A signaling role for dystrophin: Inhibiting skeletal muscle atrophy pathways

Skeletal muscle atrophy is a common comorbidity of cancer. The cellular signaling mechanisms that regulate muscle size constitute a balance of the protein breakdown pathways upregulated during atrophy, and the protein synthesis pathways that are activated during skeletal muscle hypertrophy. In this issue of *Cancer Cell*, Acharyya et al. demonstrate a new and surprising regulatory axis that is centered around dystrophin, the protein that is mutated in settings of muscular dystrophy. These data reposition dystrophin as a signaling protein and connect an important cellular complex required for the structural integrity of muscle to the pathways that modulate muscle size.

A common feature of cancer is a syndrome known as "cachexia," which is a body-wasting syndrome, and which prominently features skeletal muscle atrophy. The loss of skeletal muscle during cancer causes a profound fragility in the patient. This fragility complicates therapy and may accelerate death as a result of atrophy of the diaphragm muscle. Loss of function in the diaphragm is also the eventual cause of death in a series of conditions that have been thought to be distinct from atrophy syndromes: muscular dystrophies. In dystrophic settings, there is an obvious loss of structural patency, whereas in atrophy syndromes a similar structural perturbation has not been previously appreciated; instead, it was thought that fibers merely decrease in size, without an obvious disregulation in structure.

Components of the dystrophin glycoprotein complex (DGC) have been shown to be mutated in Duchenne/Becker forms of skeletal muscle dystrophy. The DGC helps to anchor the muscle cytoskeleton to the cell membrane via dystrophin and its binding partners; on the surface of the muscle, α-dystroglycan, which is part of the DGC, binds laminin-2 in the extracellular matrix (Ibraghimov-Beskrovnaya et al., 1992). Therefore, the DGC provides a means of communicating from the extracellular matrix to the cytoskeleton. While the DGC may have originally been thought to play a purely structural role, it had been suggested that the complex may stimulate signaling pathways and thereby function as more than a structural element, since disruption of dystroglycan binding to laminin was shown to inhibit activation of Akt signaling (Langenbach and Rando, 2002); these data were of particular interest, since Akt had been previously shown to be sufficient to mediate skeletal muscle hypertrophy (Lai et al., 2004; Rommel et al., 2001). What was unclear from the previous finding was the mechanism by which laminin was signaling; for example, laminin also stimulates activation of integrin receptors (Ignatius and Reichardt, 1988), and therefore it was quite reasonable to suppose that it was the integrin pathway, and not the DGC, that was mediating the signaling disruption. In this issue of *Cancer Cell*, a study is published that provides the critical evidence demonstrating that dystrophin itself is capable of mediating signals relevant to muscle atrophy and hypertrophy (Acharyya et al., 2005).

The signaling pathways mediating skeletal muscle atrophy have only recently begun to be defined. Protein breakdown increases during atrophy, and inhibition of the proteasome blocks these increases (Tawa et al., 1997), suggesting that ubiquitin-mediated proteolysis is required for the degradation increases seen during atrophy—this idea was also supported by recent data that showed that treatment with the proteasome inhibitor Velcade can reduce muscle loss under atrophy conditions (Krawiec et al., 2005). It is now appreciated that ubiquitination is an exquisitely modulated process—specificity of protein substrates that are targeted for breakdown

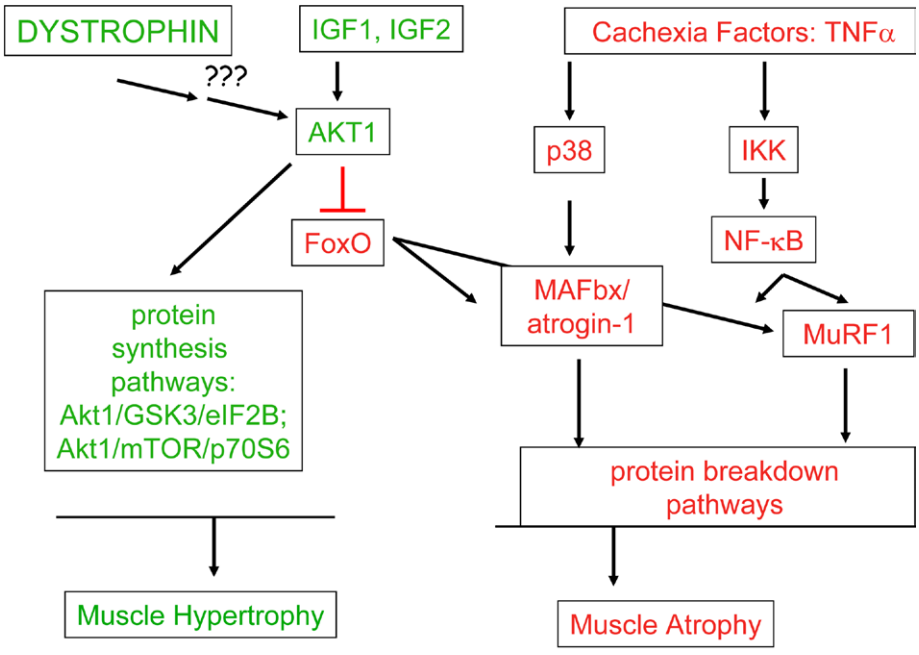

Figure 1. Signaling diagram illustrating skeletal muscle hypertrophy and atrophy signaling pathways

Modulators that increase skeletal muscle mass are in green; proteins that mediate the loss of muscle mass and that are activated during atrophy are in red. A possible position for dystrophin is illustrated, since disruption of the DGC was previously shown to result in a downregulation of Akt signaling, and since Akt is necessary to inhibit upregulation of the atrophy markers MuRF1 and MAFbx. In the current study, maintenance of dystrophin is demonstrated to block atrophy, and this effect is coincident with an inhibition of MuRF1 and MAFbx upregulation, thus giving support for the model as diagrammed. However, there are other possibilities, including a direct effect of dystrophin on the ubiquitin ligases; more study is required to conclusively demonstrate the mechanism mediating the dystrophin effect.

by the proteasome is controlled by several hundred substrate-specific E3 ubiquitin ligases. Expression of two E3 ubiquitin ligases in particular increases significantly in multiple models of skeletal muscle atrophy: MuRF1 (Bodine et al., 2001) (for Muscle RING Finger 1) and MAFbx (Bodine et al., 2001) (for Muscle Atrophy F-box; also called atrogin-1 [Gomes et al., 2001]). MuRF1 and MAFbx are more than strong markers of the atrophy process; knockout of either gene perturbs muscle atrophy—loss of MuRF1 results in sparing of functional muscle under atrophy conditions (Bodine et al., 2001), whereas MAFbx is required for the maintenance of myofiber integrity during atrophy conditions (our unpublished data).

As for signaling, MuRF1 was shown to be upregulated by the NF-κB pathway (Figure 1), which is activated by cachectic agents that can induce atrophy, such as TNFα (Ladner et al., 2003). NF-κB in muscle is also activated by disuse (Hunter et al., 2002), indicating that it might play a role in physiological pathogenesis. Activation of the NF-κB pathway was demonstrated to be sufficient to induce significant atrophy, as measured by increases in in vivo amino acid excretion and tyrosine turnover in isolated muscles (Cai et al., 2004), and blockade of the NF-κB pathway can significantly inhibit the amount of atrophy seen in physiological settings such as denervation- and tumor-induced atrophy (Cai et al., 2004).

The transcriptional upregulation of *MAFbx* and *MuRF1* that is seen under atrophy conditions can be antagonized by simultaneous treatment with the hypertrophy-inducing protein growth factor insulin-like growth factor 1 (IGF-1) (Sandri et al., 2004; Stitt et al., 2004), acting through the PI3K/Akt pathway; this finding demonstrated a novel role for Akt—in addition to stimulating skeletal muscle hypertrophy via activation of protein synthesis pathways, Akt stimulation could dominantly inhibit the induction of atrophy signaling (Figure 1). The mechanism by which Akt inhibited *MAFbx* and *MuRF1* upregulation was demonstrated to involve the FOXO family of transcription factors (Sandri et al., 2004; Stitt et al., 2004). In myotubes, FOXO transcription factors are excluded from the nucleus when phosphorylated by Akt, and translocate to the nucleus upon dephosphorylation. The translocation and activity of FOXO transcription factors are required for upregulation of MuRF1 and MAFbx, a finding that was subsequently supported by the transgenic expression of FOXO1, which resulted in atrophic phenotype (Kamei et al., 2004).

In the current study, under atrophy conditions—in this case, atrophy was stimulated by introduction of a cachexia-inducing tumor—there was a perturbation of the myofibrillar membrane, which indicated a possible loss of structural integrity in the DGC; this was supported by the finding that the resultant atrophic muscle underwent a significant decrease in dystrophin levels (Acharyya et al., 2005). The authors then asked whether wasting was enhanced by a lack of dystrophin, and therefore turned to the MDX mouse model of muscular dystrophy, in which dystrophin is mutated. While it was interesting that there was an enhanced loss of muscle mass in these animals, one might have argued that such a result was not surprising, since MDX muscle is damaged to begin with. However, the authors went further, and studied a transgenic animal that overexpressed dystrophin. Under atrophy conditions, there was significantly less muscle loss in the transgenic animal, indicating that maintenance of dystrophin levels is sufficient to inhibit a significant amount of muscle loss. The authors demonstrated that dystrophin was blocking atrophy by inhibiting the previously established atrophy pathways, since upregulation of *MuRF1* and upregulation of *MAFbx* was also inhibited in the dystrophin transgenic relative to wild-type animals, under atrophy conditions (Acharyya et al., 2005).

Since it has already been shown that Akt can block upregulation of *MuRF1* and *MAFbx*, and that disruption of the DGC can block Akt, it is appealing to speculate that this is the pathway by which dystrophin is operating in the current study; while the authors suggest this possibility, they also hold out the option that dystrophin may be modulating other signaling mechanisms, including an Akt-independent downregulation of *MuRF1* and *MAFbx*. Further studies will help to distinguish among these possibilities, but for now it is of great interest to note that proteins that play important roles in maintaining skeletal muscle structural integrity may nonetheless also be important for cell signaling, and therefore should not be dismissed as pure architecture.

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Selected reading

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