Cancer Cachexia Signaling Pathways Continue to Emerge Yet Much Still Points to the Proteasome

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Abstract

Cachexia is a life-threatening consequence of cancer that diminishes both quality of life and survival. It is a syndrome that is characterized by extreme weight loss resulting mainly from the depletion of skeletal muscle. Research from the past decades investigating the mechanisms of tumor-induced muscle wasting has identified several key cachectic factors that act through the ubiquitin-dependent proteasome system. Signaling pathways that mediate the effects of these cachectic factors have also subsequently emerged. Here, we review some of these pathways specific to myostatin, nuclear factor κ B, and the newly elucidated dystrophin glycoprotein complex. Although these molecules are likely to employ distinct modes of action, results suggest that they nevertheless maintain a link to the proteasome pathway. Therefore, although the proteasome remains a preferred choice for therapy, the continually emerging upstream signaling molecules serve as additional promising therapeutic targets for the treatment of tumor-induced muscle wasting.

Background

Cachexia is a highly debilitating condition characterized by pronounced weight loss, muscle weakness, anemia, insulin resistance, and extreme fatigue (1). This disease syndrome is tightly associated with cancer and other chronic diseases such as AIDS, heart failure, and inflammatory bowel disease. In advanced cancers, >50% of patients exhibit cachectic symptoms, and remarkably ~20% of cancer-related mortalities derive from cachexia rather than direct tumor burden (2). This wasting condition also lowers responsiveness to chemotherapy and radiotherapy, contributing to poor prognosis and a depreciating quality of life. It is also noteworthy that cachexia does not prevail in all cancer types. Whereas the incidence is low in breast and leukemia, it is highly prevalent in gastrointestinal and lung cancers, in which patients can lose up to 30% of their pre-illness weight (3).

Unlike simple starvation in which weight loss primarily derives from the depletion of fat stores, but protein content from skeletal muscle is preserved, in cachexia neither fat nor muscle is spared. This may explain why nutritional supplements alone have been found to be insufficient in reversing cachexia (2). Evidence from clinical trials has shown that such supplements transiently induce weight gain due to the accumulation of fat but are ineffective in restoring lost skeletal muscle protein. Therefore, successful anticachexia therapy will most likely depend on the use of nutritional supplementation in combination with compounds that show efficacy in either preserving or restoring lean body mass.

Although therapeutic options against this syndrome are still lacking, research in the last two to three decades has made significant progress in elucidating some of the key mediators of muscle wasting associated with cancer cachexia. These include immune and tumor-derived cytokines such as tumor necrosis factor α (TNFα; originally termed cachectin), interleukin (IL)-1, IFNγ, and IL-6, as well as tumor-specific factors such as lipid mobilizing factor and the 24-kDa sulfated glycoprotein proteolysis-inducing factor (PIF; refs. 4, 5). However, in spite of this knowledge, the heterogeneity in the expression pattern of these indicators from different cancer types and their potential synergistic mode of action have made the direct targeting of these factors challenging and yielded little clinical benefit.

Another breakthrough in the field came from the realization that the majority of these cachetic factors regulate skeletal muscle wasting by reducing the rate of protein synthesis at the level of protein translation or RNA content (6) and by stimulating protein catabolism predominantly through the activation of the ATP-dependent ubiquitin-proteasome pathway (7, 8). New findings also suggest that of the myofibrillar proteins implicated in mediating muscle atrophy and the wasting state, myosin heavy chain is a preferred substrate that can be inhibited at the RNA level or degraded through a ubiquitin-associated proteolytic process (9). In addition, there has been significant progress in identifying new signaling pathways that contribute to muscle atrophy that are potentially pertinent in cancer. These include the down-regulated insulin and insulin-like growth factor pathways that lead to Akt inactivation and reversal of muscle hypertrophy, the angiotensin system that operates through the activation of caspases, the
transforming growth factor-β family member myostatin, the p38 mitogen-activated protein kinase, Foxo, nuclear factor κB (NF-κB), activator protein-1, and p53 transcription factors, as well as a newly described pathway involving the dystrophin glycoprotein complex (DGC; refs. 10–17). Interestingly, most, if not all, of these emerging pathways (Fig. 1) seem to mediate their effects through the activation of the ubiquitin proteasome system, which is now most commonly measured through the induction of the ubiquitin E3 atrophy markers, muscle RING-finger-1 (MuRF1), muscle atrophy F-Box (MAFBx) or atrogin-1, and, more recently, E3α-II (18, 19). These findings reinforce that clinical efforts should remain focused on targeting proteasome activity but it also suggests that signaling effectors lying upstream of the proteasome merit consideration as additional therapeutic targets for the treatment of cancer cachexia. In this review, we summarize recent advances in the identification and characterization of a selected set of these signaling pathways relevant in tumor-induced muscle wasting. Due to space constraints, we apologize that we are unable to determine the prospects of the currently available inhibitors as a potential therapeutic option in the clinic.

The NF-κB family of transcription factors comprises a signaling pathway that is pivotal for various cellular processes ranging from inflammation to proliferation and apoptosis (29). Unlike most transcription factors that reside in the nucleus, NF-κB translocates from the cytoplasm to the nucleus in response to specific stimuli to regulate gene expression. NF-κB exists as a homodimer or heterodimer made up of five family members: RelA (p65), RelB, p50/p105 (NF-κB1), p52/p100 (NF-κB2), and c-Rel, of which p65/p50 is the prototypical heterodimer found in most cells. In resting conditions, NF-κB is sequestered in the cytoplasm bound to its inhibitor, IκB. NF-κB activity is regulated by the upstream IκB kinase complex that, in response to a multitude of stimuli including proinflammatory factors TNFα, IL-1β, and PIF, phosphorylates IκBs to induce its degradation and translocation of NF-κB. Transactivation of the p65 subunit can also occur through its posttranslational modifications of serine phosphorylations or lysine acetylations (30).

Research in recent years has elucidated essential functions of this pathway in skeletal myogenesis and muscle disease (31). Probably, the first hint that NF-κB was relevant in cachexia came from studies showing that activation of NF-κB by cacthectic factors TNFα or IL-1β caused a block in muscle differentiation by targeting the myogenic transcription factor MyoD (32, 33). Also deduced was that TNFα, either on its own (34, 35) or in combination with IFNγ (32), caused the reduced expression of myosin heavy chain in cultured myotubes or whole muscles and, at least with dual cytokine treatment, this decline in myosin occurred at the transcriptional level due to the added loss of the myosin transcriptional regulator MyoD (9). Although IFNγ is required for the maximal reduction of MyoD and myosin heavy chain, persistent activation of NF-κB is only mediated through TNFα, suggesting that IFNγ functions in parallel with the TNF pathway to regulate changes in myotube gene expression (36). Because IFNγ stimulates signal transducer and activator of transcription (STAT) nuclear translocation and STAT and NF-κB complexes bind to common promoters, it is likely that these parallel pathways between TNFα and IFNγ involve both transcription factors (37). More recently, TNFα/IFNγ regulation of myotube decay was also shown to occur by the MyoD-stabilizing RNA binding protein HuR (38). In the presence of these cytokines, inducible nitric oxide synthase is induced by a NF-κB-dependent mechanism that sequesters HuR away from the MyoD message, thereby causing MyoD mRNA decay. Although MyoD levels have been shown to be reduced in atrophic muscles in a rodent model of

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Myostatin is a member of the transforming growth factor-β superfamily that represents a potent signaling molecule that functions as a negative regulator of muscle mass (13). Similar to other transforming growth factor-β members, myostatin is secreted as a precursor protein that is cleaved to generate a mature ligand that signals through a type II receptor for activation of SMAD transcription factors (22). Lee (13) has shown that muscles from myostatin-null mice weigh ~100% to 200% more than their control littermates exhibiting fiber hypertrophy, as well as hyperplasia, whereas systemic overexpression of myostatin causes up to 50% loss in skeletal muscle mass resulting in severe cachexia. It was subsequently shown that myostatin modulates these effects on muscles through several mechanisms. During myogenic differentiation, myostatin can inhibit proliferation by modulating cyclin-dependent kinase-2 and p21<sup>cp1</sup> levels, as well as can repress terminal differentiation by down-regulating MyoD (23). In a cachexia condition induced from mice injected with Chinese hamster ovary cells expressing high levels of myostatin, it was shown that myostatin can still act on MyoD but, in addition, also has the capability of inducing Foxo-1 expression leading to the concomitant production of MAFBx/atrogin-1 and proteasomal activity (24).

Although never directly implicated in cancer-induced atrophy other than the Chinese hamster ovary model, unpublished results from our group show that myostatin mRNA is elevated ~2- to 3-fold in cachetic muscles from mice bearing colon-26 tumors. This regulation is similar to the increase in myostatin associated with other chronic atrophy conditions such as in AIDS, aging, and osteoarthritis (25). Based on these observations, it is reasonable to assume that chronic activation of myostatin might play a role in regulating muscle atrophy in cancer. In line with this notion, several groups have attempted to administer myostatin-blocking antibodies and peptides to prevent chronic wasting in mice, but effects were modulated with age in a muscle-specific manner (26, 27). Lee (13) has also shown that a soluble form of the activin type II receptor can act as an alternative strategy for myostatin inhibition resulting in a dramatic increase in muscle mass. Follistatin is yet another secreted protein that interacts with myostatin and antagonizes its action. Like myostatin-null mice, overexpression of follistatin results in a massive increase in muscle mass by 327% both by hypertrophy and hyperplasia, which is surprisingly even more dramatic than myostatin nulls and represents another potential therapeutic approach to target myostatin activity (28). Future studies are therefore required to define the precise contribution of myostatin in cancer cachexia and to determine the prospects of the currently available inhibitors as a potential therapeutic option in the clinic.
cancer cachexia, no changes in NF-κB activity were observed (15), suggesting that NF-κB regulation of MyoD and cachexia in response to cytokines may be more selective to regenerative myoblasts rather than mature myotubes.

Aside from TNFα and IL-1β, recent development also indicates that NF-κB is under the control of PIF, a cachectic factor that was originally identified and purified from tumors and urine samples of cachectic patients (8). Although the exact identity of the PIF receptor remains to be determined, PIF is thought to bind to receptors in the skeletal muscle membrane and initiate a host of signal transduction pathways. Wyke and Tisdale (39) showed that activation of NF-κB in C2C12 myotubes occurred through phosphorylation and degradation of IκBα, leading to nuclear translocation of the p50/p65 heterodimer. Although this activation is relatively modest in comparison with cytokine stimulation, it is sufficient to induce ATP-dependent proteasome activity because addition of the IκBαSR transdominant inhibitor of NF-κB inhibits the proteasome in response to PIF treatment. This suggests that one mode of action of PIF in cancer cachexia is through the activation of NF-κB and regulation of its downstream genes.

Interestingly, an n-3 fatty acid found in fish oil, known as eicosapentaenoic acid, has shown promising results in reversing muscle wasting by interfering with PIF-induced NF-κB activation and proteasomal degradation both in vitro and in rodent cancer cachexia models (5). The results, however, have been equivocal in cancer patients suffering from cachexia. Although initial trials with eicosapentaenoic acid reduced lean muscle loss and prolonged survival, more recent clinical trials with
either short-term treatments for 2 weeks or longer treatments for 4 or 8 weeks have not been as effective in reducing significant weight loss (40). However, in a recent double-blind, placebo-controlled randomized trial done by Fearon et al. (41) that involved more than 500 patients with either lung cancer or gastrointestinal cancers, a clinically relevant trend of weight gain was observed with a 2-g dose of eicosapentaenoic acid. It is also likely that a greater clinical benefit will derive from combinational therapies. One such possibility might be the addition of the leucine metabolite β-hydroxy-betamethylbutyrate, which, together with eicosapentaenoic acid, was recently shown to increase protein synthesis and reduce protein catabolism in an animal model of cancer cachexia (42). Noteworthy are results from a separate clinical trial that showed that although oral supplements enriched with n-3 fatty acids and antioxidants did not show a clear therapeutic advantage over control supplements in pancreatic cancer patients, a trend towards increased lean muscle mass was evident when compounds were taken in sufficient quantities (43). Such studies highlight the need for more clinical trials that may require a more homogeneous patient population with comparable stage history and tumor type to determine the optimal dose range and formulation.

Another extracellular molecule recently examined for its ability to activate NF-κB was myostatin. Although such analyses were done in vitro, findings from both our group and the Kambadur laboratory showed that myostatin-mediated inhibition of myogenesis and myotube atrophy is unlikely to occur via NF-κB (24, 44). In addition, although the myostatin promoter contains a NF-κB DNA consensus sequence, activation of NF-κB by TNFα and IL-1β in C2C12 myoblasts or myotubes does not induce myostatin expression (44), suggesting that the ability of NF-κB to regulate myogenesis and muscle turnover is most likely not through myostatin regulation. Collectively, these data suggest that NF-κB and myostatin inhibit myogenesis and regulate muscle turnover through distinct pathways.

More solid evidence for the involvement of NF-κB in tumor-induced muscle wasting has come from an elegant study done by Cai et al. (14). These investigators genetically confirmed the role of the NF-κB pathway in atrophy and showed that a muscle-specific transgenic overexpressing IκB kinase-β resulted in severe atrophy, which was mediated by enhanced proteosomal degradation of muscle proteins regulated, in part, by MuRF1. Atrophy was rescued on crossing the IκBα pathway in atrophy and showed that a dominant negative. In this study, IκBα transgenic mice with IκBα further support the role of this transcription factor in tumor-regulated muscle wasting (16, 45). Very recent evidence from Mourkioti et al. (46) showed that the converse experiment to the Shoelson study, wherein IκB kinase-β was conditionally deleted in muscle, protected against atrophy in response to denervation. Such data make a compelling argument for the contribution of NF-κB in muscle wasting. Chronic NF-κB activity has also been implicated in disuse atrophy, but, unlike the former studies, hind limb unloading of mice resulted in an activation of nonclassic p50 and Bcl-3 members of the NF-κB and IκB families (47). Genetic disruption of either p50 or Bcl-3 was further found to be sufficient to significantly rescue the atrophy phenotype associated with unloading. Although distinct in the nature of the atrophy stimulus and the NF-κB subunit composition, some of the commonalities between the two atrophic states were the involvement of IκBα, the apparent lack of regulation of proinflammatory genes, and the activation of the E3 ubiquitin ligase MuRF1 (14, 48). Given the distinct gene regulation profile by different NF-κB members, it begs the question about which NF-κB pathway is actually relevant in cancer patients with cachexia and how NF-κB mediates atrophy in such a pathophysiologic state involving a complex interplay between catabolic factors.

A more recent addition to the growing list of signaling molecules relevant in cancer cachexia is the muscular dystrophy–associated membrane complex DGC (16). The DGC is thought to form a mechanical as well as signaling link from the extracellular matrix to the cytoskeleton (49). Members of this complex, such as dystrophin and sarcoglycans, incur gene mutations that give rise to various forms of muscular dystrophies. In Duchenne muscular dystrophy, for example, mutations in dystrophin lead to reduced or absent protein expression that results in the loss of the DGC and a weakened sarcolemma. Weakened myofiber membranes, in turn, undergo contraction-induced ruptures that elicit an immune response and subsequent myofiber necrosis culminating in chronic muscle degeneration. In contrast, tumor-induced catabolic muscle fibers display little resemblance to this dystrophic phenotype, but defects in the myofiber outer membranes are detectable, and alterations in extracellular matrix proteins are also evident (16). Such changes in the membranes correlated with a reduction of the dystrophin protein and a posttranslational modification of other DGC members that occurred at around the same time as the induction of E3 ubiquitin ligase genes, MuRF1 and MAFBx/atrogen-1, but importantly before the initiation of muscle atrophy. Furthermore, genetic evidence using muscle-specific dystrophin transgenic mice showed that the restoration of DGC in a cancer condition was sufficient to decrease E3 ubiquitin ligase expression and rescue tumor-induced atrophy. In regards to clinical relevance, DGC changes correlated positively with cachexia and negatively with survival in patients with gastrointestinal cancers with varying degrees of weight loss.

Although more studies are warranted to validate these findings in cachetic patients with different types of cancers, it is nevertheless tempting to think that boosting dystrophin levels, as is currently being attempted in muscular dystrophy, may be used as an intervention against muscle wasting in cancer (50). Other questions remain, however, about how tumor factors cause the deregulation in the DGC. We speculate that this deregulation is unlikely to result solely from cytokine action because C2C12 myotubes treated with various concentrations of TNFα, IL-6, or IFNγ did not generally affect dystrophin or dystroglycan expression.\

1 Acharyya and Guttridge, unpublished observations.
that the deregulation of the DGC is not the cause of myofiber membrane alterations but rather is downstream of this alteration. If this is true, it would be predicted that alterations in the membrane and extracellular matrix occur before the changes in the DGC. At this point, it is not known when these changes are initiated and how they are mediated, but it is noteworthy that microarray analysis from various animal models of cachexia has indeed revealed a significant reduction in genes coding for extracellular matrix proteins (51). As eluted to above, it remains possible that the diminishment of these proteins might be sufficient to adversely affect the structural assembly or signaling capacity of the DGC. Support for this latter model comes from laminin α2–deficient muscles, which are known to display disrupted DGC function resulting in a severe form of muscular dystrophy (32). Progress in this area of DGC regulation, as well as others, will be required to determine its specificity to cancer cachexia and mechanism of action. Nevertheless, such studies are examples that other cachetic signaling pathways in cancer are likely to continue to emerge, and whether their mode of action resides through the regulation of ubiquitin proteasome system remains to be investigated. Such findings will not only be vital to our understanding of the role of the proteasome in cancer-induced muscle wasting but may also lead to the identification of potentially new targets that can be used for the treatment of this cancer.

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