. Altogether, these studies demonstrate that O-GlcNAc modification is a critical mediator of neuronal homeostasis and neurodegeneration.
PUBLISHED CONTENT AND CONTRIBUTIONS


E.H.J. participated in the execution of the experiments including the microarray, qPCR, IHC, and western blotting experiments, data analysis, and in the writing of the manuscript. The article, including figures, is reproduced in part within Chapter 2 with permission under the PNAS rights and permissions.


E.H.J. participated in the execution of the experiments including the peptide and protein chemoenzymatic labeling and preparation for mass spectrometry and in writing of the manuscript. The article, including figures, is reproduced in part within Chapter 6 with permission under a Creative Commons Attribution 3.0 Unported License.
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# NOMENCLATURE

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<td>A or Ala</td>
<td>alanine or adenosine</td>
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<tr>
<td>a.a.</td>
<td>amino acids</td>
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<tr>
<td>ACN</td>
<td>acetonitrile</td>
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<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>ALS</td>
<td>amyotrophic lateral sclerosis (a.k.a. Lou-Gehrig’s disease or motor neuron disease)</td>
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<tr>
<td>AMPA</td>
<td>anti-mycotic, anti-microbial</td>
</tr>
<tr>
<td>B</td>
<td>biotin/biotinylated</td>
</tr>
<tr>
<td>BCA</td>
<td>bicinechonic acid</td>
</tr>
<tr>
<td>BDNF</td>
<td>brain-derived neurotrophic factor</td>
</tr>
<tr>
<td>BEMAD</td>
<td>β-elimination Michael addition</td>
</tr>
<tr>
<td>BG</td>
<td>E11.5 basal ganglia</td>
</tr>
<tr>
<td>BM</td>
<td>bone marrow</td>
</tr>
<tr>
<td>Bp</td>
<td>base pair(s)</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>C or Cys</td>
<td>cysteine or cytosine</td>
</tr>
<tr>
<td>CBP</td>
<td>CREB-binding protein</td>
</tr>
<tr>
<td>CID</td>
<td>collision-induced dissociation</td>
</tr>
<tr>
<td>CNs</td>
<td>cortical neurons (either E15.5 or E16.5)</td>
</tr>
<tr>
<td>Cre</td>
<td>Cre recombinase</td>
</tr>
<tr>
<td>CRE</td>
<td>consensus sequence for CREB binding (Full site: TGACGTCA, Half site: TGAC/GTCA)</td>
</tr>
<tr>
<td>CREB</td>
<td>cAMP response element binding protein</td>
</tr>
<tr>
<td>CRISPR</td>
<td>clustered regularly interspaced short palindromic repeats</td>
</tr>
<tr>
<td>CuAAC</td>
<td>copper-catalyzed azide-alkyne cycloaddition</td>
</tr>
<tr>
<td>Cu(I)</td>
<td>copper (I), Cu⁺</td>
</tr>
<tr>
<td>CuSO₄</td>
<td>copper sulfate</td>
</tr>
<tr>
<td>DAPI</td>
<td>4’-6-diamidino-2-phenylindole</td>
</tr>
<tr>
<td>Dde</td>
<td>1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl</td>
</tr>
<tr>
<td>Ddv/ivDde</td>
<td>1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)isovaleryl</td>
</tr>
<tr>
<td>DE</td>
<td>differentially expressed</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s modified Eagle medium</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>dsDNA</td>
<td>double stranded DNA</td>
</tr>
<tr>
<td>DTT</td>
<td>dithiothreitol</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EGTA</td>
<td>ethylene glycol-bis(β-aminoethyl ether)tetraacetic acid</td>
</tr>
<tr>
<td>E#</td>
<td>embryonic day #</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>EOGT</td>
<td>EGF domain-specific O-linked N-acetylglucosamine transferase (extracellular)</td>
</tr>
<tr>
<td>ESC(s)</td>
<td>embryonic stem cell(s)</td>
</tr>
<tr>
<td>ETD</td>
<td>electron transfer dissociation</td>
</tr>
<tr>
<td>EtOH</td>
<td>ethanol</td>
</tr>
<tr>
<td>FBS</td>
<td>fetal bovine serum</td>
</tr>
<tr>
<td>FDR</td>
<td>false discovery rate</td>
</tr>
<tr>
<td>FLAG</td>
<td>protein tag with the sequence DYKDDDDK</td>
</tr>
<tr>
<td>FTD(P)</td>
<td>frontotemporal dementia (with Parkinsonism)</td>
</tr>
<tr>
<td>G or Gly</td>
<td>glycine or guanosine</td>
</tr>
<tr>
<td>Gal</td>
<td>galactose</td>
</tr>
<tr>
<td>GalN</td>
<td>galactosamine</td>
</tr>
<tr>
<td>GalNAc</td>
<td>N-acetylgalactosamine</td>
</tr>
<tr>
<td>GalNAz</td>
<td>N-azidoacetylgalactose</td>
</tr>
<tr>
<td>(Y289L) GalT</td>
<td>(Y289L) β-1,4-galactosyltransferase</td>
</tr>
<tr>
<td>gDNA</td>
<td>genomic DNA</td>
</tr>
<tr>
<td>GFP</td>
<td>green fluorescent protein</td>
</tr>
<tr>
<td>GlcNAc</td>
<td>N-acetylgalactosamine</td>
</tr>
<tr>
<td>GlcNAz</td>
<td>N-azidoacetylgalactosamine</td>
</tr>
<tr>
<td>HA</td>
<td>hemagglutinin tag (YPYDVPDYA)</td>
</tr>
<tr>
<td>HBSS</td>
<td>Hank’s buffered saline solution</td>
</tr>
<tr>
<td>HCD</td>
<td>higher-energy collisional dissociation</td>
</tr>
<tr>
<td>HD</td>
<td>Huntington’s disease</td>
</tr>
<tr>
<td>HDR</td>
<td>homology-directed repair/recombination</td>
</tr>
<tr>
<td>HEK293</td>
<td>human embryonic kidney cells</td>
</tr>
<tr>
<td>HEPES</td>
<td>4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid</td>
</tr>
<tr>
<td>HSV</td>
<td>replication-deficient herpes simplex virus</td>
</tr>
<tr>
<td>ICC</td>
<td>immunocytocchemistry</td>
</tr>
<tr>
<td>IF</td>
<td>immunofluorescence</td>
</tr>
<tr>
<td>IHC</td>
<td>immunohistochemistry</td>
</tr>
<tr>
<td>Ile (or I)</td>
<td>isoleucine</td>
</tr>
<tr>
<td>IRDye800</td>
<td>infrared dye 800</td>
</tr>
<tr>
<td>Leu (or L)</td>
<td>leucine</td>
</tr>
<tr>
<td>IgG</td>
<td>immunoglobulin domain G</td>
</tr>
<tr>
<td>IgM</td>
<td>immunoglobulin domain M</td>
</tr>
<tr>
<td>©KO</td>
<td>(conditional) knockout</td>
</tr>
<tr>
<td>IncRNA</td>
<td>long-noncoding ribonucleic acid</td>
</tr>
<tr>
<td>LTM</td>
<td>long term memory</td>
</tr>
<tr>
<td>LTQ</td>
<td>linear trap quadrupole</td>
</tr>
<tr>
<td>LSM</td>
<td>laser scanning microscope</td>
</tr>
<tr>
<td>LV</td>
<td>lentivirus</td>
</tr>
<tr>
<td>LWAC</td>
<td>lectin weak affinity chromatography</td>
</tr>
<tr>
<td>MAPK</td>
<td>mitogen-activated protein kinase</td>
</tr>
<tr>
<td>MEF(s)</td>
<td>mouse embryonic fibroblast(s)</td>
</tr>
<tr>
<td>MeOH</td>
<td>methanol</td>
</tr>
</tbody>
</table>
Met (or M) methionine
MS mass spectrometry
NaAsc sodium ascorbate
NFTs neurofibrillary tangles
NHEJ non-homologous end joining
NMR nuclear magnetic resonance
NPCs neural progenitor cells
nt nucleotide
O-GlcNAc O-linked N-acetylglucosamine
OGA O-GlcNAcase
(s)OGT (short isoform of) O-GlcNAc transferase
PAM protospacer motif
PARP1 poly-ADP ribose polymerase 1
PBS(T) phosphate buffered saline (Tween 20)
PC photocleavable
PC12 pheochromocytoma 12 (cell line)
PCR polymerase chain reaction
PD Parkinson’s disease
polyethylene glycol
PEG phenylalanine
Phe (or F) phenylalanine
PI3K phosphatidylinositol 3-kinase
P/S penicillin/streptomycin
PSP progressive supranuclear palsy
PTM post-translational modification
PUGNAc O-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino-N-phenylcarbamate
qRT-PCR (qPCR) quantitative reverse transcription-polymerase chain reaction
RapiGest sodium 3-[(2-methyl-2-undecyl-1,3-dioxolan-4-yl)methoxy]-1-propanesulfonate
ribonucleic acid
mRNA messenger ribonucleic acid
rRNA ribosomal ribonucleic acid
RNAi RNA interference
RT room temperature
siRNA short interfering RNA
SCX strong cation exchange
SDS sodium dodecyl sulfate
SDS-PAGE sodium dodecyl sulfate protein acrylamide gel electrophoresis
S.E.M standard error of the mean
Ser (or S) serine
ssODN single-stranded oligonucleotide
STM short term memory
TAE Tris buffered saline (pH 8.3)
TBS(T) Tris buffered saline (Tween 20)
TFA trifluoroacetic acid
THPTA: tris(3-hydroxypropyltriazolylmethyl)amine
Thr (or T): threonine
TNFα: tumor necrosis factor alpha
Trp (or W): tryptophan
Tyr (or Y): tyrosine
UDP: uridine diphosphate
(3’ or 5’)UTR: (3’ or 5’) untranslated region of RNA
UV: ultraviolet
(s)WGA: (succinylated) wheat germ agglutinin
Wnt: wingless-type MMTV integration site family member