

Muscle wasting: an overview of recent developments in basic research

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Abstract The syndrome of cachexia, i.e., involuntary weight loss in patients with underlying diseases, sarcopenia, i.e., loss of muscle mass due to aging, and general muscle atrophy from disuse and/or prolonged bed rest have received more attention over the last decades. All lead to a higher morbidity and mortality in patients, and therefore, they represent a major socio-economic burden for the society today. This mini-review looks at recent developments in basic research that are relevant to the loss of skeletal muscle. It aims to cover the most significant publication of last 3 years on the causes and effects of muscle wasting, new targets for therapy development, and potential biomarkers for assessing skeletal muscle mass. The targets include the following: (1) E-3 ligases TRIM32, SOCS1, and SOCS3 by involving the elongin BC ubiquitin-ligase, Cbl-b, cullin 7, Fbxo40, MG53 (TRIM72), and the mitochondrial Mul1; (2) the kinase MST1; and (3) the G-protein $G\alpha_i2$. D(3)-creatine has the potential to be used as a novel biomarker that allows to monitor actual change in skeletal muscle mass over time. In conclusion, significant development efforts are being made by academic groups as well as numerous pharmaceutical companies to identify new target and biomarker muscles, as muscle wasting represents a great medical need, but no therapies have been approved in the last decades.

Keyword Cachexia sarcopenia · Basic research · Muscle wasting

1 Introduction

Cachexia sarcopenia and general wasting of the musculature are related to a poor quality of life and increased morbidity/mortality [1]. They are caused by a large number of chronic diseases and the general process of aging thus affecting millions of patients and elderly [2–4]. The syndrome of cachexia is characterized as complex metabolic abnormalities that lead to the loss of body weight as a consequence of a chronic illness. A consensus statement from 2008 proposed to clinically define cachexia as a non-edematous weight loss exceeding 5 % within the previous 3–12 months in combination with symptoms characteristic for cachexia (e.g., fatigue or depression), loss of lean body mass, and biochemical abnormalities (e.g., anemia or inflammation) associated with chronic diseases [5]. In adults, a prevalence of 5–15 % has been reported in chronic heart failure (CHF) and chronic obstructive pulmonary disease (COPD), while it may be up to 80 % in advanced cancer [6]. Interestingly, an estimated 30 % of cancer patients die as a result of cachexia rather than the cancer itself [6], although the precise cause of death due to cachexia is still somewhat unclear.

In contrast to the relatively fast atrophy of skeletal muscle associated with cachexia, the syndrome of sarcopenia is characterized by a much slower decline in muscle mass and function that is directly related to the aging process and may ultimately lead to frailty and loss of independent living [7]. There is a loss of 1–2 % of muscle mass per decade of life from the fifth decade onwards, associated with a 1.5 % decline in muscle strength, potentially increasing to 3 % after the age of 60 [7]. From a histological point of view, sarcopenia is characterized by a decrease in the number and the size of the

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muscle fibers. The prevalence of sarcopenia for those over 64 years of age has been shown to be 22.6 % in women and 26.8 % in men, rising to 31.0 and 52.9 %, respectively, in those over 80 years of age [8]. It can thus be estimated that over 3 % of the total world population will be affected by sarcopenia by 2015 [8].

However, muscle wasting may also occur independently of chronic diseases and age. Disuse of muscle is a strong inducer of skeletal muscle atrophy and function that is caused by a mechanical unloading of the muscle, e.g., space flight or prolonged bed rest, and involved multiple signaling pathways [9].

The development of preventive and therapeutic strategies against cachexia, sarcopenia, and wasting disorders in general is perceived as an urgent need by health care professionals [10, 11]. Despite this great medical need, no therapies have been approved for muscle wasting or cachexia in the last decades. Nevertheless, significant efforts to identify new targets are being made by academic groups as well as numerous pharmaceutical companies [12–14].

2 Current developments in basic cachexia research

The mass of a muscle is determined by dynamic regulation of its protein balance in the muscle fibers in response to various extracellular stimuli that can be anabolic or catabolic in nature. These signals may also affect the proliferation and maturation of muscle stem cells. Potent anabolic signals in skeletal muscle are insulin, insulin-like growth factor-1 (IGF-1) [15], testosterone [16], and agonists of the β -2 adrenoreceptor [17]. Levels of IGF-1 are regulated by the ghrelin/growth hormone axis [18]. Upon binding to the receptor, both insulin and IGF-1 activate the PI3K/Akt/mTOR pathway, which induces protein synthesis while inhibiting proteolysis resulting in a hypertrophy of the muscle [19]. Interestingly, hypogonadism and low testosterone in male cancer patients has been reported to be as high as 90 %, thus making these men more susceptible to the development of muscle atrophy [20]. Indeed, a class of molecule termed specific androgen receptor modulators (SARMs) have shown to increase muscle mass in the elderly [21]. Triggers for muscle wasting are more numerous and include a general activation of the sympathetic nervous system (SNS) [22], pro-inflammatory cytokines [23, 24], angiotensin-II [25], glucocorticoids [26, 27], and members of the TGF- β family [28, 29].

3 News in catabolic signaling

In healthy individuals, muscle growth is limited by several members of the TGF- β family, namely, myostatin, activin A, and TGF- β binding to the activin IIB receptor or the TGF- β

receptor, respectively. Under disease conditions, these proteins prominently contribute to the induction of protein loss in skeletal muscle [30, 31]. Activation of either receptor induces SMAD2/SMAD3 signaling resulting in inhibition of anabolic Akt-signaling and stimulation of proteolysis [19, 30]. Cytokines like interleukine-1 (IL-1), IL-6, TNF- α , and interferon- γ lead to a receptor-mediated activation of NF κ B and FOXO in muscle [32]; the latter can also be activated by glucocorticoids [19]. Among others, the glucocorticoid receptor activates REDD1 and KLF15 expression, both inhibiting mTOR activity, KLF15 via BCAT2 gene activation, and it also directly upregulates the expression of MuRF-1 and MAFbx resulting in atrophy [33]. Interestingly, NF κ B-inhibition by targeting the IkappaB kinase complex only partially prevents cachexia [34], suggesting some redundancy in catabolic signaling, and NF κ B signaling is activated in cachectic, but not in sarcopenic, muscle [35]. Moreover, IL-1 α and TNF- α block differentiation of human myoblasts into myotubes via TGF- β -activated kinase-1 (TAK-1), which was dependent on the transcriptional induction of activin A and its subsequent signaling via activin IIB receptor and downstream activation of SMAD2/SMAD3 [36]. The transcription factors NF κ B, FOXO, and SMAD2/SMAD3 are considered to be crucial for promoting proteolysis by inducing the transcription of atrogenes including the muscle-specific E-3 ubiquitin ligases MAFbx and MuRF-1 [37]. Induced expression of these two ligases has been used in synonym with increased activity of the ubiquitin-proteasome system (UPS). While MAFbx mainly targets regulatory proteins involved in controlling protein synthesis, e.g., the initiation factor eIF3-f [38], as well as the transcription factor MyoD that is crucial to maintain the differentiated phenotype of adult fast skeletal muscle fibers [39], MuRF-1 has been shown to target the myofibrilles directly [40]. Sarcomeric proteins are degraded in an ordered process in which MuRF1 catalyzes ubiquitylation of thick filament components like myosin heavy chain [40], which becomes accessible after the thin filaments (actin, tropomyosin, troponins) and Z-band (alpha-actinin) components and have been ubiquitylated by the constitutively active E-3 ligase TRIM32, which promotes their degradation by the 26S proteasome [41]. Furthermore, the transcription factor Sox6, which plays an essential role in muscle fiber differentiation by blocking slow fiber associated gene expression, is targeted by the E-3 ligase Trip12 [42]. In C2C12 myotubes, inhibition of Trip12 or the 26S proteasome increased Sox6 protein resulting in a decrease in slow fiber-specific Myh7 expression, while an increased expression in fast fiber-specific Myh4 was detected [42]. Thus, Trip12 may play an important role in the fiber-type switch observed under atrophic conditions [43].

An additional muscle-specific mechanism of atrophy is the intracellular blunting of the anabolic IGF-1 signaling. Upon activation of the IGF-1 receptor IRS-1 is phosphorylated and induces the PI3K/ Akt/ mTOR pathway that induces protein

synthesis [19]. Previous studies have shown that a number of distinct E3 ubiquitin ligases target IRS-1 under different circumstances. SOCS1 and SOCS3 target IRS-1 in inflammation-induced insulin resistance possibly by involving the elongin BC ubiquitin-ligase [44]. Under unloading conditions, i.e., disuse, the ubiquitin ligase Cbl-b is induced and terminates IGF-1 signaling [45]. The culling 7 E3 ligase complex containing the Fbw8-substrate-targeting subunit Skp1 and the ROC1 RING finger protein is thought to play a role in cellular senescence by providing a negative feedback loop to mTOR signaling [46]. Mutated cullin 7 has also been linked to pre and postnatal growth retardation [47]. Recently, Fbxo40 has been described as a new IRS-1 targeting E3 ligases that induces rapid degradation of IRS-1 after stimulation of muscle cells with IGF-1 [48], thereby limiting overall muscle growth by de-sensitizing. Interestingly, the expression of Fbxo40 is muscle specific, being only expressed in myocytes and cardiomyocytes with increasing levels upon differentiation of the muscle cells [48]. The most recent addition to the IRS-1 targeting E3 ligases is muscle-specific mitsugumin 53 (MG53; also called TRIM72), which induces ubiquitination together with the E2 enzyme UBE2H and additionally directly targets the insulin receptor, but not the IGF-1 receptor [49]. Overall, the blunting of IGF-1/IRS-1 signaling not only leads to an inhibition of protein synthesis with concurrent induction of proteolysis, but IRS-1 itself cannot be regenerated after UPS-mediated degradation, thereby leaving the muscle cells unresponsive [29]. Although insulin resistance is noted in many patients with cancer cachexia [50], IGF-1 itself and the IGF-1 receptor are targeted in cancer patients, as they play a role in the growth of several tumors, including pancreatic cancer, which might further impair anabolic signaling in skeletal muscle [51]. However, in non-IGF-1-dependent cancers, IGF-1 supplementation may reduce muscle wasting [52]. Interestingly, some chemotherapeutic drugs state weight loss as side effects, e.g., the mTOR inhibitor temsirolimus, which increased loss of body fat, suggesting a possible end-organ metabolic effect [53].

While the UPS is considered to be the major proteolytic pathway responsible for the breakdown of muscle proteins [54], induction of FOXO1/3 by catabolic stimuli has also been linked to mitochondrial dysfunction and a subsequent loss of mitochondria in skeletal muscle contributing to impaired muscle function [55]. The mitochondrial E3 ligase 1 (Mull1) promotes the fragmentation, depolarization, and clearance of mitochondria through the autophagy-lysosome pathway termed mitophagy. Mull1 is upregulated through a mechanism involving FOXO1/3 transcription factors under catabolic conditions [56]. However, markers of mitochondrial function, e.g., pyruvate dehydrogenase function, in colon cancer patients suggest that muscle mass and mitochondrial enzyme activity are not invariably linked [57] and that the reduction muscle mitochondrial oxidative capacities may be linked to a

decrease in complex IV activity [58]. In denervation models, Mammalian sterile 20-like kinase 1 (MST1) is highly expressed in skeletal muscle and has been suggested to be a key regulator of muscle atrophy, but only affecting the fast fiber type muscle fibers by FOXO3a-mediated induction of MAFbx and LC3; the latter suggesting an activation of autophagy [59].

In addition, another member of the TGF- β family, macrophage inhibitory cytokine-1/growth differentiation factor 15 (MIC-1/GDF15), has been shown to be upregulated by 10–100-fold in some cancers resulting in anorexia by direct actions of the circulating cytokine on feeding centers in the brain [60].

In summary, novel targets identified in the last few years for anti-catabolic intervention strategies include (1) E-3 ligases TRIM32, SOCS1, and SOCS3 by involving the elongin BC ubiquitin-ligase, Cbl-b, culling 7, Fbxo40, MG53 (TRIM72), and the mitochondrial Mull1 and (2) the kinase MST1.

4 News in anabolic signaling

It has been well established that GH, IGF-1, and insulin are potent anabolic factors in skeletal muscle, promoting muscle mass gain. GH primarily regulates liver IGF-1 expression with downstream anabolic effects in skeletal muscle. Insulin and GH are also involved in fat metabolism: GH induces lipolysis and insulin promotes synthesis of fatty acids in the liver and inhibits their degradation in adipose tissue [15]. However, the GH/IGF-1 axis is controlled by various factors, including ghrelin [61], gender, age, diet, exercise, adiposity, and sleep [15]. Ghrelin and its analogues have been shown to inhibit expression of MAFbx and MuRF1 [62]. Independent of the GH/IGF-1/Akt/mTOR axis, other mechanisms inducing muscle hypertrophy have been described. Commonly used β -2 adrenoreceptor agonists originally developed for asthma, e.g., formoterol and salbutamol, have been known for some time to have anabolic properties on skeletal muscle via a direct β -2 adrenoreceptor mechanism that has even let to abuse in professional sports [63]. However, in an experimental cachexia model, formoterol treatment normalized the expression of genes involved in the homeostasis and regeneration of muscle by increasing the expression of Pax7 and follistatin, while decreasing expression of the Sirt1, ActIIB receptor, and myostatin [17, 64]. In combination with the appetite stimulant megestrol acetate, formoterol use in patients with advanced cancer cachexia was well tolerated and muscle mass and/or function were improved to a clinically significant extent in a small phase II study [65].

Aside from the β -2 adrenoreceptor, there are more G-protein-coupled receptors (GPCRs) that have anabolic effects when activated. The corticotropin-releasing factor receptor 2 (CRFR2) induces skeletal muscle hypertrophy when activated, particularly by urocortin 3, which improved muscle function and ameliorated atrophy in several rodent models,

including disuse, denervation, aging, and muscular dystrophy [66]. Binding of Wnt7a to the GPCR Frizzled7 (Frz7) directly activates the Akt/mTOR growth pathway, thereby inducing myofiber hypertrophy in myotubes. The association of the Fzd7 receptor complex with $G\alpha_s$ and PI3K was required for this mechanism and Wnt7a-Fzd7 activation of this pathway was completely independent of IGF-receptor activation [67]. The lysophosphatidic acid (LPA) receptor has been known to trigger Ca^{2+} activation in myotubes and may couple to several G-proteins. However, in human skeletal myotubes, the LPA receptor mainly couples to $G\alpha_i$, as its effects can be blocked with pertussis toxin, an inhibitor of $G\alpha_i$ -mediated signaling [68]. The effects of the LPA receptor were mediated by the $G\alpha_{i2}$ G-protein. Interestingly, the overexpression of a constitutively active mutant of $G\alpha_{i2}$ induced myotube growth by involving NFAT and PKC and inhibited TNF- α -induced atrophy by transcriptional downregulation of MuRF1. Moreover, it was also reported to enhance muscle regeneration and cause a switch to oxidative fibers, making $G\alpha_{i2}$ an interesting drug target independent of any GPCRs [68].

5 Biomarkers

A major difficulty in developing anti-wasting therapy strategies and novel drugs is the precise assessment of skeletal muscle mass and any changes during the studies. Currently, wasting assessment is limited to imaging-related quantification of muscle mass by either magnetic resonance imaging (MRI), computed tomography (CT), or dual energy X-ray absorptiometry scan (DEXA) and functional tests to quantify muscle function. Unfortunately, they are all cost-intensive and only available at big medical centers. Furthermore, these methods allow only detection of wasting, but not of patients at risk of developing muscle atrophy [69, 70]. Therefore, the identification of reliable biomarkers that can be measured in plasma and can be used in a cost-effective manner that guide diagnosis and therapy in routine clinical practice and clinical trials is an important part of further drug development [70].

Unfortunately, in the long list of biomarkers that is assessing a specific mechanism of muscle atrophy, many turn out to be not specific to muscle and only weakly associated with clinically relevant outcomes [70]. However, a number of plasma assessable, specific biomarkers to muscle mass have been identified recently. It has been proposed that serological peptide biomarkers whose tissue and pathology specificity are derived from posttranslational modification of proteins in tissues of interest and represent the most accurate tools for identifying novel, meaningful biomarkers [71]. For example, during collagen synthesis in soft lean tissue, N-terminal propeptide of type III procollagen (P3NP) is released into circulation, and its levels have been described to be associated with subsequent changes in lean mass during testosterone and

GH treatment of elderly patients [72]. Testosterone exposure may also increase skeletal muscle expression of the collagen type III gene that encodes P3NP, which was found to be elevated dose-dependently in plasma, making P3NP a biomarker candidate for muscle anabolism [73]. Another collagen fragment, the type VI collagen N-terminal globular domain epitope, which is an MMP-generated degradation fragment of collagen 6, was tested as a biomarker candidate in a small human immobilization-remobilization study [74]. However, while a correlation was found in young men at baseline and at the end of the study, none was found in elderly [74].

Myostatin seems a natural candidate for an atrophy biomarker as it directly mediates catabolic signaling and is found in plasma [28]. However, the data of a recent study in cardiac cachexia could not confirm a role of circulating myostatin as a biomarker for muscle wasting [75]; thus, more/better validation is needed. Moreover, branched chain amino acids have been suggested as markers, as they are elevated in insulin resistance, although in cancer, they may be unreliable due to the tumor metabolism [76]. Serum creatinine under steady state conditions has been suggested to serve as a reliable muscle mass biomarker, if appropriate adjustment for kidney function and dietary meat intake is undertaken [77].

A recent study used a different approach; rather than trying to identify a new protein/peptide in plasma, it utilized a heavy water-labeled tracer—creatinine-(methyl-d(3)). Its levels in urine were measured after giving rats an oral tracer dose of D(3)-creatinine to determine creatine pool size and skeletal muscle mass. It had a very high bioavailability (>99 %) with low (0.2–1.2 %) urinary spillage and a steady state after 24–48 h [78]. Creatine pool size calculated from urinary D(3)-creatinine enrichment at 72 h, significantly decreased with dexamethasone-induced skeletal muscle atrophy and correlated with lean body mass ($r=0.9590$; $c<0.0001$) [78]. This method has also been tested in humans and provided muscle mass estimates that correlated well with MRI estimates for all subjects ($r=0.868$, $P<0.0001$) [79]. Unfortunately, detection requires isotope ratio mass spectrometry (IRMS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS) technology [80], making it a very accurate tool to monitor changes in skeletal muscle mass, but with limited availability.

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