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Review

Autophagy in skeletal muscle

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ABSTRACT

Muscle mass represents 40–50% of the human body and, in mammals, is one of the most important sites for the control of metabolism. Moreover, during catabolic conditions, muscle proteins are mobilized to sustain gluconeogenesis in the liver and to provide alternative energy substrates for organs. However, excessive protein degradation in the skeletal muscle is detrimental for the economy of the body and it can lead to death. The ubiquitin-proteasome and autophagy-lysosome systems are the major proteolytic pathways of the cell and are coordinately activated in atrophying muscles. However, the role and regulation of the autophagic pathway in skeletal muscle is still largely unknown. This review will focus on autophagy and discuss its beneficial or detrimental role for the maintenance of muscle mass.

1. Introduction

Skeletal muscles are the body's agent of motion. In order to allow movement, the organization of muscle cells is highly structured so as to generate and sustain mechanical tension. The cytosol of myofibers is packed with contractile proteins that are assembled into repetitive structures, the basal unit of which is constituted by the sarcomere. Filaments of myosins are at the center of the sarcomere while filaments of actins are at the periphery. Several sarcomeres, arranged in register and surrounded by sarcoplasmic reticulum, form myofibrils. Various organelles such as mitochondria, for ATP generation, and sarcoplasmic reticulum, for calcium release are embedded among the myofibrils. This ordered assembly of contractile proteins and organelles differs from the cytosolic organization of the other cells of our body where mitochondria, endoplasmic reticulum and proteins move freely within the cytosol. Moreover, the myofiber is an enormous cell that can measure several centimeters in length and can contain hundreds of nuclei. It is therefore particularly difficult to visualize local as well as subtle but diffuse changes of mitochondrial and endo/sarcoplasmic reticulum networks as well as the dynamics of endocytic/exocytic vesicles in such rigid and long structures. Several changes occur during catabolic conditions: proteins are mobilized, mitochondrial and sarcoplasmic networks are remodeled and myonuclei are lost. In addition, the daily contractions can mechanically and metabolically damage/alter muscle proteins and organelles. For example, physical exercise requires energy whose production in mitochondria can also generate reactive oxygen species (ROS) that can have deleterious effects on many cellular components. Muscle cells therefore require an efficient system for removing and eliminating unfolded and toxic proteins as well as abnormal and dysfunctional organelles. The autophagy system is responsible for this action, generating double membrane vesicles that engulf portion of cytoplasm, organelles, glycogen and protein aggregates [1,2]. Autophagosomes are then delivered to lysosomes for degradation of their contents. Despite this important function, the role of autophagy in the control of muscle mass has only recently begun to be investigated.

2. Autophagy and muscle loss

Loss of muscle mass occurs in many conditions ranging from denervation, inactivity, microgravity, fasting to a multitude of systemic diseases such as cancer, sepsis, AIDS, diabetes, cardiac and renal failure [3]. In all of these catabolic conditions protein breakdown is enhanced and exceeds protein synthesis resulting in myofiber atrophy [4]. The activation of the major proteolytic

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systems requires a transcription-dependent program. Comparing gene expression in different models of muscle atrophy lead to the identification of a subset of genes that are commonly up- or down-regulated in atrophying muscle [5–8]. These common genes are thought to regulate the loss of muscle components and were thus designated atrophy-related genes or atrogenes [4,9]. Among these genes there are several belonging to the autophagy-lysosome system. Importantly, two autophagy genes, LC3 and Gabarap, are among the upregulated atrogenes which encode for proteins that are degraded when autophagosomes fuse with lysosomes [10–12]. Together, these data strongly suggest that autophagic flux is increased during atrophy and that this increase requires a transcriptional regulation to replenish components that are lost. Indeed, previous evidence has shown that lysosomal degradation contributes to protein breakdown in denervated muscle [13,14]. However, advances in the field were particularly slow perhaps owing to the initial difficulties of autophagosome detection within the complex structure of the myofiber. Development of biochemical and imaging tools to follow autophagosome formation greatly improved the characterisation of autophagy in atrophying muscles [15]. Mizushima et al. generated transgenic mice expressing LC3 fused with GFP [16]. LC3 is the mammalian homolog of the yeast Atg8 gene and is critical for membrane commitment and growth [17]. Activation of autophagy can be now easily visualized by the appearance of bright GFP-positive spots within myofibrils and beneath the plasma membrane of myofibers. Morphological analyses demonstrate the activation of the autophagy system during fasting in skeletal muscle. This animal model is also extremely useful for comparing the size of autophagosomes in different tissues under basal and fasted conditions. Interestingly, fasted skeletal muscle shows the smallest vesicles when compared to autophagosomes of liver, heart and pancreatic acinar cells [16]. Thus, the small size of autophagosomes could be another important aspect that, in the past, had limited the detection of autophagosomes in muscle. By using these tools we have only recently begun to unravel the contribution of autophagy to muscle loss. For example it is now known that myofiber atrophy induced in vivo by overexpression of constitutively active FoxO3 requires autophagy. Knocking down the critical gene LC3 by RNAi partially prevents FoxO3-mediated muscle loss [10]. Other genetic models have also confirmed the role of autophagy during muscle atrophy. Oxidative stress, induced by expression of mutant $SOD1^{G93A}$ specifically in skeletal muscle, causes muscle atrophy and weakness mainly via autophagy activation. The reduction of autophagic flux by expressing shRNAs against LC3 spares muscle mass in $SOD1^{G93A}$ transgenic mice [18]. However, since the preservation of muscle mass was studied by morphological observations, we do not know whether this protection is also functional and therefore, beneficial for preserving muscle force. This aspect would be important to address in the next years for the development of appropriate therapeutic approaches against weakness. Increased oxidative stress has also been reported to occur during denervation and hindlimb suspension. During these disuse conditions neuronal NOS (nNOS) moves from the sarcolemma, where it is bound to the dystrophin-glycoprotein complex, to the cytosol. Free cytosolic nNOS induces oxidative stress and enhances FoxO3-mediated transcription of the atrophy-related ubiquitin ligases, atrogin 1 and MuRF1, causing muscle loss [19]. A similar mechanism has been recently described in another model of muscle atrophy. When DHPR, a L-type Ca^{2+} channel, is reduced in skeletal muscles of adult animals by RNAi, it also triggers atrophy via nNOS relocalization and FoxO3 activation. However, in this genetic model of muscle atrophy, the genes that are upregulated by FoxO3 are the autophagy genes LC3, VPS34, Bnip3 and the lysosomal enzyme cathepsin L. Morphological studies of LC3 immunolocalization and ultrastructural observations by electron microscopy confirm the induction of autophagosomes when DHPR is reduced [20].

Recent data suggest that autophagy may also contribute to sarcopenia, the excessive loss of muscle mass that occurs in the elderly [21]. During ageing there is also a progressive deterioration of mitochondrial function and activation of autophagy. Forced expression of PGC1α, the master gene of mitochondrial biogenesis, in skeletal muscles of old mice ameliorates loss of muscle mass and prevents the age-related increase of autophagy [22]. Thus, autophagy activation has been reported in acute conditions of muscle loss as well as in chronic and long-lasting situations of muscle debilitation and weakness.

Interestingly, recent findings suggest that autophagy activation might be critical not only for protein breakdown and muscle atrophy but also for myofiber survival. In fact mutations that inactivate Jumpy, a phosphatase that counteracts the action of VPS34 for autophagosome formation and reduces autophagic flux, is associated with centronuclear myopathy [23]. Therefore unbalanced autophagy might be the pathogenic mechanism that causes positional alteration of myonuclei and, consequently, triggers myofiber degeneration. Together, these findings strongly suggest that excessive autophagy, similar to the ubiquitin-proteasome system, is detrimental to muscle mass.

3. Autophagy and muscle mass maintenance

Autophagosomes have been found in almost every myopathy and dystrophy studied so far and are characteristic of a group of muscle disorders named Autophagic Vacuolar Myopathies (AVM) [24]. However, it is unclear whether autophagy is detrimental and part of the mechanisms that induce muscle degeneration or whether it is a compensatory mechanism for cell survival. The features of protein aggregation, abnormal mitochondria and distension of endo/sarcoplasmic reticulum that are typical of many acquired and genetic muscle diseases suggest an impairment, more than an exacerbation, of autophagic flux. For instance, protein aggregates that are positive for ubiquitin and p62/SQSTM1 proteins have been recently described in muscle of patients affected by sporadic Inclusion Body Myositis as well as in different tissue-specific autophagy knockout mice [25–27]. To clarify the role of basal autophagy we have generated two conditional knockout mice for the critical Atg7 gene to block autophagy specifically in skeletal muscle [28]. The expectation was to preserve muscle mass and eventually to gain more contractile proteins and to improve muscle strength. Surprisingly, suppression of autophagy is not beneficial and instead triggers atrophy, weakness and several features of myopathy. A similar atrophic phenotype has also been observed in muscle-specific Atg5 knockout mice, another genetic model to block autophagy [29]. Deletion of Atg7 gene causes accumulation of protein aggregates, appearance of abnormal mitochondria and of concentric membranous structures that assemble between the myofibrils or just beneath the sarcolemma, induction of oxidative stress and activation of Unfolded Protein Response. Together, these pathological conditions lead to myofiber degeneration [28] (Fig. 1). Another interesting aspect is the accumulation of polyubiquitinated proteins in detergent soluble and insoluble fractions of autophagy-null muscles. This finding has been reported in other tissue-specific autophagy knockout mice which also show an increase of polyubiquitinated proteins and accumulation of concentric membranous structures [26,27,30,31]. These observations, together with the evidence that proteasome activity is not seriously impaired in Atg7 null muscles, suggest that some ubiquitinated proteins are specifically targeted to lysosomal degradation via autophagy. We actually do not know which signals establish whether polyubiquitinated proteins are degraded via proteasome