REVIEW

The role of myostatin in muscle wasting: an overview

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Abstract Myostatin is an extracellular cytokine mostly
expressed in skeletal muscles and known to play a crucial
role in the negative regulation of muscle mass. Upon the
binding to activin type IIB receptor, myostatin can initiate
several different signalling cascades resulting in the
upregulation of the atrogenes and downregulation of the
important for myogenesis genes. Muscle size is regulated
via a complex interplay of myostatin signalling with the
insulin-like growth factor 1/phosphatidylinositol 3-kinase/
Akt pathway responsible for increase in protein synthesis in
muscle. Therefore, the regulation of muscle weight is a
process in which myostatin plays a central role but the
mechanism of its action and signalling cascades are not
fully understood. Myostatin upregulation was observed in
the pathogenesis of muscle wasting during cachexia
associated with different diseases (i.e. cancer, heart failure,
HIV). Characterisation of myostatin signalling is therefore a
perspective direction in the treatment development for
cachexia. The current review covers the present knowledge
about myostatin signalling pathways leading to muscle
wasting and the state of therapy approaches via the
regulation of myostatin and/or its downstream targets in
cachexia.

Keywords Myostatin · Muscle wasting · Cachexia

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1 Introduction

Cachexia is a syndrome occurring at terminal stages of
diseases such as cancer, chronic heart failure, chronic
kidney failure or AIDS [1]. This syndrome is characterized
by loss of body weight as a consequence of pathological
changes in different metabolic pathways. It leads to
increased morbidity and mortality irrespective of the
underlying disease. A major role in the development of
cachexia is played by the loss of muscle mass accompanied
by the loss of fat [2]. Such muscle hypotrophy is the result
of multiple alterations at the molecular level, e.g. the
disturbance in the balance between protein degradation and
protein synthesis [1]. Proinflammatory cytokines such as
interleukin-1, interleukin-6 and tumour necrosis factor-α
(TNFα) were shown to play an important role in the
development of muscle wasting [3]. However, there is no
established treatment for cachexia based on the regulation
of their signalling. Many different peptides have received
therapeutic interest over the last decade, including ghrelin,
leptin, melanocortins and growth hormone [4–6]. In 1997,
the role of another extracellular factor in the negative
regulation of muscle mass, referred to as myostatin, was
discovered [7]. Myostatin upregulation was found in the
pathogenesis of cancer, HIV, heart failure associated
cachexia and aging [8–12]. It has become one of the main
targets in the investigation of the regulation of muscle mass.

2 Myostatin

Myostatin, also known as growth/differentiation factor-8 (GDF-8) is a member of tumour growth factor β (TGF-β) family [7]. This protein is a homodimer with a molecular weight of 25 kDa and a disulfide bond between the monomers at the C-terminal regions [7]. Myostatin circulates in the blood in a latent form with an additional non-covalently bound propeptide at the N-terminus. Proteolytic cleavage of the propeptide by the bone morphogenetic protein (BMP)-1/tolloid family of metalloproteinases is necessary for activation of protein function [13].

The role of myostatin in skeletal muscle was discovered using the method of gene disruption in mice. *Mstn* null animals showed significant increase in muscle mass (up to two-fold) and decrease of fat tissue compared to the wild type [14, 15]. Similar effects were observed in the presence of natural mutations of *Mstn* in cattle, sheep, dogs and humans [16–19] and upon the inhibition of the protein function in adult mice [20]. At the same time, over-expression of *Mstn* led to the reduction of muscle mass suggesting myostatin to be a negative regulator of skeletal muscle growth. During embryogenesis, myostatin is exclusively expressed in skeletal muscle [7] to control the differentiation and proliferation of the myoblast, but in adulthood, it is not only restricted to skeletal muscle but also detected in other tissues (e.g. heart, adipose tissue, mammary gland) [7, 21–25].

The expression of myostatin in the healthy heart is low and mostly detected in Purkinje fibres but not in cardiomyocytes [22]. After myocardial infarction, increased expression of myostatin was observed in sheep, but its expression was restricted to the damaged zone [22]. Moreover, George et al. recently published new data suggesting the increase of myostatin levels in the failing heart [26]. The authors also showed an increase of its latent complex in circulation and expression of BMP-1 that could explain the development of cachexia in patients with heart failure [26]. These data are in accordance with the recent research of Heineke et al. who suggested that myostatin produced by cardiomyocytes could stimulate muscle wasting in heart failure [27].

The role of myostatin in the regulation of adipose tissue is not well understood. Myostatin and its receptor (type IIB activin receptor, ActRIIB) were shown to be expressed in adipose tissue at low levels [7, 14]. Several studies showed the negative effect of myostatin on preadipocyte differentiation and proliferation [28, 29]. Contrary, an effect of promotion of the differentiation leading to the increased adipogenesis was observed in pluripotent mesenchymal cells [30, 31]. Moreover, *Mstn* null mice had a decreased amount of adipose tissue [14, 32]. It is still unclear whether the effect of myostatin on adipose tissue is the direct result

of regulation or it is an indirect consequence of skeletal muscle growth. Reduced adiposity was observed in different transgenic mouse models of muscle hypertrophy [33–35]. Thereby the decreased amount of adipose tissue in *Mstn* null mice could be an indirect drawback of significant increases in muscle mass. Such an effect can be mediated through leptin—adipose-specific hormone regulating food intake and energy homeostasis. Its level was shown to be lower in *Mstn* null mice [32]. Therefore, additional experiments are required to determine the role of myostatin in adipose tissue.

3 Binding to the cell

Myostatin is an extracellular cytokine, and as many other members of the TGF-β family, it mediates the signal through activin receptors [36]. Active myostatin mostly binds to the ActRIIB [36] and engages the signalling cascade leading to the inhibition of myoblast differentiation and proliferation (Fig. 1). ActRIIB can mediate other signalling pathways with diverse affinity for ligands—high for activin A and GDF11, low for BMP-2 and BMP-7 [38]. These ligands are responsible for several cell responses by activating different members of the Smad family of transcription factors.

Activin receptors are transmembrane threonine/serine kinases divided in two types. Type I receptor (ALK 4 and ALK 5 for myostatin [28]) has the unique GS domain located closely to the intracellular space and adjacent to the kinase domain which is absent in the second type (ActRII). Binding of myostatin with ActRIIB causes its assembly with type I receptor and phosphorylation of its GS domain. Therefore, the signal of myostatin is mediated through activated complex of two receptors [39].

Myostatin signalling through ActRIIB is crucial for the regulation of muscle growth. The natural defect in ActRIIB sensitivity in humans leads to a significant increase in muscle mass [40]. The same effect was observed in the experiments that used blockade of the murine receptor [40]. In the characterization of the ActRIIB role in myostatin signalling, the soluble form of this receptor (sActRIIB) is used. sActRIIB is a fusion protein of the receptor extracellular domain with immunoglobulin Fc. It acts as a decoy receptor for myostatin. Healthy mice treated with sActRIIB showed a 60% increase in muscle mass, just 2 weeks after treatment initiation [40]. Surprisingly, in mice treated with antimyostatin antibodies (JA-16) or myostatin propeptide known to bind circulating myostatin, the hypertrophy of muscle was lower compared to sActRIIB [41, 42]. Moreover, in *Mstn* knockout mice treated with the sActRIIB, an increase in muscle mass by 15–25% was observed [40]. This suggests the presence of at least one

more ligand that binds to the receptor thereby regulating the mass of skeletal muscle. Souza et al. proposed that such ligands could embrace BMP-11, activin A, B and AB, but this hypothesis requires more evidence [43]. However, it could explain why myostatin blockade in clinical trials had no significant effect on muscle weight [44]. The results obtained in cancer models of Lewis lung carcinoma and B16F10 melanoma are in agreement with this theory. It was shown that myostatin knockout mice had a more pronounced tendency to develop muscle wasting than wild-type animals. This finding suggests that myostatin is not the only ligand providing the signal leading to muscle wasting. On the other hand, the same study showed that myostatin could prevent muscle wasting to a certain degree [45].

Recently, Zhou et al. moved a significant step forward in development of a novel therapy [46]. The authors demonstrated that sActRIIB prevents or even reverses development of cancer cachexia. Their research showed the connection between the development of cancer cachexia and the activation of activin receptor. Blockade of ActRIIB led to the regeneration of muscle and cardiac mass [46]. These data suggest a fundamental role of ActRIIB-mediated signalling pathways in the induction of muscle wasting during cachexia and can be used in the development of treatments against cachexia.

4 Myostatin extracellular regulation

Myostatin circulates in the blood in a latent complex with non-covalently bound propeptide at the N-terminus [13, 36, 47], crucial for the correct folding of the protein [20]. The propeptide was shown to bind the active myostatin in vivo and in vitro, and its overexpression in mice results in an increase of muscle mass [36, 48].

Specific inhibitors can prevent binding of myostatin to ActRIIB in serum. One of them is follistatin, an extracellular cysteine-rich glycoprotein with a structure not similar to members of TGF-β family. Follistatin binds myostatin and inhibits its activity by preventing its binding to the receptor [11, 36]. Overexpression of follistatin in vivo leads to muscle hypertrophy similar to the one observed in *Mstn* null mice [36]. Mice with homozygous mutation in the *Fst* gene showed a decrease in muscle mass suggesting an important role of follistatin in the regulation of myogenesis [49]. Follistatin-like 3 protein has more than 30% homology with follistatin and was also shown to bind circulating myostatin [41].

Another inhibitor of myostatin is growth and differentiation factor-associated serum protein 1 (GASP-1). Unlike members of the follistatin family, GASP-1 does not have affinity to activin [42]. The structure of GASP-1 is different from previously described inhibitors: With the exception of one follistatin domain, it has additional domains typical for protease inhibitors. Interestingly, GASP-1 can also bind to the myostatin propeptide and possibly regulate the activation of myostatin through proteolytic cleavage [42]. Moreover, myostatin can be inactivated upon covalent binding to latent TGF-β binding protein 3 (LTBP3). This inactive complex of myostatin/LTBP3 is used for myostatin storage in the extracellular matrix [50]. Taken together, myostatin is regulated by at least four different inhibitors via binding of the active or latent form. The mechanisms of myostatin inhibition still remain elusive, and the crosstalk between the different intrinsic inhibitors is still unclear. The variety of inhibitors emphasizes the importance of a strict myostatin regulation in order to avoid muscle damage and wasting. The schematic mechanism of myostatin extracellular signalling is presented in Fig. 1.

5 Intracellular response

Skeletal muscle mass is maintained as a consequence of two main molecular mechanisms: protein synthesis and protein degradation. Despite the profound knowledge of the

Fig. 1 Myostatin pathway. Myostatin is synthesized and secreted by muscle cell; it signals through the activin IIB/ALK 4/5 heterodimer to activate different pathways resulting in the decrease in muscle growth and differentiation (with permission from [37])
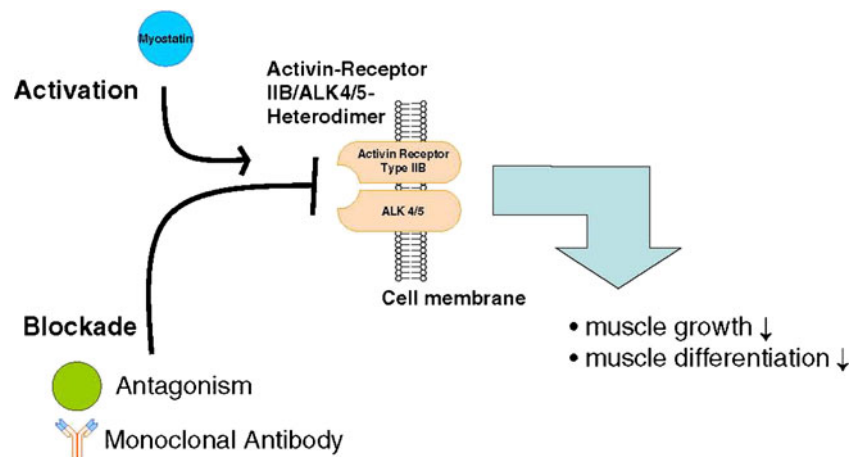
role of myostatin in the regulation of muscle growth, many details of the molecular mechanisms of its action are poorly understood. It has been suggested that binding of myostatin to the ActRIIB results in the phosphorylation of two serine residues of Smad2 or Smad3 at COOH domains. This leads to the assembly of Smad2/3 with Smad4 to the heterodimer that is able to translocate to the nucleus and activate transcription of target genes [51–53]. One of the known downstream targets of Smad signalling is MyoD, a transcriptional factor that is involved in skeletal muscle development and takes part in the repair of damaged skeletal muscle [54–57]. Downregulation of *myoD* expression was shown in vitro and in vivo during cachexia, possibly via TNFα through the induction of the NF-κB pathway. Interestingly, myostatin downregulates *myoD* in an NF-κB-independent way [58, 59]. Myostatin also inhibits Pax3 expression, which is possibly an upstream target of MyoD [58, 60, 61]. Moreover, Smad signalling targets other genes such as *myf5* and *myogenin*, known to be important for myogenesis [62].

Interestingly, other Smad proteins (Smad6 and Smad7) work as agonists. They compete for the binding to the activin type I receptor and thereby inhibit signalling of TGF-β family members [63, 64]. Myostatin was also shown to activate Smad7 transcription. Smad7, in turn, is able to inhibit the association of Smad2/3 with Smad4 [65, 66]. Therefore, the Smad pathway is regulated by feedback control [67].

Myostatin signalling via Smad has been intensively investigated, but it is not the only feasible signalling pathway. TGF-β family members were shown to activate mitogen-activated protein kinases (MAPKs), particularly p38 and extracellular signal-regulated kinase 1/2 (ERK1/2) [68–71]. p38 MAPK is responsible for the cell response to stress factors and was shown to be activated by myostatin via TGF-β activated kinase 1 (TAK-1)/MAPK kinase (MAPKK) cascade. Its signalling results in the downregulation of myogenesis-related genes, but it does not target Smads [72]. The role of ERK1/2 in the regulation of muscle mass is controversial. On the one hand, there are studies confirming that ERK1/2 takes part in the process of satellite cell proliferation that is necessary for the maintenance of skeletal muscle weight [73] and induces protein synthesis under physiological conditions [74, 75]. On the other hand, there are experiments showing an opposite effect of ERK1/2 activation [76–79]. Thus, the data suggest that increased activity of ERK1/2 leads to the differentiation inhibition in several cell types [76, 77]. At the same time, myostatin significantly activated ERK1/2 in C2C12 cells. Similar effects were observed in mice during systematic administration of myostatin [78]. Taken together, it seems likely that myostatin mediates its signal at least partially through ERK1/2 activation. Therefore, different responses through ERK1/2 could be caused by different levels of myostatin corresponding to normal and pathological conditions. MAPK cascade normally involves the activation of Ras/Raf/MEK1. To check whether myostatin uses the same pathways to activate ERK1/2, some experiments were done. Using an inhibitor of MEK1 in C2C12 cells, Yang et al. showed that this kinase is involved in the myostatin-induced activation of ERK1/2 [78]. Moreover, such inhibition of MEK1 leads to the rescue of cell differentiation, which means that MEK-1/ERK1/2 play a role in differentiation suppression by myostatin. The presence of dominant negative form of Ras was shown to positively influence MEK1/ERK1/2 through the downstream activation of Raf. Therefore, myostatin activates ERK1/2 via Ras/Raf/MEK1 pathway [78].

6 Cross talk of myostatin and IGF-1

One of the main positive regulators of muscle growth is insulin-like growth factor 1 (IGF-1). Under normal conditions, IGF-1 signalling seems to be dominant and blocks the myostatin pathway [80]. However, an inhibition of IGF-1 was observed when myostatin is overexpressed [81, 82]. IGF-1 can prevent TGF-β family-mediated apoptosis [83], and it was shown that in the absence of IGF-1, the level of apoptosis in C2C12 cells treated with myostatin increased. Yang et al. speculate it may in part explain the ability of myostatin in regulating cell cycle but not apoptosis in normal conditions [84].

The mechanism by which IGF-1 regulates myostatin signalling includes the inhibition of transcription factors responsible for the induction of atrogenes via phosphorylation with phosphatidylinositol 3-kinase (PI3K)/Akt pathway. Akt plays a significant role in different metabolic processes in the cell, particularly in the hypertrophic response to insulin and IGF-1 [85, 86]. Akt is the crossing point between IGF-1/myostatin pathways. The results of several studies suggest that the genetic loss of myostatin leads to the increase in Akt activity in skeletal muscle in vivo and in vitro [82, 87]. In contrast, a decreased level of phosphorylated Akt is associated with incubation of myotubes with myostatin [58]. It is likely that in the conditions of muscle wasting, myostatin can switch Akt/ mammalian target of rapamycin (mTOR) pathway, responsible for protein synthesis, to inhibition of protein synthesis involving FoxO, GSK-3β or other unknown patterns leading to the loss of muscle mass. These pathways will be discussed below in more detail.

Akt/FoxO The class of Forkhead box O (FoxO) transcription factors is involved in the regulation of energy metabolism. FoxOs take part in the formation of skeletal

muscle and adipose tissue as major organs for energy distribution [88, 89]. During myoblast proliferation, FoxO1 and FoxO3 are localized in the cytoplasm in the latent, phosphorylated form. Upon initiation of differentiation, FoxO translocates to the nucleus where it binds to DNA and regulates transcription. Akt regulates the activity of FoxO1 and FoxO3 by phosphorylating them and thereby retaining them in the cytoplasm [58]. In myotube culture, the level of FoxO1 in the nucleus was shown to increase after treatment with myostatin [58, 90], together with FoxO3 resulting in the induction of the muscle-specific E3-ubiquitin ligases atrogin-1 (MAFbx) and MuRF-1 [91, 92]. Expression of constitutively active FoxO1 in transgenic mice led to decrease in myoblast differentiation and a reduction in muscle weight [93]. In muscle, FoxOs are known to interact with Smad3 and Smad4 inducing the protein degradation. Recently, it was shown that FoxO1 and Smad synergistically increase the expression of myostatin mRNA and its promoter activity in C2C12 myotubes but via different pathways [94]. Taken together, myostatin-mediated signalling activates FoxO, and it leads to the upregulation of proteasome ubiquitin ligases MuRF-1 and atrogin-1, which participate in protein degradation.

Akt/GSK-3β Cycline D1 is an important component for G1 phase of cell cycle which is also regulated via PI3K/Akt pathway. In C2C12 myoblasts treated with myostatin during the phase of active cell growth, a decrease in cycline D1 levels was shown, suggesting that myostatin targets cycline D1 for proliferation inhibition. In favour of this hypothesis, overexpression of cycline D1 was shown to rescue cell cycle. The activation of GSK-3β via dephosphorylation at Ser 9 increases proteolysis of cyclin D1 [95] in the response to myostatin-mediated dephosphorylation of Akt at Ser 473. Usage of IGF-1 or active Akt treatment blocks myostatin induced arrest of cell proliferation [84]. Thus, one more pathway activated by myostatin suggests that inhibition of Akt plays a dramatic role in the providing of myostatin signalling.

Akt/mTOR/p70s6K This pathway is important for the differentiation of myoblasts and hypertrophy of myotubes. mTOR is a kinase downstream of Akt, which phosphorylates 4E-BP1 and p70s6k thereby inducing initiation of protein synthesis [85, 96]. In *Mstn* knockout mice, an increase in Akt/mTOR/p70s6K signalling was observed [87]. There are two mTOR complexes called TORC1 and TORC2, containing the proteins RAPTOR and RICTOR, respectively [97]. Activation of TORC1 leads to protein synthesis, while TORC2 plays a significant role in the feedback Akt phosphorylation leading to the blockade of FoxO signalling [98]. Inhibition of RAPTOR was shown to play an important role in myostatin signalling, whereas

RICTOR is necessary for the cell differentiation itself. Inhibition of RAPTOR amplifies myostatin actions such as Smad phosphorylation [80].

Additional data confirming the connection between myostatin and Akt pathways were received in experiments with hypoxic muscles. The expression of myostatin was shown to be upregulated under these conditions [99]. At the same time, the Akt/mTOR pathway was downregulated in the rats with chronic hypoxia [100].

In conclusion, signal transduction of myostatin is a complex process involving activation and inhibition of several cellular signalling pathways. These pathways result in the downregulation of the expression of myogenic factors, decrease in protein synthesis and activation of proteasome–ubiquitin ligases. The schematic picture of myostatin signalling is presented in Fig. 2. There are a lot of questions still connected with the order of activation and precise importance of each pathway and its contribution in the development of muscle wasting.

7 Treatment

Myostatin has become a main target for the development of drugs for cachexia and muscle wasting diseases. Despite the experiments using antimyostatin substances that were conducted, there are no drug discoveries against muscle wasting in cachexia so far. The information about compounds that were involved in clinical trials is scarce, but none of the known substances had a positive effect. MYO-029 is recombinant human myostatin-specific antibody that did not show a significant improvement in muscle strength and function in adult muscular dystrophies [44]. The other compound developed by Amgen, AMG 745, has an unknown composition, but its testing was stopped after phase I clinical trials [102]. The problem of using antimyostatin compounds is likely due to the ability of the ActRIIB to bind other ligands from the TGF-β family resulting in a redundancy of myostatin.

The impact of sActRIIB use in the regulation of muscle mass is intensively studied at the moment. Recently, very positive results were published by Zhou et al. [46] in C-26 tumour-bearing mice, where the use of sActRIIB resulted in the prolongation of life and reversal of muscle wasting during cachexia. In this study, a positive effect on cardiac muscle was also seen. The results of this study provide a major step forward in establishing novel cachexia therapies.

The significance of these results for the treatment of cardiac cachexia was shown by Heineke et al. [27], who observed that myostatin produced from cardiomyocytes is
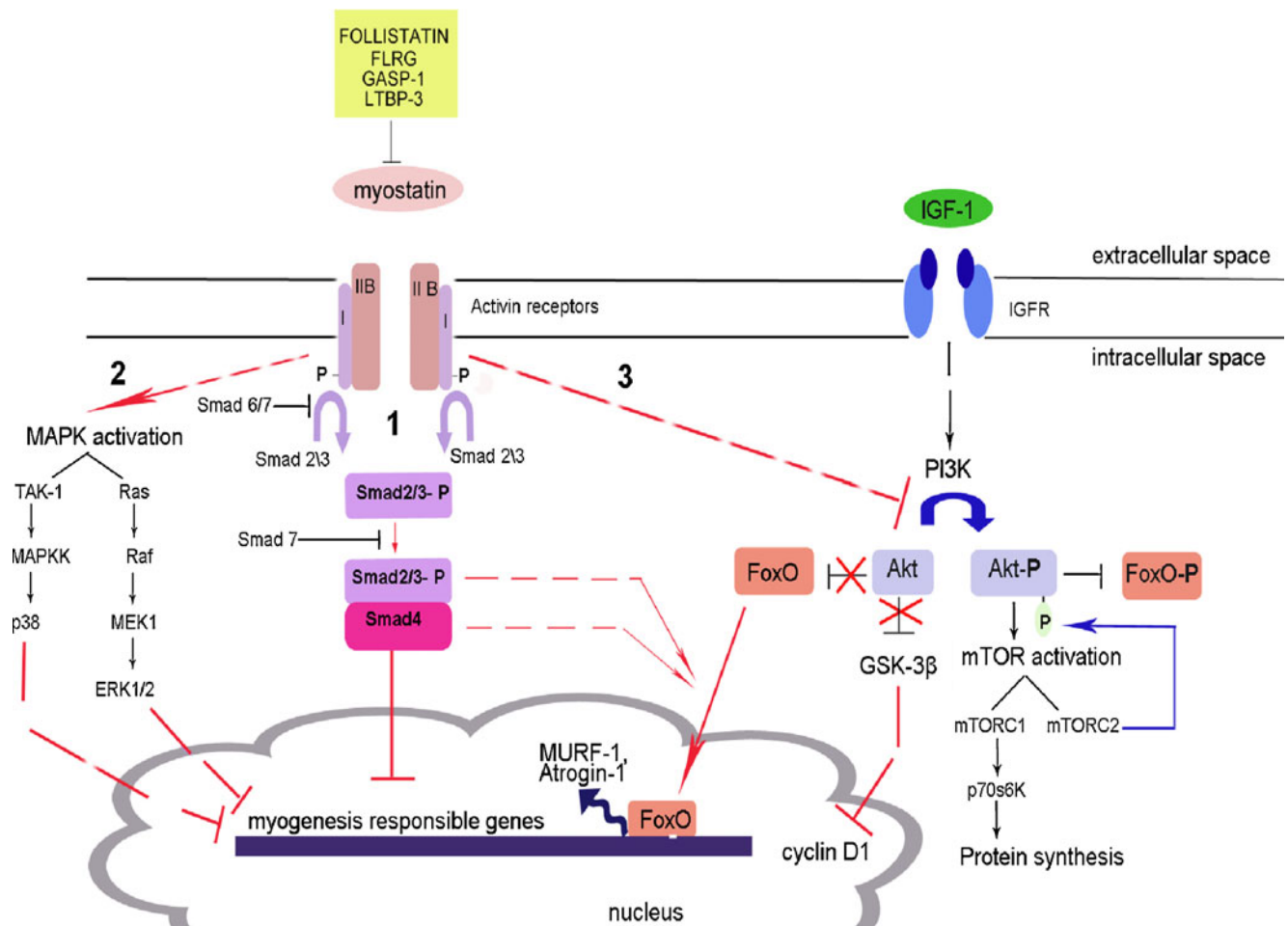
Fig. 2 Different pathways of myostatin signalling. → The activation of the process, -| the inhibition of the process, – – the presence of intermediate steps either unknown or omitted in the figure. *1* Canonical pathway of Smad activation. Myostatin binds to ActRIIB and induces its assembly with activin type I receptor. Subsequent phosphorylation of Smad2/3 leads to its binding with Smad4 and translocation of the complex to the nucleus where it blocks the transcription of genes responsible for the myogenesis. Smad6 and Smad7 compete for the binding with activin type I receptor. Smad7 can also prevent the formation of the Smad 2/3 and Smad4 complex. *2* MAPK activation. The activation of MAPKs is mediated via myostatin using different pathways: TAK-1/MAPKK for p38 MAPK or Ras/Raf/MEK1 for ERK1/2. It leads to the blockade of genes responsible for myogenesis. *3* Inhibition of Akt signalling. Akt phosphorylation occurs in the response to insulin and IGF-1. In normal case, active Akt induces mTOR signal leading to the protein synthesis; at the same time, it inhibits FoxO by phosphorylation. In the pathological conditions, dephosphorylated Akt does not inhibit FoxO. It leads to the accumulation of FoxO in the nucleus where it binds to the DNA and induces the transcription of E3 ubiquitin ligases MURF-1 and Atrogin-1. Smad3 and Smad4 possibly participate in FoxO signalling (adapted from [51, 84, 90, 101])

released into the circulation in heart failure and that myostatin subsequently activated muscle wasting. The deletion of *Mstn* from cardiomyocytes and inhibition of myostatin protein using antibodies in mice prevented muscle wasting in heart failure [27].

8 Conclusion

Myostatin is a negative regulator of myoblast proliferation and differentiation. Normally it functions to regulate hypertrophy of muscles, but a role in the induction of muscle loss was observed in muscle wasting diseases and

cachexia associated with severe illnesses. The mechanism of myostatin signalling is complex and involves the activation of several downstream pathways. Additional studies are required to elucidate the cross talk between the signalling cascades and their regulation. The experiments with myostatin propeptide and antimyostatin antibodies showed a positive effect on regulation of muscle mass in different models of wasting but lacked efficacy in phase I clinical trials. The most promising target in terms of cachexia treatment seems to be the activin type II receptor. The blockade of this receptor led to a significant increase in muscle mass and even its restoration in cancer cachexia. Therefore, targeting myostatin and its receptor represent a

promising direction in the development of effective treatments for cachexia and muscle wasting diseases.

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