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Inflammation Modulates the Activation of Autophagy in C2C12 Myotubes

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Abstract

Autophagy is a cellular housekeeping process by which proteins and damaged organelles are targeted for engulfment by autophagosomes ultimately culminating in degradation within the lysosomes. Chronic inflammation has been shown to disrupt normal physiological homeostasis leading to increased risk of cardiovascular disease, type 2 diabetes mellitus and muscle atrophy. The purpose of this study was to investigate whether inflammation disrupts the activation of autophagy in C2C12 myotubes. Myoblasts were grown in 10% fetal bovine serum supplemented DMEM until confluent and then differentiated for 5 d in 2% FBS-DMEM. Autophagy was monitored by Western blotting for changes in p62/sequestosome 1 (p62/sqstm1) and in the lipidation status of microtubule-associated protein light chain 3 (LC3) where the protein separates into LC3-II and LC3-I by electrophoresis. Changes in the ratio of LC3-II to LC3-I represent a method to monitor initiation of autophagy. Rapamycin (25-50 nM) for 48 hr successfully initiated autophagy as observed by a decrease in p62/sqstm1 and increase in LC3-II/LC3-I. Incubation of cells with lipopolysaccharide (LPS) to activate the inflammatory process for 48 hr reduced cell diameter and this decrease was partially attenuated by rapamycin (50 nM). LPS disrupted the activation of autophagy by rapamycin. Exposure to LPS for 48 hr increased p62/sqstm1 levels, but this was abolished with co-incubation of LPS and rapamycin; however, LC3-II/I was enhanced by co-incubation with LPS and rapamycin. To further investigate the components of the inflammatory process, cells were exposed to interleukin-6 (IL-6). Acute IL-6 exposure for 3 hr at 10 ng/mL or 50 ng/mL modulated basal autophagy as observed by an increase in the ratio LC3-II/LC3-I. In addition, chronic IL-6 exposure disrupted the initiation of autophagy by rapamycin. B-cell lymphoma 2 (Bcl-2) associated athanogene 3 (Bag3) is a molecular chaperone that plays a role in shuttling proteins to the proteasome but also in targeting proteins for degradation through autophagy. We observed that in our experiments Bag3 levels decreased concurrently with rapamycin treatment, but frequently decreased as p62 increased when exposed to LPS, and vice versa. Furthermore, Bag3 co-localizes with LC3 in primary hepatocytes further indicating a role for Bag3 in the targeted autophagy process. In conclusion, autophagy is disrupted by components of the inflammatory process in C2C12 myotubes. Future investigation may include the protective influence of autophagy in the process of atrophy.

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