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Article

Autophagy Impairment in Muscle Induces Neuromuscular Junction Degeneration and Precocious Aging

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SUMMARY

The cellular basis of age-related tissue deterioration remains largely obscure. The ability to activate compensatory mechanisms in response to environmental stress is an important factor for survival and maintenance of cellular functions. Autophagy is activated both under short and prolonged stress and is required to clear the cell of dysfunctional organelles and altered proteins. We report that specific autophagy inhibition in muscle has a major impact on neuromuscular synaptic function and, consequently, on muscle strength, ultimately affecting the lifespan of animals. Inhibition of autophagy also exacerbates aging phenotypes in muscle, such as mitochondrial dysfunction, oxidative stress, and profound weakness. Mitochondrial dysfunction and oxidative stress directly affect acto-myosin interaction and force generation but show a limited effect on stability of neuromuscular synapses. These results demonstrate that age-related deterioration of synaptic structure and function is exacerbated by defective autophagy.

INTRODUCTION

Aging is accompanied by numerous tissue phenotypes. Changes that occur in postmitotic organs are considered hallmarks of aging. A common feature of aged organisms is functional alteration and gradual loss of muscle mass and force. A deficit of muscle innervation due to motor-neuron loss is believed to have a major contribution to age-dependent muscle wasting, also called sarcopenia. This age-acquired deficit in muscles contributes profoundly to reduced quality of life in elderly people and predisposes them to an increased risk of morbidity, disability, and mortality (Visser and Schaap, 2011). The etiology of sarcopenia is multifaceted and involves several intrinsic and extrinsic factors. Despite the clinical, social, and economic relevance of sarcopenia, the precise mechanisms for the age-related loss of muscle mass and function are not yet fully understood. Age-related changes in muscle are complex, with key features including myofiber atrophy, profound weakness that is partially independent of muscle mass loss, myofiber degeneration, accumulation of dysfunctional mitochondria, and increased oxidative stress. Until recently, it was thought that age-associated atrophy and weakness were secondary to motor-neuron loss in the brain or in the spinal cord. However, this hypothesis has been recently challenged. In fact, little neuronal death occurs in most areas of the aging nervous system, and there is no decline of lower motor neurons during aging (Chai et al., 2011; Morrison and Hof, 1997). Conversely, it is emerging that neuromuscular junctions (NMJs) and their interactions with myofibers are greatly altered during aging, resulting in a loss of muscle innervation (Chai et al., 2011; Valdez et al., 2010). Oxidative stress and decreased release of trophic factors are independent influences on NMJ integrity and contribute to denervation (Jang et al., 2010, 2012; Jang and Van Remmen, 2011). However, to date, it is not known whether myofiber denervation is due to deleterious changes in muscle cells themselves, in neurons, or both.

Two lifestyle adaptations, caloric restriction and exercise (Jang et al., 2012; Valdez et al., 2010), have been consistently demonstrated to extend lifespan and, in parallel, to mitigate

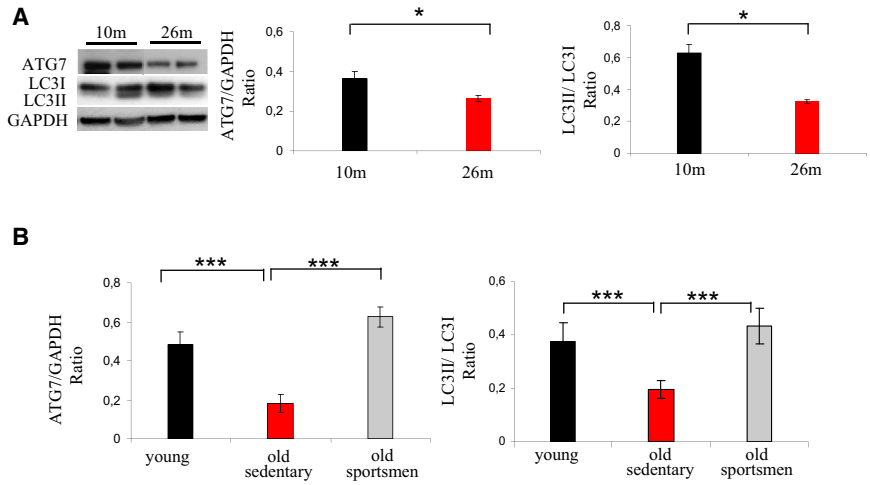

Figure 1. Autophagy Is Impaired during Aging

(A) Representative immunoblotting for the critical E1-like enzyme, ATG7, LC3II, and LC3I on muscle extracts from gastrocnemius of 10- and 26-month-old mice. The graphs show the quantification of ATG7 protein and the LC3II/LC3I ratio. Values are mean ± SEM; n = 4 of each condition; *p < 0.05.

(B) Aging reduces whereas exercise maintains expression of ATG7 and LC3 lipidation in humans. The graphs show the quantification of ATG7 protein and LC3II/LC3I ratio revealed by immunoblotting on muscle biopsies. Values are mean ± SEM; n = 6 young; n = 10 elderly, sedentary men; and n = 4 senior sportsmen; ***p < 0.0001.

age-related alterations of NMJ. Macroautophagy, hereafter named autophagy, is activated by both of these conditions in skeletal muscles and other tissues (Grumati et al., 2010, 2011a, 2011b; He et al., 2012; Rubinsztein et al., 2011; Wohlgemuth et al., 2010). Autophagy is a highly conserved homeostatic mechanism used for the degradation and recycling of bulk cytoplasm, long-lived proteins, and organelles through the lysosomal machinery (Mizushima and Komatsu, 2011). The autophagy machinery generates double membrane vesicles (autophagosomes) that engulf and sequester target cellular components (Mizushima and Komatsu, 2011). Autophagosomes are then delivered to and fuse with lysosomes to degrade their contents. Therefore, autophagy can be defined as a process of cytosolic renovation. In mammals, the relationship between autophagy inhibition and aging is still largely phenomenological and correlative. Conversely, robust genetic evidence in worms and flies clearly demonstrates this relationship. Deficient expression of ATG1, ATG7, ATG8, BECN1 (Beclin1), and SESTRIN1 (which is also required for basic autophagy) shortens the lifespan of the fruit fly *D. melanogaster* and of the nematode *C. elegans* (Lee et al., 2010b; Rubinsztein et al., 2011; Simonsen et al., 2008). On the other hand, increased autophagy contributes to longevity, and mutation of essential Atg genes prevents the gain of longevity (Meléndez et al., 2003). A recent report shows that aging can be controlled by a single tissue; overexpression of FOXO transcription factor or its target EIF4EBP1 in fly skeletal muscle abolishes the age-associated decline in autophagy and increases longevity (Demontis and Perrimon, 2010). We previously identified FOXO3A as a regulator of autophagy (Mammucari et al., 2007, 2008; Zhao et al., 2007), and genetic variations in the human FOXO3A have been linked to longevity in multiple population studies (Kenyon, 2010). The role of autophagy in sarcopenia in mammals is still controversial. Both impaired and excessive autophagy have been associated with aging sarcopenia (Wenz et al., 2009; Wohlgemuth et al., 2010). However, loss-of-function experiments that support the involvement of autophagy in aging sarcopenia are still lacking. Here, we show that specific inhibition of autophagy in skeletal muscle leads to abnormalities in motor neuron synapses that ultimately lead to denervation whereas accumulation of dysfunctional mitochondria triggers oxidation of contractile elements, causing a decrease in force generation.

RESULTS

Autophagy Declines during Aging

Age-related muscle loss is believed to result from a general decline of protein synthesis or an enhancement of protein breakdown. To study autophagy in aging, we monitored the expression of autophagy markers, such as LC3 (MAP1LC3A) and the critical E1-like enzyme, ATG7, in mice and humans. Aged mice showed a decline of ATG7 protein and, concurrently, of LC3 lipidation when compared to young animals (Figure 1A). Then we tested whether this decrease of the autophagy system occurs in humans. An important decrease of ATG7 protein and LC3II was found in muscle biopsies of elderly sedentary (sarcopenic) subjects (Figure 1B). Because exercise activates autophagy and counteracts most age-related characteristics, including the decline of muscle mass (Zampieri et al., 2014), we tested whether long-life regular exercise in humans was able to prevent the reduction of these autophagy markers. ATG7 protein and LC3II were maintained in muscle biopsies of senior sportsmen that had been previously shown to have better muscle mass and strength than elderly sedentary subjects (Zampieri et al., 2014).

Autophagy Inhibition Affects Neuromuscular Synaptic Morphology and Function

To mimic the decrease of ATG7 in aging, we characterized muscle-specific autophagy-deficient ($Atg7^{-/-}$) mice that we have generated (Masiero et al., 2009). The absence of autophagy caused a significant reduction of animal survival (Figure 2A). Morphological analyses of aged mice showed higher levels of atrophy, center-nucleated fibers, and inflammation in $Atg7^{-/-}$ muscles compared to age-matched controls (Figures 2B and S1A). Fiber size was affected in $Atg7^{-/-}$, and fibers displayed great variability in dimension. Some fibers atrophied to a level that made them hardly detectable (Figure 2C). These small fibers did not express the typical markers of regeneration