Do neurogenic and cancer-induced muscle atrophy follow common or divergent paths?

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Abstract

Skeletal muscle is a dynamic tissue capable of responding to a large variety of physiological stimuli by adjusting muscle fiber size, metabolism and function. However, in pathological conditions such as cancer and neural disorders, this finely regulated homeostasis is impaired leading to severe muscle wasting, reduced muscle fiber size (atrophy), and impaired function. These disease features develop due to enhanced protein breakdown, which relies on two major degradation systems: the ubiquitin-proteasome and the autophagy-lysosome. These systems are independently regulated by different signalling pathways, which in physiological conditions, determine protein and organelle turnover. However, alterations in one or both systems, as it happens in several disorders, leads to enhanced protein breakdown and muscle atrophy. Although this is a common feature in the different types of muscle atrophy, the relative contribution of each of these systems is still under debate. Here, we will briefly describe the regulation and the activity of the ubiquitin-proteasome and the autophagy-lysosome systems during muscle wasting. We will then discuss what we know regarding how these pathways are involved in cancer induced and in neurogenic muscle atrophy, highlighting common and divergent paths. It is now clear that there is no one unifying common mechanism that can be applied to all models of muscle loss. Detailed understanding of the pathways and proteolysis mechanisms involved in each model will hopefully help the development of drugs to counteract muscle wasting in specific conditions.

Key Words: muscle atrophy, cancer cachexia, neurogenic muscle atrophy, ubiquitin-proteasome, autophagy.

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Skeletal muscle is a highly dynamic tissue that modifies its size to respond and adapt to a large variety of stimuli. In physiological conditions, a balance between the biosynthetic and catabolic systems maintains muscle mass. Stimuli, which alter the homeostatic balance determine the predominance of one system over the other, leading to either muscle hypertrophy or atrophy. Skeletal muscle atrophy occurs in a variety of pathological settings, including disuse, cachexia, denervation or diabetes and it is characterized by a decrease in myofiber size, mainly due to loss of organelles, cytoplasm, and proteins, and a reduction in muscle function.[1] A major contribution in understanding muscle atrophy came from studies on gene expression profiling performed independently by the groups of Goldberg and Glass, in the late 20th century. By comparing gene expression profiles in different models of muscle atrophy they identified a group of genes that are commonly up- or down-regulated in atrophying muscle. The commonly up- or down-regulated genes were believed to be important for loss of muscle components and were called atrophy-related genes or "atrogenes".[2,3] Together, these findings marked a major advance in the field of muscle wasting and indicated that muscle atrophy is an active process controlled by specific signalling pathways and transcriptional programs. Among the identified genes, several belong to the major cellular degradation systems, the ubiquitin-proteasome (UPS) and the autophagy-lysosome. Both were found to be primarily regulated by the phosphoinositide 3-phosphate (PI3K)-AKT-mammalian target of rapamycin (mTOR) signalling pathway, which promotes protein synthesis, while suppressing protein degradation, and by the FOXO transcription factors, which can directly induce the transcription of both autophagy- and UPS-related genes.[4–7] Upon

phosphorylation by AKT, FOXO is retained in the cytoplasm, and becomes inactive.[8] Among the FOXO family members, FOXO-1 and -3 appear to be mostly involved in muscle wasting, since they are activated in all types of atrophy and both induce members of the UPS and autophagy machineries. Their activity is tightly regulated at post-transcriptional level: phosphorylation by AKT, deacetylation by sirtuin1, ubiquitylation, or binding to JunB or to PGC1α inhibits FOXO3 activity, preventing muscle wasting.[4,9–13] On the other hand, phosphorylation by mammalian sterile 20-like protein-1 (MST1) or 5'-AMP activated protein kinase (AMPK) activates FOXO-3, and promotes proteolysis and muscle wasting.[14–16] Another member of this family, FOXO-4, appears to mediate TNFα-induced atrophy-related gene expression, in an AKT-independent manner.[17] Interestingly, a recent study proposes that FOXO-4 activity is regulated by the With-no-lysine (K) (WNK) kinase-1, instead, and that a WNK1-FOXO-4 axis is involved in the physiological regulation of skeletal muscle mass maintenance.[18] Other transcription factors can also be important in causing muscle atrophy in specific settings, including SMAD-2 and -3, glucocorticoid receptors, and Nuclear Factor-κB (NFκB), whose inhibition can reduce or block different types of atrophy.[8,19–22] The precise roles of these transcription factors in regulating the expression of muscle atrophy-related genes is still unclear, but it appears that they cooperate with or act on FOXO-transcription factors, the principal mediators of muscle atrophy.

Although it is now clear that the activation of "atrogenes" leads to protein breakdown, the pathways activated, and in turn, the specificity of the downstream targets might be different in different settings. Below we discuss the possible similarities and differences between cancer-induced and neurogenic muscle atrophy, focussing on the UPS and autophagy systems.

Cancer-induced cachexia is a complex, systemic, metabolic syndrome characterized by severe muscle loss. Pro-inflammatory cytokines, such as TNFα, IL1β and IL6, contribute to muscle wasting and protein breakdown, acting either directly or systemically. On the other hand, neurogenic muscle atrophy might develop primarily due to the disruption of the NMJ-dependent signalling pathways required for muscle maintenance.[23–27] One similarity is that both cancer-induced and neurogenic muscle atrophy, are associated with increased UPS and autophagy activity. However, the specificity and the relative contribution of downstream targets in the resulting muscle loss in these different settings, is still unclear.

The Ubiquitin-proteasome system in cancer-induced and in neurogenic muscle atrophy

The UPS is one of the major systems governing protein degradation, and, in physiological conditions, ensures protein turnover and quality control. Degradation requires tagging of the protein by covalent binding to the multiple ubiquitin molecules, and breakdown of the tagged substrate by the 26S proteasome. This process involves at least three classes of enzymes: the ubiquitin-activating E1, the ubiquitin-conjugating E2 and the ubiquitin-ligase E3. The major muscle-specific members of the ubiquitin machinery first identified as consistently increased in muscle atrophy were atrogin-1/MAFbx and MuRF1, belonging to the E3 class of ubiquitin-ligases.[2,28] These enzymes are strongly up-regulated in a wide range of conditions associated with muscle wasting, including cancer, diabetes, glucocorticoid or cytokine treatments as well as in denervation. Several studies suggest that, while numerous stimuli can activate both atrogin-1 and MuRF-1, the downstream pathways affected may be different for each protein. For example, MyoD appears to be a preferential substrate of atrogin-1.[29] When atrogin-1 was overexpressed, polyubiquitination of MyoD was observed, while knock-down of atrogin-1 reversed endogenous MyoD degradation; moreover, the expression of a MyoD mutant resistant to ubiquination, prevented muscle atrophy in vivo.[30] Furthermore, Atrogin-1 was found to interact with transcription factors, components of the translational machinery, soluble enzymes, mitochondrial proteins, as well as with sarcomeric proteins, including myosins, desmin and vimentin.[31] On the other hand, MuRF-1 mainly interacts with structural proteins such as myosin heavy chain proteins.[32,33] Additionally, MuRF-1 degrades myosin light chain 1 and 2 during denervation and fasting conditions.[34] Several studies using tumour-bearing animal models have suggested that accelerated proteolysis and muscle wasting in cancer cachexia is mainly due to the up-regulation of members of the proteasome subunits expression, increased UPS activity, as well as the overexpression of both atrogin-1 and MuRF-1.[35–38]

Proinflammatory cytokines, such as TNFα can induce the expression of genes involved in UPS activity, and knockdown of atrogin-1 by small interfering RNA (siRNA) protected C2C12 muscle cells from the adverse effect of TNF-α.[38] Another signalling pathway suggested to be involved is the Akt. It has been shown that cachexia-associated loss of Akt-dependent signalling in human skeletal muscle was associated with decreased activity of regulators of protein synthesis and a disinhibition of protein degradation.[39] Indeed, FoxO-dependent transcription appears to play a central role in controlling diverse gene networks in skeletal muscle during cancer cachexia, since its inhibition prevented muscle atrophy in a tumour-bearing mouse model.[40] Moreover, transcriptome analysis of upregulated gene transcripts that required FoxO, revealed enrichment of the proteasome, AP-1 and IL-6 pathways, and included several atrophy-related transcription factors.[41] In cachectic tumour-bearing mice, circulating IL-6 levels are associated with suppressed muscle protein synthesis

and mTORC1 signalling.[42] Indeed, it was reported that salidroside, a phenylpropanoid glycoside in Rhodiolarosea L, alleviates cancer cachexia symptoms via activation of the mTOR signalling, and prevents TNFα-induced down-regulation of mTOR in C2C12 cells,[43] suggesting that promoting mTOR signalling and ribosomes biogenesis counteracts cancer-induced muscle atrophy. However, investigations in humans have so far failed to be conclusive, and results on the level of UPS activity in different type of cancer patients have been contradictory.[44–47] The likely reason for the conflicting findings could be the fact that the kinetics of cancer development and signalling in humans are different compared to mice.

In contrast, during denervation, the mTOR pathway was unexpectedly found to be activated, and the expression of genes related to myogenesis were markedly increased, while that of myostatin, a known muscle growth inhibitor, was decreased.[48] However, de novo ribosomal RNA synthesis and the levels of ribosomal RNAs were dramatically decreased in denervated muscle, suggesting that ribosome biogenesis is strongly controlled by factors other than the mTOR pathway. On the other hand, denervation atrophy was not protected by ActRIIB treatment, yet resulted in an upregulation of the pro-growth factors AKT, SGK and components of the mTOR pathway. Thus, these studies suggested that denervation atrophy is not only independent from AKT, SGK and mTOR activation, but also has a different underlying pathophysiological mechanism compared to other types of muscle atrophy.[49]

Despite the recognized role of the Atrogin-1 and MuRF-1 E3 ligases in muscle atrophy, there is as yet no evidence suggesting their requirement in animal models of cancer cachexia. On the other hand, their role in neurogenic muscle atrophy, was previously explored by Moresi V et al, showing an up-regulation of Atrogin-1 and MuRF-1 in muscle upon denervation, via up-regulation of myogenin expression.[50] By preventing the denervation-dependent induction of myogenin expression, up-regulation of Atrogin-1 and MuRF-1 was also prevented and muscle loss counteracted, highlighting a crucial role of these enzymes in neurogenic muscle atrophy. However, atrogin-1/MAFbx or MuRF1knockout mice are only partially resistant to denervation-induced atrophy. Together, these data suggest either that the expression of one of these ligases is enough to induce neurogenic muscle atrophy, or that additional factors are required to mediate denervation-induced muscle loss. Also, in other types of muscle atrophy models, such as fasting or glucocorticoid treatment, targeting one or the other E3 ligases, either genetically or pharmacologically, were not conclusive, arguing against the exclusive role of these enzymes in muscle loss.[51–53] These observations prompted researchers to search for other ubiquitin ligases that contribute to sarcomeric protein breakdown. Among them TRAF6, another E3 ligase, was identified.

Interestingly, muscle-specific TRAF6 knock out mice are resistant to muscle loss induced by either denervation, cancer or starvation.[54–56] This protection appears to depend on both direct and indirect effects on protein breakdown. Another novel ubiquitin ligase MUSA-1 belongs to the SCF complex, and its expression is highly induced in a tumour-bearing mouse model. While there is no direct evidence for its role in cancer-mediated muscle loss, its knockdown by RNA interference significantly reduced neurogenic muscle atrophy.[53] In addition to the ubiquitin ligases, several proteasome subunits, as well as some de-ubiquinating enzymes, have been shown to be strongly upregulated in muscle during cancer cachexia and/or denervation,[57,58] however their contribution to muscle atrophy is still under investigation, and will not be discussed here.

The autophagy-lysosome system in cancer-induced and in neurogenic muscle atrophy

The other major proteolytic system involved in muscle atrophy is the autophagy-lysosome system. In the autophagy system, small ubiquitin-like molecules (LC3, GABARAP, GATE16, and Atg7) are transferred from the conjugation system to membranes which then grow and commit to become a double-membrane vesicle (autophagosome) that engulfs portions of the cytoplasm leading to the proteolysis of long-lived proteins and organelles.[59] In contrast to the proteasome-ubiquitin E3 ligases or "atrogenes", discussed above, whose expression is clearly induced to initiate the atrophy program, whether and to what extent autophagy contributes to muscle loss in different conditions, is not clear yet. Autophagy, also referred as macroautophagy, is an important physiologic mechanism that ensures recycling of damaged organelles and macromolecules, thus maintaining tissue homeostasis. It is therefore conceivable that alterations in autophagy, either an increase or a decrease, can exacerbate muscle loss during catabolic conditions. The activation of autophagy was initially regarded as one of the catalytic mechanisms leading to muscle atrophy in several conditions, such as cancer cachexia, starvation, disuse and denervation.[27,60–62] However, reduced autophagy was also associated with some muscle disorders, such as the UCMD and Bethlem myopathies or the Duchenne muscular Dystrophy, as well as sarcopenia.[63–66] Indeed, treatment with chloroquine, a lysosome inhibitor, induced a severe myopathy (e.g., chloroquine myopathy) due to generalized lysosome impairment. Similarly, mouse models lacking Atg5 or Atg7, or with compromised autophagy, undergo generalized myofiber degeneration due to accumulation of protein aggregates, appearance of abnormal mitochondria, induction of oxidative stress, and activation of unfolded protein response.[67,68] Re-activation of autophagy, through low-protein diet, counteracted muscle defects in both animal models and patients, highlighting the important role of intracellular clearance for muscle maintenance.[69]

The autophagy machinery in muscle atrophy, like the proteasome system, is controlled by the FoxO protein family, predominantly by the FoxO3 member.[6,68,70,71] FoxO3 activation increases the expression of many autophagy-related genes in myotubes, and its inactivation is incompletely compensated by the other factors in preventing muscle loss, suggesting that FoxO3 is the most critical factor for the atrophy programme. Interestingly, FoxOs inhibition did not result in severe myopathy, unlike chloroquine treatment or Atg7 depletion, suggesting that FoxOs acts electively on the autophagy-induced muscle atrophy program.[68] This finding implies the existence of a subset of FoxO-dependent downstream targets regulating autophagy in muscle wasting, without interfering with the physiological clearance. The identification of those targets will no doubt be useful for the design of novel strategies aiming to selectively prevent autophagy-dependent muscle loss in specific settings. Although speculative at this stage, the FoxO-dependent downstream targets could be different depending on the stimulus. Since FoxO factors do not act alone to induce atrophy related genes, but cooperate with other factors, such as NFκB, SMADs etc, which might be activated in response to a specific atrophy stimulus, this possibility is very likely. Intriguingly, the lists of FoxO-dependent genes during denervation and fasting do not overlap completely[68]. Studies on the molecular pathways involved in neurogenic muscle atrophy and on the contribution of the autophagy machinery in this setting, have produced conflicting results. Some studies reported that autophagy is suppressed in muscle early after denervation.[72] while, according to others, it is triggered seven days after denervation, primarily targeting damaged mitochondria,[73,74] Other studies reported the activation of autophagy markers three days after denervation, as a possible mechanism leading to muscle wasting.[68] However, in contrast to fasting, deletion of FoxO 1,2,3 only partially prevented neurogenic muscle atrophy and the autophagy system was mildly affected.[68] Interestingly, exercise, known to induce autophagy in muscle, was recently found to prevent neurogenic muscle atrophy, maintaining proteostasis in muscle.[75,76] So far, none of the above studies conclusively determined whether autophagy contributes to neurogenic muscle atrophy or not. To this end, a recent study by the group of Moresi, published in the previous issue of EJTM, addressed this question more closely.[77] They found that the expression of autophagy markers is induced as early as eight hours following denervation and increased over time. The observed increase appeared to be independent from FoxO activation.[68,77] Indeed, both Akt and mTOR active/phosphorylated forms were higher in denervated muscle after 7 days, compared to control muscle. As mentioned before, Akt is known to promote protein synthesis, and its activation in this setting might reflect an attempt to counteract muscle loss; on the other hand,

the denervation-induced mTOR activity may counteract Akt pathway activation, or even increase proteasome level, as previously suggested.[78,79] Interestingly, since mTOR is known to inhibit autophagy, its activation in denervated muscle suggests that it has no major effect on autophagy in neurogenic muscle atrophy, in contrast to muscle atrophy driven by other stimuli. Despite the signalling pathways involved in autophagy activation during denervation, whether autophagy contributes to neurogenic muscle atrophy is still unclear. Interestingly, Pigna et al. showed that further inducing autophagy following denervation, by either intermittent fasting or rapamycin treatment, did not alter denervation-induced muscle atrophy. Conversely, Tang et al. showed that treatment with higher doses of rapamycin prevented muscle atrophy, mostly by preventing E3-ubiquitin ligases upregulation, in a FOXO-dependent manner. Indeed, rapamycin treatment of mice restored Akt activity, suggesting that the denervation-induced increase in mTORC1 activity was producing a feedback inhibition of Akt. To further complicate the story, autophagy was shown to be rather suppressed in denervated muscles, due to a constitutive activation of mTORC1.[72] Further studies are needed to conclusively understand whether and, at which stage of the process, autophagy is required for neurogenic muscle atrophy.

Likewise, whether and how autophagy can contribute to cancer-induced muscle atrophy is also controversial. As mentioned above, autophagy was found to be activated in cachectic muscle.[47,80–83] Nevertheless, although acute systemic inhibition of autophagy in tumour-bearing mice significantly ameliorated tumour growth, it failed to prevent muscle and fat loss, suggesting that autophagy is required for muscle maintenance.[84,85] It is important to stress here that cancer cachexia is a systemic multifactorial disorder, and autophagy activation in muscle could be required as a metabolic response to inflammatory, nutritional or energy stresses induced by tumour growth. Interestingly, exercise has emerged as a possible therapeutic strategy to counteract cancer cachexia and prolong life span in cancer patients.[85–88] Exercise is known to physiologically activate autophagy in muscle, which appears to mediate the beneficial effects of physical activity on glucose homeostasis.[62,89,90] Together, these data suggest that systemic activation of autophagy might preserve tumour cell survival, and its inhibition may reduce tumour growth; at the same time, autophagy is required in muscle to ensure clearance of dysfunctional organelles, and thus maintain energy homeostasis.

Conclusions and Perspectives

Significant advances have been made during the past decade in our understanding of the mechanisms that control muscle wasting. A major breakthrough has been the identification of the ubiquitin proteasome and autophagy lysosome systems, as the principal cellular degradation systems involved in protein breakdown in

muscle, and the transduction pathways controlling their activity. However, it is now clear that, although very similar, there is no common mechanism that applies to all models of muscle wasting. As discussed above, activation of the UPS system appears a common feature in both cancer-induced or in neurogenic muscle atrophy, although specific downstream targets might differ, and still need to be conclusively identified. Moreover, while autophagy is emerging as a selective degradation system, given the conflicting findings thus far, studying the degraded proteins or organelles of the autophagic process as wells as the kinetics of their disposal would be more informative.

In summary, a more detailed understanding of the relative contribution of the degradative systems and the downstream targets involved in cancer-induced or in neurogenic muscle atrophy is needed to facilitate the development of novel therapeutics for the prevention of muscle loss.

Author's contributions

All authors contributed to critical analysis of the literature and to writing the manuscript.

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Conflict of Interest

None of the authors have conflicts of interests.

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