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REVIEW

Recent progress in elucidating signalling proteolytic pathways in muscle wasting: Potential clinical implications☆

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Abstract *Aims:* Muscle wasting prevails with disuse (bedrest and immobilisation) and is associated with many diseases (cancer, sepsis, diabetes, kidney failure, trauma, etc.). This results first in prolonged hospitalisation with associated high health-care costs and second and ultimately in increased morbidity and mortality. The precise characterisation of the signalling pathways leading to muscle atrophy is therefore particularly relevant in clinical settings.
Data synthesis: Recent major papers have identified highly complex intricate pathways of signalling molecules, which induce the transcription of the muscle-specific ubiquitin protein ligases MAFbx/Atrogin-1 and MuRF1 that are overexpressed in nearly all muscle wasting diseases. These signalling pathways have been targeted with success in animal models of muscle wasting. In particular, these findings have revealed a finely tuned crosstalk between both anabolic and catabolic processes.
Conclusions: Whether or not such strategies may be useful for blocking or at least limiting muscle wasting in weight losing and cachectic patients is becoming nowadays a very exciting clinical challenge.

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Background

The ubiquitin—proteasome system (UPS) is the most complex proteolytic machinery in eukaryotic cells. There are two sequential steps in this pathway: first, a polyubiquitin chain of at least four ubiquitin moieties is covalently attached to the substrate; second, this degradative signal is recognised by the 26S proteasome, which breaks down the targeted protein into peptides [1]. The lysosomal, and sometimes the Ca^{2+}-activated and the caspase proteolytic systems are coordinately activated with the UPS in various muscle wasting conditions [2—4]. The UPS is clearly recognised as the major actor in the control of muscle proteolysis and mass since only proteasome (but not other proteinase) inhibitors suppress the enhanced proteolysis and the specific breakdown of myofibrillar proteins in different catabolic conditions (for an example see Ref. [5]). However, recent elegant work has shown that the lysosomal route also plays a critical role in the control of muscle mass [6].

Skeletal muscle atrophy occurs upon disuse (immobilisation and bedrest) and various diseases (cancer, sepsis, diabetes, kidney failure, trauma, etc.). An increased proteolysis is mainly responsible for this wasting [2], including in humans [3]. An obvious goal is to propose new strategies that may prevent or at least limit muscle loss in clinical settings. Elucidating precise mechanisms responsible for this wasting may contribute to achieve this goal.

Major signalling pathways of muscle proteolysis

Various factors including malnutrition, impaired physical activity and the production of catabolic molecules (i.e., glucocorticoids and/or cytokines that are overproduced in multiple catabolic states) upregulate rates of muscle proteolysis [2]. Conversely, anabolic stimuli (i.e. branched-chain amino acids (BCAAs), moderate exercise, insulin, insulin growth factor 1 (IGF1), etc.) down-regulate protein breakdown [2]. The precise signalling pathways responsible for these adaptations start to be identified. Two muscle-specific ubiquitin protein ligases E3, which target specifically a limited number of proteins for breakdown by the proteasome, are overexpressed in many different catabolic conditions [7,8]. These E3 ligases are called Muscle Atrophy F box (MAFbx)/Atrogin-1 and Muscle Ring Finger 1 (MuRF1). In addition, mice knockout for either E3 were partially resistant to muscle atrophy [7]. Consequently, MAFbx/Atrogin-1 and MuRF1 are critical for enhanced proteolysis in muscle wasting (although this is now challenged for MAFbx/Atrogin-1, see below). These findings led to rapid discoveries. The expression of MAFbx/Atrogin-1 and MuRF1 was demonstrated to be controlled by FoxO transcription factors and the nuclear factor κB (NFκB) signalling pathway [9—12]. These observations form the basis of our current understanding of the regulation of proteolysis. We summarise here recent data giving further insights into the precise mechanisms of this process.

Coordinate activation of the UPS and lysosomal pathway in catabolic states

The lysosomal pathway is coordinately activated with the UPS in various muscle wasting diseases (for an example see Ref. [3,13] for a review). In-depth studies unambiguously identified FoxO3 as a critical transcription factor responsible for these adaptations, both in cultured muscle cells and in rodent atrophying muscles. Using various approaches, it was shown that FoxO3 induces autophagy and the expression of many Atg (autophagy-related) genes in myotubes, muscle fibres and skeletal muscle [14,15]. This transcriptional regulation involves the direct binding of FoxO3 to specific sites in promoters of Atg, and in particular of Bnip3, which is an important regulator of autophagy. Conversely, the knockdown of FoxO3 prevents autophagy induced *in vivo* by starvation.

Adenosine monophosphate-activated protein kinase (AMPK) is also involved in enhanced proteolysis through a FoxO3-dependent increased expression of MAFbx and MuRF1 [12] and activation of autophagy. Mitochondrial fission and dysfunction lead to the activation of AMPK/FoxO3 and to the enhancement of lysosomal and proteasomal proteolysis *via* an amplification loop in which FoxO3 induces mitochondrial fission [12].

The expression and autoubiquitination of tumour necrosis factor-α receptor adaptor protein 6 (TRAF6), a protein involved in receptor-mediated activation of several signalling pathways, increase in atrophying muscles [16]. TRAF6 mediates the activation of Jun kinase 1/2, p38 mitogen-activated protein kinase, AMPK and NFκB, and induces the expression of MAFbx/Atrogin-1, MuRF1 and Atg genes in skeletal muscle. Conversely, depletion of TRAF6 rescues myofibrillar proteolysis and preserves muscle fibre size and strength upon catabolic treatment.

Altogether, FoxO3 [6], AMPK [12] and TRAF6 [17] regulate the activation of autophagy and of the UPS in muscle atrophy (Fig. 1).

Crosstalk between muscle proteolysis and protein synthesis

The size of muscle cells is timely regulated by signalling networks that determine the balance between overall rates of protein synthesis and degradation. The insulin/IGF-1/Akt/mammalian target of rapamycin (mTORC1) pathway stimulates myofibre growth and muscle protein synthesis and inhibits protein breakdown. In situations of positive nitrogen balance both insulin and IGF1 act through phosphoinositide-3-kinase (PI3K) to phosphorylate Akt, which in turn stimulates protein synthesis and phosphorylates FoxO. The latter cannot enter the nucleus. Thus, the transcription of MAFbx/Atrogin-1 and MuRF1 is blocked resulting in low muscle proteolysis [9] (Fig. 1). By contrast, when negative nitrogen balance prevails, there are either low levels of insulin and/or IGF1 or resistance to their anabolic effects, or both. Thus, PI3K cannot phosphorylate Akt so that protein synthesis is inhibited. Simultaneously, non-phosphorylated FoxO transcription factors enter the nucleus and stimulate the transcription of MAFbx/Atrogin-1, MuRF1 and Atg genes resulting in elevated rates of proteolysis. Therefore, Akt controls both processes of protein turnover [9] (Fig. 1).

AMPK also plays an important role in the crosstalk between proteolysis and protein synthesis, according to the energy status of the cell. The enzyme not only activates