InACTIVatIng cancer cachexia

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Summary of and comment on a recent Cell paper entitled ‘Reversal of cancer cachexia and muscle wasting by ActRIIB antagonism leads to prolonged survival’ (Zhou et al., 2010).

Cachexia is a debilitating muscle- and fat-wasting condition that is associated with multiple diseases, including cancer, diabetes, AIDS and tuberculosis, as well as injuries. Estimates reveal that up to 80% of all cancer patients suffer from cachexia, which not only leads to a decrease in the quality of life due to impaired mobility but also accounts directly for ~20% of cancer-related deaths (Fearon, 2008; Tisdale, 2009). Death from muscle wasting occurs mostly as a consequence of impaired respiratory and heart muscle function. The highest incidence of cachexia and associated weight loss is seen in patients with solid tumors, such as those with pancreatic, gastric or lung cancers: for instance, 85% of pancreatic cancer patients exhibit such a high degree of cachexia that it is one of the diagnostic features of the disease (Fearon, 2008; Tisdale, 2009). In a study of lung cancer patients who lost 30% of their pre-illness body weight, it was found that their body composition changed dramatically with respect to body fat mass (which decreased by 85%) and skeletal muscle protein mass (which decreased by 75%) (Preston et al., 1987). Such drastic losses in the amount of skeletal muscle and body fat have potentially large implications on whole body metabolism.

Under normal conditions, adult skeletal muscle retains its mass owing to a balance between the signals that regulate muscle hypertrophy (increased muscle protein synthesis) and atrophy (increased muscle proteolytic degradation). Stimuli such as exercise or steroids can shift this balance towards muscle hypertrophy, whereas muscle disuse or disease states such as cachexia can cause muscle atrophy. Studying how this balance is maintained normally and how it is disrupted in disease conditions will be crucial for understanding and developing treatment regimes for skeletal muscle cachexia. Some of the signaling molecules that are important in regulating this balance have been identified and are discussed below. Currently, there are no efficient treatments that specifically target skeletal muscle cachexia, although drugs such as steroids that increase appetite can be administered, but with limited success on reducing muscle wasting.

Mice are an excellent model for the study of cancer cachexia, as several mouse models of cancer exhibit this muscle wasting condition (Matzuk et al., 1994; Lazarus et al., 1999). Furthermore, human cancer cells can be transplanted onto immunocompromised nude mice, and such heterologous models are helpful for studying how human cancer cells might cause muscle wasting in vivo (Cannon et al., 2007). Moreover, several pathways leading to cachexia have been well characterized in mice (discussed below). Finally, the availability of mice carrying mutations in genes that are associated with cachexia make mice an optimal model organism for investigating this condition.

Multiple studies indicate that muscle atrophy during cachexia is primarily regulated by the ubiquitin-proteasome proteolytic pathway, and that two muscle-specific E3 ubiquitin ligases, atrogin-1 (also known as MAFbx and F-box only protein 32) and MuRF1, play crucial roles in this process (Zhao et al., 2007; Cohen et al., 2009). Because these E3 ubiquitin ligases function downstream in the pathway that leads to muscle atrophy, an important focus has been to identify upstream signaling molecules that lead to activation of atrogin-1 and MuRF1.

One such upstream factor is the Akt signaling pathway: its capacity to inhibit the activity of FOXO transcription factors was shown to regulate the expression of the genes encoding atrogin-1 and MuRF1 (Sandri et al., 2004; Stitt et al., 2004). However, other modes of protein degradation, such as lysosomal pathways or even cell death, might also be involved in cachexia. In addition, pro-inflammatory cytokines such as TNFα and IL-6 have also been suggested to promote cancer cachexia (Tisdale, 2009).

Another signaling pathway that has long been recognized to affect skeletal muscle atrophy-hypertrophy balance is the transforming growth factor-β (TGFβ) pathway. This signaling pathway comprises numerous extracellular ligands (such as activin A, myostatin, nodal), transmembrane receptors [such as activin receptor type IIB (ActRIIB), TGFβ receptor type III] and intracellular signal transducers (SMAD proteins) (Schmierer and Hill, 2007). TGFβ signaling is initiated when an extracellular ligand binds its type II receptor dimer, which in turn leads to the recruitment of a type I receptor dimer to form a heterotetrameric activated receptor complex that enables recruitment and activation of cytoplasmic SMAD proteins, eventually leading to the regulation of gene transcription (Schmierer and Hill, 2007). Previous studies demonstrated that signaling through the activin receptor is crucial for regulating muscle atrophy-hypertrophy balance, with increased signaling being thought to promote cachexia. For instance, expression of a dominant-negative activin type II receptor, ActRIIB, in mice results in skeletal muscle hypertrophy (Lee and McPherron, 2001; Lee et al., 2005). Moreover, loss-of-function mutations in myostatin, which binds ActRIIB, lead to skeletal muscle hypertrophy, as observed in ‘double-muscled’ cattle, dogs and humans that carry such mutations (McPherron et al., 1997). Conversely, overexpression of myostatin in adult mice leads to muscle wasting (Zimmers et al., 2002). Loss of inhibin, another TGFβ family ligand, which competes with activin to bind ActRIIB and therefore is an antagonist to activin signaling, in mice can cause both tumor formation and cachexia, providing further evidence for the role of the TGFβ signaling pathway in regulating the balance between muscle atrophy and hypertrophy (Matzuk et al., 1994). However, although several lines of evidence link TGFβ signaling...
(specifically activin signaling) to skeletal muscle cachexia, it is not clear which downstream pathways are activated and whether the ubiquitin-proteasome pathway is activated in response to activin signaling.

In a recent study (Zhou et al., 2010), the authors defined the mechanism by which ActRIIB and its ligands regulate cachexia by studying multiple mouse models of cancer. They also investigated the potential therapeutic effects on cancer cachexia of blocking signaling through the ActRIIB signaling pathway. To antagonize the ActRIIB pathway, they engineered a decoy receptor containing the extracellular portion of human ActRIIB fused to the Fc region of IgG2, designated sActRIIB (soluble ActRIIB-Fc). The authors administered this decoy receptor to tumor-bearing mice to antagonize the ActRIIB pathway in four distinct mouse models of lethal cancer cachexia: colon26 (C26) murine adenocarcinoma-bearing mice, inhibin-α knockout mice (which develop gonadal tumors) and immunocompromised nude mice bearing either human G361 melanoma or TOV-21G ovarian carcinoma xenografts.

Administration of the sActRIIB decoy receptor to tumor-bearing mice prevented further skeletal muscle wasting and also completely and rapidly reversed weight to normal levels, leading to a striking increase in survival compared with tumor-bearing control animals that did not receive sActRIIB. The effect of sActRIIB treatment was restricted to recovery of skeletal muscle, and did not affect fat loss. In addition, the treatment did not affect the size or weight of the tumor, demonstrating that the effect of sActRIIB was specific to skeletal muscle cachexia and had no effect on the tumor itself. The authors found that, depending on the type of tumor, the levels of two ligands of ActRIIB – myostatin or activin (or both) – are elevated in tumor-bearing mice, and that administration of sActRIIB brings them back to normal levels. The finding that elevated serum levels of the pro-inflammatory cytokines TNFα, IL-6 and IL-1β in tumor-bearing mice were not decreased after treatment with sActRIIB.

**Fig. 1. The activin signaling pathway in skeletal muscle.** Schematics of the activin pathway under normal conditions (A), cancer cachexia (B) and treatment with the sActRIIB decoy receptor in cancer cachexia (C) are shown. (A) Myostatin and activin, activating ligands of the activin pathway, and the inhibitory ligand inhibin are able to bind the ActRIIB receptor, causing a balanced activation of the pathway so that SMAD2 is phosphorylated, which causes FOXO3A to be activated by dephosphorylation, inducing the activation of muscle-atrophy-associated E3 ubiquitin ligases atrogin-1 and MuRF1 to steady-state levels. The hypertrophy signals mediated by the muscle satellite cells and other factors are also at steady state levels, leading to a balance between muscle atrophy and hypertrophy under normal conditions. (B) However, during cancer cachexia, higher levels of myostatin and activin hyperactivate the pathway, resulting in increased levels of phospho-SMAD2 and dephosphorylated FOXO3a, causing overinduction of atrogin-1 and MuRF1, resulting in the balance shifting towards muscle atrophy. (C) Treatment with sActRIIB during cancer cachexia causes myostatin and activin to be sequestered by this decoy receptor, leading to reduced availability of these ligands to activate the ActRIIB receptor, which leads to decreased activin signaling, thereby preventing muscle atrophy. It is not clear whether inhibin binds sActRIIB. An increase in proliferation by the satellite cells in this situation shifts the balance towards muscle hypertrophy.
indicates that the observed recovery from cachexia did not occur owing to changes in the levels of these cytokines.

To directly test the role of activin signaling on cachexia, Chinese hamster ovary (CHO) cells expressing high levels of activin A, a ligand for ActRIIB, were transplanted intra-muscularly into immunocompromised mice (Zhou et al., 2010). This resulted in cachexia characterized by weight loss, anorexia, muscle wasting and death. If an anti-activin-A neutralizing antibody was administered to these animals, the cachexia and death could be prevented, further indicating that the activin signaling pathway is crucial in mediating skeletal muscle cachexia. This result using a neutralizing antibody that blocks activin signaling at the ligand level validates the results obtained using sActRIIB to block signaling at the receptor level.

To identify the mechanism by which activin signaling leads to increased muscle protein catabolism and thereby to cachexia, the authors compared ubiquitin-proteasome pathway activation in muscles from cachectic animals with and without treatment with sActRIIB. Interestingly, they found that the levels of the muscle-specific E3 ubiquitin ligases atrogin-1 and MuRF1, as well as ubiquitin, were elevated in atrophying muscles. Importantly, this induction of muscle-atrophy-related gene expression was eliminated upon sActRIIB treatment. They also found that the level of phospho-SMAD2, a downstream transcription factor in the activin signaling cascade, was increased in cachetic muscles but could be brought back to normal by sActRIIB treatment. Because the myostatin-activin-SMAD cascade has been shown to activate FOXO transcription factors, among which FOXO3a is a crucial activator of muscle-atrophy-related gene expression, the authors investigated the level of active FOXO3a in cachetic skeletal muscle from control and sActRIIB-treated animals. They found that there was an increase in the amount of total FOXO3a and decrease in that of inactive FOXO3a in cachetic skeletal muscle, and that sActRIIB treatment reduced this effect. Thus, activation of the activin signaling pathway during cachexia results in upregulation of FOXO3a activity, which in turn induces the ubiquitin-proteasome proteolytic muscle-atrophy-related gene expression, resulting in muscle wasting (Fig. 1).

Because sActRIIB treatment not only prevents muscle atrophy but also rescues and causes hypertrophy of muscle, the authors investigated whether adult muscle stem cells (called satellite cells) played a role in the observed hypertrophy response. Satellite cells are normally quiescent and start proliferating only in response to injury or a hypertrophy cue. However, in cachetic mice treated with sActRIIB, a dramatic increase in proliferation of the satellite cells was observed, thereby explaining the skeletal muscle hypertrophy response. Thus, blocking the activin signaling pathway prevents muscle atrophy and also initiates a hypertrophy response mediated by the skeletal muscle satellite cells.

This study establishes the link between the TGFβ family activin signaling pathway and muscle-atrophy-associated activation of the ubiquitin-proteasome proteolytic system. In addition, it also indicates that the activin signaling pathway might be a therapeutic target for reversing muscle wasting in cancer cachexia. It will be important to assess the effect of sActRIIB on other TGFβ family ligands for its specificity, as well as to investigate off-target effects of downstream signaling on other processes in which the TGFβ pathway is involved, before this decoy receptor can be developed as a viable treatment for cancer cachexia. Future studies are required to find the pathways that regulate loss of fat mass in cancer cachexia, and to verify whether the same pathway operates in skeletal muscle cachexia associated with diseases other than cancer, and during injury.

REFERENCES


