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## **Coming back: autophagy in cachexia**

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## **Purpose of the review**

Cachexia is a complex syndrome characterized by body weight loss, tissue wasting, systemic inflammation, metabolic abnormalities and altered nutritional status. One of the most prominent features of cachexia is the loss of muscle mass, mainly due to increased protein degradation rates. The review is aimed at discussing the involvement of autophagy in the pathogenesis of muscle wasting in cachexia.

## **Recent findings**

Modulations of muscle mass in the adult reflect an imbalance between protein synthesis and degradation rates. Muscle depletion in cachexia is associated with increased protein breakdown, mainly involving the pathways dependent on ubiquitin-proteasome and autophagy-lysosomes. This latter, in particular, was considered not relevant for a long time. Just in the last years autophagy was shown to contribute to the pathogenesis of muscle wasting not only in myopathies due to intrinsic muscle defects, but also in muscle depletion associated with conditions such as sepsis, chronic obstructive pulmonary disease, glucocorticoid treatment, cancer cachexia, aging.

## **Summary**

The present review highlights that both excess or defective autophagy are relevant to the onset of muscle depletion, and draws some considerations about possible therapeutic intervention aimed at modulating autophagy in order to improve muscle trophism (see Video, Supplementary Digital Content 1).

## **Key words**

cachexia, autophagy, muscle wasting, lysosomal proteolysis, cancer, aging, COPD

## **Introduction**

Improved clinical management and therapies markedly increased life expectancy in patients affected by chronic pathologies such as diabetes, cancer, cardiomyopathies, autoimmune or neurological diseases. However, these patients frequently develop cachexia, a syndrome characterized by body weight loss, skeletal muscle and adipose tissue depletion, metabolic abnormalities, systemic inflammation, impaired nutritional status and reduced ability to perform everyday activities (1). In spite of this negative impact, the occurrence of cachexia in patients affected by chronic diseases is frequently undervalued and only recognized late in the disease course, when the few therapeutic options available are useless. Recently, considerable efforts have been made to convey on this issue the due attention of the medical audience. By all evidence, clinicians should be aware that, to be effective, the interventions on cachexia must be implemented as early as possible and monitored with adequate laboratory tools.

Among the most impressive features of cachexia stands the body weight loss, which negatively correlates with survival, tolerance to treatments and quality of life (1). Adipose tissue and skeletal muscle depletion largely account for body wasting in cachectic patients, though these compartments are not equally affected. While fat loss may be a late phenomenon, muscle depletion is a quite constant and early feature in chronically ill patients. For this reason, the present review will focus on the relevance of autophagy to the pathogenesis of muscle wasting in cachexia, though this degradation pathway is likely to play crucial roles also in other compartments, among which liver and adipose tissue.

## **Muscle wasting**

The regulation of muscle protein mass depends on the balance between protein synthesis and degradation rates. Muscle wasting in cachexia is frequently associated with increased protein breakdown rates, while the contribution of reduced protein synthesis rates is less clear. Several experimental data indeed suggest that when the degradation rate is higher than normal, total protein is lost irrespectively of the synthesis rate. In fact, protein synthesis is connoted by first order kinetics and degradation by zero order rates. In other words, anabolic stimuli are likely to be ineffective unless associated with lowered protein catabolic rates.

Among the proteolytic systems operating in the skeletal muscle, only those depending on ubiquitin-proteasome or autophagy-lysosomes extensively degrade their substrates. Increased expression and/or activity of components of the ubiquitin-

proteasome pathway were reported in muscle atrophy of both systemic (fasting, cancer, sepsis) or local (denervation, immobilization) origin (2). Some years ago, two muscle-specific ubiquitin ligases, were identified: atrogin-1, which appears mainly involved in the degradation of proteins contributing to cell proliferation, differentiation and survival, and MuRF1, the substrate of which mainly are structural proteins (2). Since their identification, several other ubiquitin ligases have been recognized to play a role in muscle protein degradation, including TRAF6, Trim32, Nedd4-1, Fbxo40 (2), and the newly identified MUSA1 (3, 4).

The contribution of the autophagic-lysosomal proteolysis to the pathogenesis of muscle atrophy was neglected for many years, mainly on the basis of reports showing that lysosomal protease inhibitors could not reduce protein degradation in isolated muscles (5). However, evidence was also provided suggesting that activation of lysosomal proteolysis was occurring in muscle atrophy, such as increased expression and/or activity of lysosomal proteases in septic rats or in experimental and clinical cancer cachexia (6).

### **Autophagy and muscle: a new era**

Two main reasons account for the delay in recognizing the involvement of autophagic-lysosomal proteolysis in muscle wasting. The first mainly was a conceptual one in that the skeletal muscle was long believed to be unable of a prompt autophagic response to stress conditions such as starvation, rather ascribed to the liver. Only in the last ten years the occurrence of autophagy in the skeletal muscle was clearly documented in 24 h fasted mice transgenic for green fluorescent protein (GFP)-LC3 (6). The second reason relies on the tools available to study autophagy, that were very poor until a decade ago. A guideline to autophagy assessment indeed became available only a few years ago (6).

Autophagy may occur by different modalities: macroautophagy, microautophagy, selective macro- and microautophagy (7). Macroautophagy (autophagy, from now on) relies on the sequestration of portions of cytoplasm and/or organelles into double-membrane vesicles (autophagosomes) that subsequently fuse with lysosomes and release the inner membrane into the lumen for degradation. The sequestered material is broken down and the resulting degradation products made available for recycling (6). Autophagic sequestration was long considered as a random process, mainly aimed at providing substrates in conditions of nutrient starvation. In the last years, however, autophagy was recognized to play a critical role in the selective removal of damaged organelles such as

peroxisomes or mitochondria (pexophagy and mitophagy, respectively) as well as in the degradation of damaged or misfolded proteins (6).

The morphological aspects of autophagy have been well described long ago. By contrast, the molecular machinery operating this degradation pathway was identified quite recently (2). About fifteen years ago a class of genes named Atg (Autophagy-related genes) was identified in yeast, and their mammalian orthologs found and functionally characterized. These gene products (Ulk1/Atg1, beclin-1/Atg6, Atg12-Atg5 conjugate, LC3/Atg8, among others) are involved at different steps in autophagosome formation. While Atg12 and Atg5 are constitutively present in mammalian cells, LC3 expression and lipidation are inducible, typically by starvation. Other important components of the autophagic machinery are p62/SQSTM1, a ubiquitin-binding protein, and Bnip3, which is involved in mitophagy (6). Expression level and intracellular localization of some of these proteins can be used to monitor autophagy, in particular beclin-1 as an indicator of autophagy induction, LC3B-I conversion to its lipidated LC3B-II form to measure autophagosome abundance, and p62/SQSTM1 as an indicator of substrate sequestration and subsequent degradation. However, these markers *per se* do not give an estimate of the autophagic rate, since their levels can vary as a function of increased autophagic sequestration or of reduced lysosomal degradation, or of both. This point can be settled by measuring the levels of autophagy markers in the presence of drugs that block either autophagosome formation or their fusion with lysosomes (colchicine; (2)), or lysosomal proteolysis (chloroquine, E64, pepstatin A; (2)).

Physiologically, autophagy occurs at basal rates, but can be markedly accelerated when cells face environmental stresses. In this regard, fasting was long known to activate autophagy as a compensatory mechanism to cope with the lack of nutrients. Just recently, however, the underlying mechanisms began to be unraveled (8), showing that upon fasting reduced sirtuin-1 levels lead to FoxO activation via decreased deacetylation, resulting in induction of atrophy-related genes (atrogin-1, MuRF1, autophagy genes). Consistently, sirtuin-1 overexpression inhibits FoxO and rescues starvation-induced muscle atrophy (8). Besides fasting, other experimental conditions are known to induce autophagy in the muscle, such as denervation atrophy, in which genes involved in autophagy are hyperexpressed, mainly due to FoxO activation. In denervated muscles, atrophy has been alternatively reported to depend on decreased sirtuin-1 levels (8), or increased expression of mammalian Ste20-like kinase (MST)-1, a member of the Hippo signaling pathway (9), or on activation of the BMP-dependent signaling (3).

The energy balance in the cell is one of the main autophagy regulators, autophagic sequestration being activated when reduced energy availability is detected by sensors such as the mammalian target of rapamycin (mTOR) or AMP-activated kinase (AMPK), acting as compensatory mechanisms that favor cell survival. Defective or excess autophagy are equally detrimental for the cell, and may contribute to the pathogenesis of several diseases. Excess autophagy may deprive the cell of components necessary to normal metabolism, while autophagic sequestration rates below the physiological level have been proposed to lead to accumulation of protein aggregates and dysfunctional organelles (2, 6). When deregulated autophagy takes place in the skeletal muscle, myofibers are poised either into a hypercatabolic state (excess autophagy) or into a condition of engulfment (defective autophagy); in both cases loss of homeostasis, atrophy and functional impairment might occur.

### **Autophagy and pathology: muscle wasting and cachexia**

The relevance of autophagy to muscle homeostasis is demonstrated by reports showing that several myopathies such as some types of muscle dystrophies, Pompe disease, Danon disease, etc. are associated with altered autophagic rates (6). In particular, reduced autophagic capacity appears to occur in many of these myopathies, resulting in accumulation of unwanted substrates. From this point of view, strategies aimed at increasing autophagy, such as exercise or AMPK activation, may prove beneficial (reviewed in(10)).

Data emerging in the last few years clearly show that the autophagic-lysosomal proteolysis is also involved in the pathogenesis of muscle wasting in cachexia.

Induction of autophagy occurs in the skeletal muscle of tumor-bearing animals, as shown by high LC3B-II levels; these further increase when autophagosome fusion with lysosomes is inhibited by treatment with colchicine, demonstrating that the sequestration rate is enhanced (11). Of interest, overtly cachectic tumor bearers cannot survive colchicine administration, suggesting that the autophagic-lysosomal degradation is a critical process when host metabolism is markedly compromised. Quite surprisingly, the increased autophagic sequestration in the tumor host muscle is associated with high p62 levels, suggesting an accumulation of unprocessed autophagosomes, that possibly depends on exhaustion of the lysosomal clearance capacity. Consistently, cathepsin B and L activity is decreased in the muscle of tumor-bearing animals at the end of the experimental period, in parallel with increased atrogin-1 mRNA levels and calpastatin

degradation (11). Reduced activation of mTORC1, a protein complex that plays a crucial role in the regulation of autophagy, and increased expression of molecules involved in the autophagic degradation have also been reported to occur during the progression towards cachexia in the ApcMin/+ mice (12).

Muscle autophagy is also induced in cancer patients. Bnip3 mRNA and LC3B-II protein levels increase in muscle biopsies of lung cancer patients with respect to control subjects, while gene expression of the muscle-specific ubiquitin ligases is not modified. Such modulation of autophagy gene expression is associated with increased circulating proinflammatory cytokines and muscle NF- $\kappa$ B activation (13). Finally, a quite recent report (14) shows that, while no differences in proteasome, calpain and caspase 3 activities can be observed in the skeletal muscle of esophageal cancer patients compared to controls, both cathepsin B and L activities and LC3B-II levels are increased.

Induction of autophagy in cancer hosts is likely associated with the negative energy balance that arises from reduced food intake, mitochondrial damage and dysmetabolism (11). Not all the newly formed autophagosomes are processed, however, on one side resulting in persistent lack of anabolic substrates, on the other in the accumulation of damaged proteins and organelles, eventually leading not only to myofiber reduction in size, but also to impaired function.

Cachexia is a frequent feature in patients affected by chronic obstructive pulmonary disease (COPD). In particular, muscle atrophy in these patients was found associated with upregulation of components of the ubiquitin-proteasome-dependent proteolytic system (15). A recent study, however, shows that neither atrogin-1 nor MuRF1 are upregulated in stable COPD patients, irrespective of the low muscle mass (16). The involvement of autophagy is not clear, some data showing that both beclin-1 and LC3 mRNA levels do not differ between COPD patients and controls, while some others, from a different set of patients, show increased beclin-1, LC3B-II and p62 protein levels, suggesting that the system is induced (17).

Lipopolysaccharide (LPS) treatment is an experimental condition widely used to mimic the metabolic alterations occurring in septic patients. A recent study shows that activation of muscle protein breakdown by LPS requires an intact glucocorticoid signaling. Indeed, the induction of autophagy genes such as Bnip3, Gabarapl and cathepsin L is significantly reduced in LPS-treated mice not expressing the glucocorticoid receptor (18). Therefore, this receptor-dependent signaling appears involved in the regulation of muscle autophagy. Consistently, dexamethasone-induced muscle atrophy is associated with



increased expression of beclin-1 and LC3B-II, suggesting an enhanced autophagic flux, whereas reduced p62 levels point to ready degradation of sequestered proteins (11).

In a recent study (19), muscle depletion owing to burn injury was found associated with marked autophagy activation, that persists for up to 7 days after burning. The enhanced LC3B-I to LC3B-II conversion further increases when the animals are treated with the lysosomal protease inhibitors pepstatin A and E64; whether the latter effect really reflects an accelerated autophagic flux (19) is not clear. Muscle wasting and enhanced autophagy can be partially rescued by treating burned animals with tadalafil, a phosphodiesterase 5 inhibitor (19).

Finally, autophagy does not appear to exceed basal levels or is even less active in aging-related sarcopenia, since beclin-1 and LC3B-II levels are comparable in aged vs young animals; by contrast, p62 is increased, suggesting an accumulation of substrates due to insufficient autophagic sequestration (11). Similarly, in a putative experimental model of aging, the GSK-3 $\alpha$  knock-out mice, reduced muscle autophagy has been reported and this finding accounted for by increased mTORC1 phosphorylation appears to account for this finding (20). These observations are intriguing, since impaired autophagy was earlier reported to occur in the liver of aged animals and more recently in the skeletal muscle as well. Noteworthy, muscle-specific Atg7 knock-out mice, unable to activate autophagy in this tissue, develop changes that closely resemble aging sarcopenia (2).

## **Conclusions and perspectives**

The role of autophagy in the pathogenesis of muscle atrophy can vary, depending on the situation. As an example, muscle wasting occurs in both cancer hosts and aged individuals, but autophagy is induced just in the former, to compensate for cancer-induced negative nitrogen and energy balance (Figure 1). By contrast, defective autophagy contributes to aging sarcopenia (see 2). Taking such differences into consideration, to address autophagy as a therapeutic target is not an easy task. Indeed, local interventions might be useful for intrinsic myopathies, though modulating autophagy in the skeletal muscle could affect other compartments. In this regard, muscle-specific Atg7 knock-out mice show reduced fat mass and are resistant to diet-induced obesity and insulin resistance (21). By contrast, autophagy inhibition is unlikely to improve muscle wasting of systemic origin such as the one occurring in cachexia, also in view of the multifactorial nature of this syndrome. On this line, protocols aimed at counteracting muscle wasting in cachexia should down-regulate autophagy by impinging on the inducing stimuli rather than

on the process itself. In this regard, integrated treatments designed to improve the negative nitrogen and energy balance, the hypoanabolic response and the hypercatabolic state would be advisable.

### **Key points**

- autophagic-lysosomal degradation is crucial to maintain the homeostasis in the skeletal muscle.
- both defective or excess autophagy may play a role in causing muscle wasting in cachexia;
- depending on the situation, therapeutic interventions aimed at directly interfere with autophagy might be detrimental for the whole organism as well as for the skeletal muscle;
- an integrated approach involving strategies targeting autophagy-inducing stimuli, such as the negative nitrogen and energy balance are likely the best choice to manage with cachexia

### **Conflict of interest**

The authors declare that no conflict of interest exists.

Figure 1. Contribution of defective or excess autophagy to the pathogenesis of muscle wasting.

Basal autophagy is active in the muscle of healthy adults, contributing to tissue homeostasis. The reduced metabolic efficiency that characterizes aging results in a slow but progressive accumulation of protein aggregates and dysfunctional organelles, that is not supported by concomitantly increased autophagy (6); the final event is myofiber energy deficit, mainly because of mitochondrial dysfunction, and consequent atrophy. By contrast, autophagy is induced above control levels in cancer cachexia (11), in order to get rid of waste substrates, but mainly because the muscle is experiencing an energy shortage, due to reduced food intake, mitochondrial abnormalities (22) and dysmetabolism. Such enhanced autophagic flux results in an increased number of autophagosomes that eventually exceeds the lysosomal clearance capacity, significantly contributing to myofiber atrophy, loss of integrity and function. The results available in the literature suggest that a similar mechanism may be involved also in cachexia associated with other chronic diseases, though a conclusive demonstration is still lacking.

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Supplementary Digital Content 1: videoabstract

