Chapter 4: General discussion, perspectives and conclusion

General discussion

Our work shows that deletion of Mstn gene protects skeletal muscle from glucocorticoid-induced atrophy, partially through inhibition of proteolysis. In addition, inhibition of Mstn by FS causes muscle hypertrophy, through satellite cells recruitment and increased protein synthesis. We also show that Act, another FS ligand, may contribute to the FS hypertrophic effect. Taken together, these observations indicate that inhibition of Mstn and Act may constitute a promising approach to combat muscle atrophy.

Role of Myostatin in the glucocorticoids-induced muscle atrophy

In the first part of our work, we have demonstrated that Mstn gene deletion prevents muscle atrophy caused by glucocorticoid.

The absence of muscle atrophy in Mstn KO animals is associated with blockade of the upregulation of atrogens expression and proteasomal activity normally induced by glucocorticoid administration. This observation suggests that the protective effect on muscle mass is partly due to the inhibition of muscle proteolysis. These data agree with the observation that Mstn activates the ubiquitin-proteasome pathway, one of the major proteolytic system in the skeletal muscle. Indeed, Mstn has been shown to increase the expression of several atrogens together with the ubiquitination of muscle proteins in myotubes (1). Our work however did not include a direct measure of muscle proteolysis, which is a critical point because changes in atrogens do not necessarily parallel the rate of proteolysis. Another mechanism by which the absence of Mstn could prevent the glucocorticoid-induced muscle atrophy is the stimulation of the muscle protein synthesis. Indeed, Mstn deletion or inhibition has been associated with a higher rate of protein synthesis (2) probably mediated through the upregulation of the mTOR/p70S6K pathway (3;4). Since inhibition of p70S6K is thought to be one of the major mechanisms by which glucocorticoid cause muscle atrophy (5;6), it is possible that the prevention of muscle atrophy by Mstn gene deletion results from the maintenance at normal levels of p70S6K phosphorylation which could blunt the inhibition of protein synthesis caused by glucocorticoids. In our experiment, however, we did not investigate the possibility for Mstn deletion to blunt the glucocorticoid inhibitory effect on muscle protein synthesis nor phosphorylation of mediators of the mTOR/p70S6K pathway. Further works will be therefore necessary to evaluate the role of protein synthesis in the anti-

atrophic effect of the Mstn blockade. This is particularly important, since the weight of evidence suggests that Mstn affects myofiber size more by affecting protein synthesis rather than degradation (7). Besides protein synthesis and proteolysis, muscle cell differentiation contributes also to muscle growth. Actually, both glucocorticoid and Mstn are known to inhibit muscle cell differentiation. More specifically, glucocorticoids and Mstn have been shown to downregulate the expression/activity of the myogenic regulatory factors MyoD and myogenin (1;8-14). As glucocorticoids are known to stimulate the expression of Mstn, it was tempting to hypothesize that this upregulation of Mstn could mediate the inhibitory effect of glucocorticoids on muscle cell differentiation. In our experiment we observed however that the expression of these two myogenic factors is downregulated in response to glucocorticoids even in the absence of Mstn. This observation indicates therefore that the inhibitory effect of glucocorticoids on these myogenic factors and probably on muscle cell differentiation is not mediated by Mstn. Moreover, these data confirm that our Mstn KO mice are still sensitive to glucocorticoids treatment. The protective effect of Mstn gene deletion towards muscle atrophy caused by glucocorticoid has to be compared with the protective effect of IGF-I overexpression observed in the same model of muscle atrophy (15). Together, these studies suggest that Mstn and IGF-I act on a common pathway regulating the muscle mass development, a pathway which is impaired by glucocorticoids. Recent observations have confirmed this hypothesis. Indeed, while IGF-I stimulates muscle growth by activating the phosphorylation of Akt (16), Mstn causes atrophy by inhibiting the phosphorylation of this key mediator (7). Whether Mstn deletion prevents glucocorticoid-muscle atrophy by preventing down regulation of Akt phosphorylation, as IGF-I overexpression does, has not been assessed yet. Although Mstn and IGF-I share common pathways to regulate muscle development, their effects might be additive as suggested by the observation that muscle hypertrophy caused by transfection of dnALK4, the co-receptor activated by Mstn, is enhanced in transgenic mice overexpressing caAkt compared to WT animals (17).

Role of satellite cells in the follistatin-induced muscle hypertrophy

In the second part of our work, we have demonstrated the crucial role of satellite cells in the muscle hypertrophy caused by FS overexpression.

This finding is in agreement with the well-established inhibitory effect of Mstn on the proliferation of SC (18). Indeed, several pieces of evidence strongly support the role of SC proliferation in the muscle hypertrophy induced by Mstn inhibition (19-23). First, the

percentage of BrdU-positive SCs isolated by Percoll gradient is higher in muscle from Mstn KO mice than from WT mice (21). Similarly, the number of Brdu-positive SCs is higher in single myofibers isolated from Mstn KO mice than from WT mice (23). Second, SCs isolated from Mstn KO mice proliferate faster than SCs isolated from WT mice (20;21). Moreover, addition of recombinant Mstn to SCs isolated from Mstn KO mice reduces the proliferation rate to that of SCs isolated from WT mice (20;21). Similarly, addition of recombinant Mstn to myofibers isolated from WT muscles results in reduced SC proliferation as measured by BrdU incorporation (22). Third, the absence of Mstn also promotes the migration of SCs from single isolated myofibers to the wells. Conversely, this migration of SCs is inhibited by Mstn addition in a dose-dependent manner (21). Finally, a higher proportion of SC nuclei, highlighted by CD34 immunostaining, is found among total nuclei of myofibers isolated from Mstn KO mice compared to WT mice (21;23). However, the total number of myonuclei per fiber was not assessed in these studies.

Nevertheless, this view has been recently challenged by a very recent publication of H Amthor *et al.*. Using Pax-7 immunostaining, these authors reported a smaller number of SC per myofiber isolated from Mstn KO mice compared to WT mice (24), a result which contrasts to those obtained by R Kambadur's group. It should be kept in mind however that the results reported by H Amthor were obtained on EDL muscle in contrast to those obtained by R Kambadur *et al*. which assessed the tibialis anterior muscle. More importantly, the different mode of expression of the results, number of SCs per 100 myonuclei (for R Kambadur) vs per fiber (for H Amthor), might explain the discrepancy between the two authors. Indeed, the number of myonuclei reflects the size of fiber, only if the myonuclear domain remains unchanged. However, the coexistence of decreased number of myonuclei per fiber, as observed by H Amthor, together with enlarged area and length fiber indicates that the absence of Mstn causes an increase in the size of the myonuclear domains. All together, the decrease in SCs number per fiber together with the increase of the myonuclear domains observed in KO Mstn mice led H Amthor *et al.* to conclude that the muscle hypertrophy observed in Mstn KO animals does not require SC recruitment.

In his work, however, H Amthor has made several observations which contrast to previous publications. First, he could not demonstrate any inhibitory effect of Mstn on proliferation of SCs distributed around isolated myofibers from WT animals. It is however possible that the production of endogenous Mstn, present in WT animals, blunts the response to exogenous Mstn. Indeed, Wagner *et al* was able to demonstrate an inhibitory effect of Mstn on the

proliferation on SCs only in cells isolated from KO mice (20). Second, he observed that the muscle hypertrophy of Mstn KO mice results only from fiber hypertrophy (24) without any increase in the number of fibers. The animal model that he used is the one established in the strain C57BL/6 by Se-Jin Lee and Alexandra McPherron (25). In their original description, McPherron *et al* described a muscle hypertrophy resulting mainly from an increased muscle fiber number (+86%) (hyperplasia) and to a lesser extent from an increased fiber size (hypertrophy). Accordingly, they observed a 50% increase in the DNA content of the skeletal muscles of these animals (25). These observations make it likely that such muscle hypertrophy requires SCs recruitement. However, the muscles studied by McPherron did not include the EDL, which is precisely the only one studied by Amthor *et al*.. It is not known if the relative contribution of fiber hyperplasia versus fiber hypertrophy in the muscle size increase induced by Mstn gene deletion is dependent on the nature of the muscle.

The contribution of SCs proliferation to the muscle hypertrophy seems to be dependent on the stimulus used to induce muscle growth. Indeed, while the inhibition of the SC proliferation capacity by gamma irradiation totally prevents the muscle hypertrophy induced by muscle overloading (26-28) and strength training (29), it decreases only 50% of the muscle hypertrophy induced by IGF-I overexpression (30) or reloading after hindlimb suspension (31). At the extreme, muscle hypertrophy induced by administration of beta-agonists such as clembuterol occurs without any increase in DNA content and myonuclear number, making the role of SC in this hypertrophy unlikely (32).

In our experiments, the implication of satellite cells in the FS-induced muscle hypertrophy is suggested by several observations. First, the increase in DNA and PCNA expression attests of increased cell proliferation. Second, the parallel increase in DNA and protein contents reflects a constant myonuclear domain and indirectly supports the role of SCs proliferation in this hypertrophy. Third, the marked increase in neonatal MHC expression, a marker of differentiation of SCs into new myofibers, indicates increased myogenesis. Unfortunately, these parameters have not been assessed by Amthor *et al*. precluding any comparison with our data. Furthermore, the possibility for FS to stimulate SCs proliferation has been previously well established in myotubes (19), in embryo (12) and in transgenic animals (33). Since we show that the effects of FS are not only mediated through Mstn inhibition, it is still possible that the effects of FS on SCs we observed do not result from the inhibition of Mstn, but of other ligands such as Act. Indeed, the muscle hypertrophy caused by injection of soluble ActRIIB receptor, which can bind Mstn and Act, is also associated with a parallel increase in

DNA and protein (34). It is also possible that the increased myonuclear domain observed by H Amthor *et al.* is due to an increased protein synthesis which exceeds or precedes the SC input. Indeed, Welle *et al*. using the same Mstn KO mice have reported both an increase in myonuclear domain (CSA/myonucleus ratio) and an increased SC proliferation (myonucleus /myofiber ratio) in Mstn KO compared to WT mice (35). This, observation might reconciliate data at a first glance contradictory.

Besides satellite cells, protein synthesis also plays a crucial role in the FS-induced hypertrophy. Indeed, as we observed, FS is still able to stimulate muscle growth, albeit to a lesser extent, when the proliferative capacity of SCs has been previously destroyed by gamma irradiation. Our observation suggests therefore that FS causes muscle hypertrophy by stimulating protein synthesis, a hypothesis recently demonstrated *in vivo* (3). Indeed, infusion of FS in neonatal rats has been shown to stimulate muscle protein synthesis together with increased in S6K phosphorylation. The increase in MHCIIb mRNA that we observed agrees with this conclusion. Because mTOR is an integrator of growth factor signal that controls protein synthesis and cell size, its role in the hypertrophy caused by Mstn inhibition has been recently explored. Sartori *et al*. recently demonstrated that the muscle fiber hypertrophy induced by the transfection of a dominant-negative ActRIIB, which blocks the actions of Mstn and Act among others, is inhibited by 33% when animals are treated with rapamycin (17), a specific inhibitor of mTOR. In a preliminary experiment, we have also observed the same degree of inhibition by rapamycin in FS-induced muscle hypertrophy (data not shown). These observations suggest therefore that the muscle hypertrophy induced by Mstn inhibition, and in particular by FS, is only partially dependent on the protein synthesis stimulated by mTOR signalling. The fact that mTOR does not play a major role in the protein synthesis stimulated by Mstn inhibition is supported by the observation made by Welle et al. showing that rapamycin does not prevent the acute stimulation of protein synthesis induced by an anti-Mstn antibody (4). Interestingly, this conclusion contrasts with what has been observed in other models of muscle hypertrophy. For example, the muscle hypertrophy induced by IGF-I is totally prevented by rapamycin (16;36). The hypertrophy caused by clembuterol is partially or totally mTOR-dependent, depending of the muscle (37). Finally, the contraction-induced increase in muscle protein synthesis in humans is also blocked by rapamycin (38). Further studies will have to decipher the mediators responsible for the increased protein synthesis caused by Mstn inhibition.

Follistatin ligands involved in the follistatin-induced muscle hypertrophy

In our work, we have demonstrated that FS-induced muscle hypertrophy results from the inhibition of not only Mstn but also Act.

Before starting this work, several pieces of evidence had already suggested that Mstn was not the only target of FS in the skeletal muscle. First, the muscle hypertrophy observed in FS transgenic animals is greater than in Mstn KO animals (33). Second, a quadrupling of muscle mass is observed in Mstn KO animals carrying a FS transgene, compared with a doubling of muscle mass induced by the lack of Mstn alone (39). All together, these observations pinpoint the fact that Mstn is not the only ligand whose inhibition leads to muscle hypertrophy in animals overexpressing FS. In fact, FS is also able to bind with high affinity and to inhibit other members of the TGF-β family, including Act and GDF-11 (40-42). For several reasons, GDF-11 has often been considered as the alternative target of FS in the muscle. First, GDF-11 is a TGF-β family member closely related to Mstn with 90% amino acid identity and same high affinity for FS (25;43;44). Second, GDF-11 binds ActRIIB, which is thought to mediate the action of Mstn on muscle cells (45-47). Third, GDF-11, which is expressed among other tissues in the skeletal muscle, is able, as Mstn, to inhibit myogenesis either in chick limb (48) or in murine and human myoblasts (47;49). However, our observations do not support the role of GDF-11 inhibition as one of the mechanisms for the FS-induced muscle hypertrophy. Indeed, the fact that overexpression of FSI-I, a FS mutant with conserved affinity for Mstn and GDF-11, lost its ability to stimulate muscle growth in Mstn KO mice suggests that GDF-11 does not play a major role. In accordance with our results, recent data have shown that deletion of GDF-11 specifically in skeletal muscle does not affect muscle size, fiber number, or fiber type (50). This unexpected conclusion prompted us to investigate the possibility for other FS ligands to mediate the FS-induced hypertrophy. The smaller hypertrophic effect of FSI-I, a FS mutant characterized with reduced affinity for Act, compared to the native FS highlights the role of Act inhibition in the FS hypertrophic effect. Moreover, the ability of Act to regulate muscle mass has been confirmed by our observation that ActA when transfected in the muscle causes muscle atrophy. This is to our knowledge the first demonstration of the muscle atrophic effect of Act *in vivo*. Although we did not explore the mechanisms of muscle atrophy caused by Act, it is likely that Act shares with Mstn the ability to downregulate the Akt pathway after binding to ActRIIB and stimulation of Smad 2/3 (7;49). Our data do not exclude a role of other ligands in the FS hypertrophic effect. Indeed, some BMPs, in particular BMP2 and 7, have been shown to

inhibit differentiation of human myoblasts (49). Nevertheless, the FS affinity for these ligands and their potency seem to be lower than Activin, GDF-11 and Mstn (49).

Besides its powerful effect on skeletal muscle, FS is also able to bind and inhibit Mstn and Act in the plasma. Overproduction of Act (alpha-inhibin KO animals) or Mstn (animals implanted with CHO cells overexpressing Mstn) in the blood flow is associated with a cancer cachexia-like wasting syndrome characterized by decreased food intake together with loss of both muscle and fat mass (51;52). This cachexia syndrome is thought to be mediated by the ActRIIA present on the liver cells (51;53). Interestingly, this wasting syndrome, in particular the muscle mass loss, has been shown to be reversed by overexpression or administration of FS (54;55). The relevance of these observations to the human species is questionable, as circulating Mstn concentrations are 10-fold lower in humans than in mice (56). Although the regulation and the role of circulating Mstn have not yet been demonstrated in humans, the Act circulating concentrations have been shown to increase in acute inflammatory situations as sepsis (57;58). The tissue or organ responsible for this increased Act production has not yet been unravelled. Interestingly, inhibition of Act action by FS injection has been reported to decrease mortality caused by endotoxin injection in mice (58). Again, the mechanisms responsible for this beneficial effect are still unexplored. Whether FS might represent a new tool to prevent cachexia is, at this stage, far too speculative.

Myostatin and IGFs interactions

Although Mstn and IGFs are two growth factors regulating skeletal muscle mass, their relationships in the control of muscle mass developement have not yet been studied.

Our work suggests that the upregulation of the growth factor IGF-II might be involved in the muscle hypertrophy induced by the FS-mediated Mstn inhibition. Indeed, IGF-II has been shown to stimulate both mitogenesis and myogenesis through its binding and activation of the IGF type 1 receptor (59-61). Furthermore, increased IGF-II expression has been observed in the muscles of Mstn KO mice compared to WT mice (62-65). Finally, Mstn inhibits the Akt pathway (Amirouche 2009), a pathway responsible for the muscle hypertrophy induced by IGFs. In line with these results, we showed that FS-induced hypertrophy is associated with increased level of IGF-II mRNA. Although this upregulation of IGF-II might suggest a direct negative effect of Mstn on the expression of this gene, no such evidence is yet available. Recently, Marshall *et al* showed that Mighty or Akyrin-1, a novel promyogenic factor downregulated by Mstn could play a key role in the signalling pathway downstream of Mstn.

In these experiments, overexpression of Mighty in C_2C_{12} cells resulted in myotube hypertrophy partially mediated by increased IGF-II expression (64). This observation suggests that increased muscle IGF-II might contribute to the hypertrophy caused by Mstn inhibition both in Mstn KO mice and FS-overexpressing muscles. This hypothesis is going to be tested soon in our laboratory using IGF-II KO mice.

Clinical perspectives

Despite its clear interest for the better understanding of the factors controlling muscle mass development, our research work is also associated with some clinical perspectives.

Clinical applications of Myostatin inhibition

Decline in muscle mass and strength is a major clinical feature of inherited myopathies and of common acquired muscle atrophies associated with aging, disuse, glucocorticoids and cancer. Preventing muscle wasting by promoting muscle growth has been proposed as a possible therapeutic approach. In this context, blockade of Mstn or its catabolic pathway represents a novel strategy to combat muscle loss in genetic and acquired myopathies. Several works have already demonstrated the beneficial effects of Mstn inhibition in the *mdx* mouse model of Duchenne muscular dystrophy. The treatment with Mstn antibody, the administration of Mstn propeptide, and the intra-muscular injection of an adeno-associated-virus coding for Mstn binding proteins all improve the pathophysiology in *mdx* mice (66-68). In patients affected by Duchenne dystrophy, the first clinical trial has verified the safety of a treatment with a Mstn monoclonal antibody (MYO029). Nevertheless, the efficiency of such treatment has not yet been demonstrated since the Mstn antibody administered at low doses did not induce functional improvement or significant hypertrophy in this study (69). However, preliminary results obtained on single myofiber isolated from these patients seem encouraging (70). In fact, improvement was seen in single muscle fiber contractile properties following treatment with MYO029 compared to placebo (70). Further works will be necessary to investigate the efficiency of a treatment based on Mstn inhibition in inherited human myopathies. Furthermore, if Mstn inhibition has showed a promising effect in *mdx* mice, mitigated results obtained in models of limb-girdle muscular dystrophy and amyotrophic lateral sclerosis suggest that the beneficial effect of Mstn blockade are limited in severely affected or late-stage disease (71-73). Mstn inhibition might therefore benefit muscular dystrophy when instituted early or in relatively mild disease. Among acquired myopathies, some data suggest

that the muscle atrophy associated with aging could benefit from Mstn inhibition. Indeed, the prolonged absence of Mstn leads to increased muscle mass and improves muscle healing in young and in senescent mice (20;22;23). As the muscle mass and strength condition the quality of life for old patients, the functional increase offered by Mstn inhibition could be really interesting. In the same vein, our work showing that invalidation of Mstn gene prevents the muscle atrophy caused by glucocorticoids suggest that Mstn inhibition could have beneficial effects in pathologies where glucocorticoids play a major role. Cachexia, starvation or sepsis are all conditions in which treatment with glucocorticoid receptor antagonist attenuates muscle atrophy, indicating that glucocorticoids are in part responsible for this muscle loss (74). Blockade of Mstn could therefore limit the muscle wasting observed in these situations. In contrast, in muscle loss associated with disuse, the anti-atrophic effect of Mstn inhibition seems to be ineffective. Indeed, it has been demonstrated that Mstn KO mice lose more skeletal muscle mass than WT animals during hindlimb suspension (75). Some hypotheses can be raised to explain the absence of the protective effect of the Mstn deletion in this catabolic situation. First, the muscle atrophy caused by unloading affects mainly slow-twitch muscle fibers (MCHI), where Mstn is poorly expressed. These slow-twitch fibers might be therefore less sensitive than the fast-twitch to the protective effects of the inhibition of Mstn, explaining the lack of protective response in the unloading model of muscle atrophy. Second, muscle atrophy caused by hindlimb suspension is thought to be related to the activation of the NFkB, a transduction pathway that seems not to be affected by Mstn (1). The potential of Mstn inhibition to prevent muscle weight loss has been also recently tested in cancer cachexia. On one hand, it has been shown that Mstn inhibition by siRNA leads to an increase in muscle mass in cancer cachectic mice (76). On the other hand, Mstn blockade by stimulation of FS expression by inhibitors of histone deacetylases does not counteract cancer-induced muscle atrophy (77). Therefore, further studies will be necessary to demonstrate a beneficial effect of Mstn blockade in this cachexia model.

Tools to inhibit Myostatin in humans

To inhibit Mstn activity during postnatal life, several approaches have been tested: administration of an anti-Mstn antibody, the Mstn propeptide, or the soluble ActIIB receptor, injection of short interfering RNAs targeting Mstn or infection with an adeno-associated virus coding for inhibitory binding proteins. All of these approaches have shown a beneficial effect on muscle mass. However, the efficiency of these techniques to stimulate muscle growth is

not necessary similar. Indeed, the Mstn blockade using Mstn propeptide led to morphological and functional benefits which seem to exceed those obtained with Mstn antibody in the same conditions (67). In the same way, among the inhibitory binding proteins of Mstn, FS seems to be the more effective to induce muscle hypertrophy. Indeed, when tested in the same experiment, FS induced a greater muscle increase compared to FLRG and GASP-1 (68). As we observed, the potent hypertrophic effect of FS could be explained by its ability to bind more than one ligand of the ActRIIB. Conversely, the fact that FS binds and inhibits both Mstn and Act could have some disadvantages. Actually, as Act exerts diverse functions in tissues other than skeletal muscle, its inhibition by FS could lead to potential side effects in particular on reproductive performance and fertility. Consequently, some FS mutants displaying minimal Act-inhibiting activities have been developed and have shown promising results (78;79). Nevertheless, as we observed with the FSI-I mutant, the loss of Act inhibition capacity is automatically associated with a reduction of the hypertrophic effect on muscle.

Functional benefits of Myostatin inhibition

From a clinical point of view, the main goal of such therapies is to increase not only muscle mass, but more importantly, muscle function. Several studies showed that deletion or inhibition of Mstn is associated with increased muscle strength as assessed by exercise performances. Indeed, in *mdx* mice, the muscle hypertrophy induced by Mstn gene deletion is associated with a greater grip strength, which is consistent with an increased functional muscle mass (20). Moreover, in healthy mice and in rodent models of myopathy, the postnatal inhibition of Mstn by injections of Mstn antibody or Mstn propeptide results in greater performances in grip strength and in rotarod tests (66;67;72;80-82). With the same functional tests, it has also been demonstrated that the FS overexpression by transgenesis or gene transfection increases the strength of healthy and *mdx* mice (68;78). Accordingly, *mdx* mice treated with trichostatin A, a pharmacologic agent which stimulates the FS expression in skeletal muscle, perform better than untreated *mdx* mice in a treadmill test and in a swimming experiment (83).

Although data clearly demonstrate that the muscle size increase induced by Mstn inhibition is associated with increased physical performances, *ex vivo* analyses of the muscle physiological properties show conflicting results. In fact, one report has shown that the excessive muscle growth caused by Mstn deletion results in impaired force generation (84). In this study, authors reported that the large muscle mass of the Mstn KO mice relative to WT mice is not

associated with an increase in maximum tetanic force generation. These data contrast with a previous study showing that the tetanic force is increased in the muscle of Mstn KO mice compared to their WT littermates (85). Moreover, treatment of dystrophic mice with anti-Mstn antibody, Mstn propeptide or trichostatin A has been shown to increase the muscle force production during twitch and tetanic contraction (66;67;82;83), indicating that the muscle weight gain caused by Mstn inhibition is associated with increased muscle strength. Nevertheless, when normalized for muscle cross-sectional area, the force production of the muscles (specific force) is reduced in Mstn KO mice compared to WT animals (84;85). Whereas specific force output in *mdx* mice remained decreased after treatment with anti-Mstn antibody (66), treatment with Mstn propeptide resulted in an increase of specific force output (67). The discrepancy between these observations could be partially explained by difficulties with the measurement of whole muscle specific force. These difficulties include differences in intramuscular fiber orientation or pennation and the presence of intramuscular connective tissue. These confounding variables are eliminated by using single muscle fibers to study contractile properties. This technique has been used to assess the contractile properties of muscles from dystrophic patients treated with a Mstn antibody. In this study, an improvement was seen in single muscle fiber contractile properties after 6 months of treatment (70).

Altogether, these results suggest that although the increase in muscle strength is not proportional to the degree of muscle hypertrophy, the absolute muscle force and the physical performances seem to be systematically improved by Mstn inhibition. This functional improvement in muscle produced by Mstn blockade could benefit to patients with muscular disorders.

Conclusion

In conclusion, this work demonstrates the interest of Mstn inhibition as a potential therapeutic approach in muscle wasting disease. Since Mstn gene deletion prevents the muscle atrophy caused by dexamethasone and as glucocorticoids are involved in muscle atrophy observed in many catabolic conditions, Mstn inhibition may be helpful in the treatment of disease-related muscle loss.

Among Mstn inhibitors, FS seems to be a promising tool because of its powerful hypertrophic effect on skeletal muscle mass. As we showed, its anabolic action results from inhibition of both Mstn and Act, which negatively regulate muscle growth.

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