Quantitative Proteomic Analysis of Altered O-GlcNAcylation During the Metaphase to Anaphase Transition

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Abstract

Post-translational modifications are essential in regulating the function, stability, and localization of proteins. One such modification is the O-GlcNAc modification. O-GlcNAc is the attachment of a single N-acetylglucosamine moiety to serine and threonine residues on nuclear and cytoplasmic proteins. O-GlcNAc is dynamically cycling on Ser/Thr residues and is often reciprocal with phosphorylation. The modification is dynamically processed on proteins by the enzymes O-GlcNAc transferase (O-GlcNAc addition) or O-GlcNAcase (O-GlcNAc removal). O-GlcNAc is highly abundant PTM (>1700 identified proteins), but is difficult to study when compared to phosphorylation due to inadequate and limited tools. Our objective is to explore enrichment of O-GlcNAc modified peptides during mitosis. We employed SILAC (Stable Isotope Labeling of Amino Acids in Cell culture) labeling to quantitate differences in cells with either an O-GlcNAc transferase or O-GlcNAcase gain of function. Next, these samples were combined, proteolytically digested, fractionated to enrich for O-GlcNAc and compared to phosphorylation. By mass spectrometry analysis, we have now identified numerous proteins and post-translational modifications altered by aberrant O-GlcNAcylation. Using multiple bioinformatic and systems biology tools allowed a comprehensive analysis of the interplay between multiple post-translational modifications providing valuable insight into how these modifications control cellular functions such as mitotic progression with implications to cancer, type II diabetes and cardiovascular disease. This project was funded by NIH-NCRR grants P41 RR010888/ GM104603, S10 RR015942, S10 RR020946, S10 RR025082 and NIH-NHLBI contract N01 HV00239.