

Identification of Nutrient Sensitive Signaling Pathways via Analysis of O-GlcNAcylation

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Abstract : The majority of glucose metabolism proceeds through glycolytic pathways such as glycolysis or pentose phosphate pathway, however, about 5% is shunted through the hexosamine biosynthetic pathway, producing uridine diphosphate N-acetyl glucosamine (UDP-GlcNAc). This precursor can then be incorporated into complex oligosaccharides decorating the cell surface or remain as an intracellular post-translational-modification (PTM) of serine/threonine residues (O-GlcNAcylation, OGN), which has been identified on over 4,000 cytosolic or nuclear proteins. Intracellular OGN has major implications on cellular processes, typically by modulating protein localization, protein-protein interactions, protein degradation, and gene expression. Additionally, OGN is known to have an extensive cross-talk with phosphorylation, be in a competitive or cooperative manner. Unlike other PTMs there are only two cycling enzymes that are capable of adding or removing the GlcNAc moiety, O-linked N-acetyl glucosamine Transferase (OGT) and O-linked N-acetyl glucosamidase (OGA), respectively. The activity of OGT has been shown to be sensitive to cellular UDP-GlcNAc levels, even changing substrate affinity. Owing to this and that the concentration of UDP-GlcNAc is related to the metabolisms of glucose, amino acid, fatty acid, and nucleotides, O-GlcNAc is often referred to as a nutrient sensing rheostat. Indeed OGN is known to regulate several signaling pathways as a result of nutrient levels, such as insulin signaling. Dysregulation of OGN is associated with several disease states such as cancer, diabetes, and neurodegeneration. Improvements in glycomics over the past 10-15 years has significantly increased the OGT substrate pool, suggesting O-GlcNAc's involvement in a wide variety of signaling pathways. However, O-GlcNAc's role at the receptor level has only been identified in a case-by-case basis of known pathways. Examining the OGN of the plasma membrane (PM) may better focus our understanding of O-GlcNAc-effected signaling pathways. In this current study, PM fractions were isolated from several cell types via ultracentrifugation, followed by purification and MS/MS analysis in several cell lines. This process was repeated with or without OGT/OGA inhibitors or with increased/decreased glucose levels in media to ascertain the importance of OGN. Various pathways are followed up on in more detailed studies employing methods to localize OGN at the PM specifically.

Keywords : GlcNAc, nutrient sensitive, post-translational-modification, receptor

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