Defining the mechanism of O-GlcNAc signaling in stress-induced autophagy and cardioprotection.
The Department of Biological Chemistry at the Johns Hopkins University School of Medicine, Baltimore, MD 21205. kfahie1@jhmi.edu

Ischemic heart disease causes significant morbidity and mortality worldwide. Therapies for treating myocardial infarction and ischemia reperfusion injury (IR/I) have remained elusive. Current research demonstrates that the dynamic modification of intracellular proteins by O-linked β-N-acetylglucosamine (O-GlcNAc) confers cardioprotection in models of IR/I. However the molecular mechanism(s) by which O-GlcNAc promotes cardiac survival are unknown. In this study we investigated the hypothesis that O-GlcNAc regulates the cardioprotective process of autophagy. As many mammalian expression technologies (RNAi, viral transduction) induce autophagy, we generated a series of tools that facilitate conditional control of cellular O-GlcNAcylation. Utilizing destabilizing domain technology, we generated chemically inducible constructs that regulate the protein levels of the enzymes that catalyze the addition and removal of O-GlcNAc, the O-GlcNAc transferase (OGT) and the O-GlcNAcase. These constructs and their catalytic mutants have been stably transfected into cells and characterized. We show that enhancing global O-GlcNAcylation increases autophagic flux, whereas reducing O-GlcNAc levels, via O-GlcNAcase overexpression, suppresses peroxide-induced autophagy. Consistent with these observations, we demonstrated that AMP kinase signaling, which promotes autophagy, is upregulated when O-GlcNAc levels are increased pharmacologically. To tease out the mechanisms by which O-GlcNAc regulates autophagy we assessed the O-GlcNAc-modification state of key regulators of autophagy. AMPK, ULK1 and p62/sequestosome are O-GlcNAc modified or associate with O-GlcNAc modified proteins. Importantly, AMPK appears to be directly O-GlcNAc modified in a stress-dependent manner and associates with OGT. These data suggest that O-GlcNAc may regulate autophagy by modifying and regulating AMPK directly. Together, our data suggests that O-GlcNAc regulates numerous points in the autophagic pathway and may promote cardioprotection through the upregulation of autophagy.