Chemo-genetics Define a Role for O-GlcNAcylation in Regulating Autophagy

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Abstract

The modification of intracellular proteins by the monosaccharide O-linked N-acetylglucosamine (O-GlcNAc) was first described in 1984. Found on more than 1,000 proteins localized to the nucleus, cytoplasm, and mitochondria, O-GlcNAc is thought to regulate proteins in a manner analogous to protein phosphorylation. Since it is added and removed by the enzymes which add and remove O-GlcNAc, O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), respectively, or in response to various intracellular stimuli. Over 1,000 proteins localized to the nucleus, cytoplasm, and mitochondria, O-GlcNAc is thought to regulate proteins in a manner analogous to protein phosphorylation. As prolonged expression of these proteins is ultimately toxic, we have generated a series of inducible constructs. We have focused on a method on induction regulated post-transcriptionally, we have also shown that the O-GlcNAcase activity is regulated by dynamic O-linked GlcNAc levels in the cell by modulating the expression of OGT and O-GlcNcase, the enzymes which add and remove O-GlcNAc. As a result, we have generated a series of inducible constructs, allowing us to assess the effect of O-GlcNAc levels on the induction of autophagy.

Chemo-genetics

Why Chemo-Genetics?

Common methods for modulating the expression of proteins such as viral transduction, RNAi, and transient transfection result in the induction of autophagy. To avoid this non-specific induction we have aimed to make cells which stably express OGT and O-GlcNcase. As prolonged expression of these proteins is ultimately toxic, we have generated a series of inducible constructs. We have focused on a method on induction regulated post-transcriptionally, we have also shown that the O-GlcNAcase activity is regulated by dynamic O-linked GlcNAc levels in the cell by modulating the expression of OGT and O-GlcNcase, the enzymes which add and remove O-GlcNAc. As a result, we have generated a series of inducible constructs, allowing us to assess the effect of O-GlcNAc levels on the induction of autophagy.

My Strategy

We have generated a number of constructs with which we can fine tune O-GlcNAc levels in the cell by modulating the expression of OGT and O-GlcNcase, the enzymes which add and remove O-GlcNAc. As a result, we have generated a series of inducible constructs, allowing us to assess the effect of O-GlcNAc levels on the induction of autophagy. We have also demonstrated that when you increase the levels of O-GlcNAc transferase (OGT) and thiamin G (TMG), you see the induction of autophagy. This data suggest that stress-induced O-GlcNAcylation may promote stress-induced autophagy. Future directions will focus on identifying the mechanisms by which OGT and O-GlcNcase regulate autophagy. We will start by examining the effect of modulating O-GlcNcase phosphorylation of Unc1, a key regulatory protein in the induction of autophagy. Other experiments will include using a catalytically dead OGT and O-GlcNcase to demonstrate that is the action of these proteins, rather than their protein-protein interactions, which regulate autophagy. Finally we would like to assess the importance of autophagy to O-GlcNAc-mediated cellular protection. To do this we will utilize small molecular inhibitors of autophagy (3MA) and a dominant-negative ATG5.

Relevant References

1. Taylor et al., Gene Therapy. 2007 14(3):R161-77
2. Li et al., Circ Res 2005 97(5):599-605
6. Zachara et al., Circu. 2007 100(5):1054-61