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REVIEWS

Myostatin: Twenty Years Later

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Abstract—In recent years, there has been an increasing interest in myostatin, a hormone that inhibits the growth and differentiation of muscle tissue. This interest is associated with an increase in the amount of data on the spectrum of the myostatin functioning. Myostatin, which has been known since 1997, belongs to the family of transforming growth factor β (TGF-β) and is a paracrine factor of skeletal muscle myocytes. It turned out that myostatin also affects the satellite cells and muscle fibroblasts, and its functions are not only to limit growth, but also to remodel skeletal muscles, which is necessary for muscle adaptation to physical training. Recent studies show that myostatin can play a significant role in musculoskeletal and cardiac cachexias in various pathologies, including cancer. It has been found that myostatin can be produced not only by skeletal muscle cells, but also by adipocytes and cardiomyocytes. It has been shown that, in cardiac pathology, the level of myostatin production increases in cardiac tissue. It is suggested that an increase in the myostatin production in the heart is necessary to prevent myocardial hypertrophy, which develops in some cardiac diseases. In this review, we examined the myostatin functions, as well as aspects of myostatin gene expression, mechanisms of its biosynthesis, its effect on various intracellular targets and transcription factors, and the regulation of its production. The importance of myostatin functions, as well as its involvement in pathological processes, allows us to consider this hormone as a promising target in therapeutic studies.

Keywords: myostatin, skeletal muscle, cardiac muscle, physical training, heart failure

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Metabolic processes in muscles are extremely energy-intensive. To save energy, a strict correspondence between the size of the muscles and their work is maintained. In the case of skeletal muscles, among the regulatory factors is the exercise stress that changes the balance of the local muscle signaling connections and the sensitivity of the muscles to them and systemic hormones involved in the regulation of the build-up and loss of muscle strength. The main systemic regulators of muscle mass are divided into positively acting androgens and negatively acting glucocorticoids. These systemic regulators act not only directly on the muscle cells of various levels of differentiation, but also modify the production of paracrine signaling muscle connections, one of which is myostatin. Myostatin reduces both the number and size of the muscle cells present in the body. In the absence of exercise stress muscle tissue secretes more myostatin. This indicates that the main physiological effect of myostatin is the adaptation of muscle mass to a decrease in exercise stress in order to save energy. The disruption of myostatin production is directly related to a large number of diseases of high social significance, which increases the interest in this hormone. The authors are primarily interested in the role of this protein in humans, since the advantages of using myostatin as a pharmaceutical agent are obvious:the increase in strength applied by sportsmen to achieve new results, treatment of diseases associated with inadequate development or damage of muscle tissue.

The year of myostatin discovery was 1997. The initial success was achieved with the help of genetic engineering, namely, gene knockout. In the laboratory headed by Professor Si-Jin Lee in the John Hopkins University, homozygous mice for the knocked out gene of the growth differentiation factor 8 (GDF-8) were produced. As a result of experiment, a 2.5-fold increase in all skeletal muscles occurred in these mice. There was a hyperplasia of muscle fibers, as well as their hypertrophy. These animals were viable, the problems with fertility were also not observed. As a result of these experiments it has been proved that the GDF-8 protein is responsible for the negative regulation of the skeletal muscle growth. Therefore, it was named myostatin. The results obtained are valid not only for mice; many mammals, including humans, experience an increase in muscle mass and strength when the myostatin gene is switched off.

Myostatin is expressed by skeletal muscle cells during the entire embryogenesis, beginning with the myotomes of developing somites. In the tissues of adult animals, myostatin is mainly expressed by skeletal muscles and to a much lesser degree in myocardial and adipose tissue. When myostatin is knocked out, a

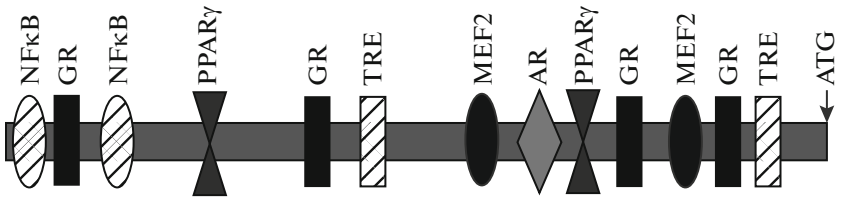

Fig. 1. Some GSEs in the myostatin gene promoter for nuclear factor κB (NFκB), glucocorticoid receptor (GR), peroxisome proliferator-activated receptors gamma (PPARγ), thyroid hormone receptor (TRE), myocyte enhancer factor-2 (MEF2), androgen receptor (AR), and ATG start codon [3].

sharp increase in muscle mass two to three times in comparison with the wild type is observed in mice [1]. In the adult age, during strength training, myostatin takes part in the adaptation of skeletal muscles and tendons to the exercise, since it facilitates their remodeling. At the moment, it is known that the signaling pathway activated by myostatin is performed via its interaction with membrane activin receptors, this leads to the activation of the MAP kinase cascades and the accumulation of Smad proteins in the nucleus, which activate the expression of certain genes [2].

The ability of myostatin to limit the growth of muscle mass attracted attention to it as a potential target for therapeutic intervention in the degenerative diseases, trauma, and other pathologies of the muscular system, as well as for application in sports medicine and sports. Myostatin plays a role in the cardiac pathology. Thus, in heart failure (HF), cardiomyocytes begin to express myostatin, which leads to inhibition of the growth of cardiomyocytes and provokes cardiac cachexia and progression of HF. Many studies show that such action of myostatin causes serious complications in various types of HF such as myocardial infarction, hypervolemia, ischemic and dilated cardiomyopathies, etc. Probably, inhibition of myostatin effects may become a promising technique for treating muscle and cardiac atrophy in cardiac pathology, and also in other types of diseases that lead to muscle exhaustion.

MYOSTATIN GENE

Myostatin was found and identified in 1997 by McPherron et al. as one of the members of the superfamily of TGF-β growth and differentiating factors [1]. The myostatin gene is localized in the centromeric region of the second chromosome and contains three exons and two introns. It has one single reading frame and encodes a protein consisting of 376 amino acid residues. The myostatin-encoding sequence has features conservative in all other members of the TGF-β family, including the *N*-terminal signaling sequence for secretion, the proteolytic processing site, and the C-terminal domain containing nine conserved cysteine residues [1].

The C-terminal region of myostatin is characterized by a high degree of homology with other members of the TGF-β superfamily. Homology is the most prominent between myostatin and GDF-11 factor, which is 90% of the amino acid residues in the C-terminal region and allows these factors to form a separate subgroup in the TGF-β superfamily.

A number of specific binding sites were found in the promoter of the human myostatin gene: three TATA-*box* sites, CCAAT-*box* site, and five octameric sequences homologous to the homeodomain protein POU binding sites. Twelve E-box sites responsible for binding the myogenic factor of differentiation 1 (MyoD), two sequences homologous to the binding sites of the myocyte enhancer factor 2 (MEF2), binding site of receptor γ activated by a peroxisome proliferator, and the nuclear factor NF-κB binding site were also identified (Fig. 1). Several hormone response elements (HREs) to the androgen receptor (ARE), five HREs to the glucocorticoid receptor (GRE), three HREs to the thyroid receptor (TRE), and cAMP-dependent binding sites (CRE) were also found [3]. Cloning of the regulatory region of the sheep myostatin gene also showed a muscle-specific Mt binding site, HRE for progesterone (PRE), as well as several sensitive elements for octamer-binding factor 1 (Octamer), activator protein 1 (AP1), and growth factor independence 1 zinc finger protein (Gfi-1B) [4].

MYOSTATIN BIOSYNTHESIS

Like other members of the TGF-β family, myostatin synthesis occurs as an inactive precursor protein that becomes a biologically active molecule only after three consequent proteolytic cleavages.

Immediately after translocation to the endoplasmic reticulum, the myostatin precursor, prepromyostatin, forms a homodimer connected by disulfide bridges. The first step in biosynthesis is proteolytic cleavage of 24 amino acid signal sequence which is necessary for the localization of prohormone in the endoplasmic reticulum. As a result of the first cleavage, promyostatin that is directed to the secretory pathway is formed (Fig. 2). Promiostatin is further cleaved by the protein convertases of the furin family in the RSRR (Arg-Ser-Arg-Arg) site corresponding to 240–243 amino acids of promyostatin. In this case, the N-terminal (27.7 kDa) and the C-terminal (12.4 kDa) fragments are formed. The C-terminal fragment exists in the

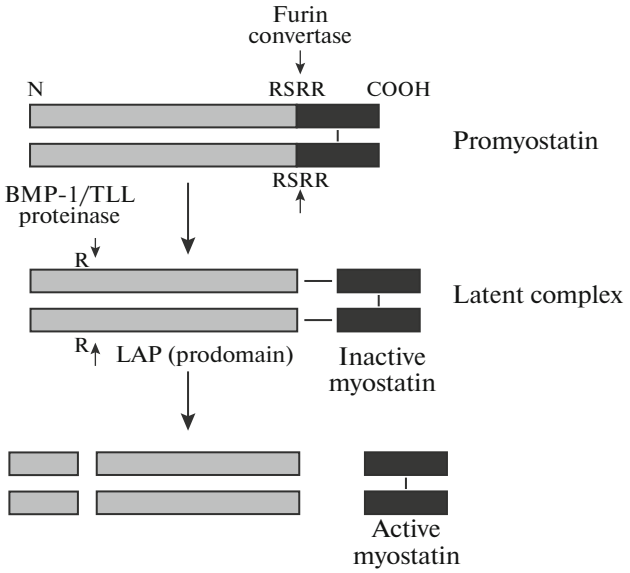

Fig. 2. Myostatin processing [64] with changes. Explanations are given in the text.

form of a dimer connected by a disulfide bridge and remains non-covalently bound to the N-terminal fragment. It is the C-terminal dimer that is the biologically active molecule of myostatin. The N-terminal fragment is an inhibitory prodomain or latency associated peptide (LAP) with a molecular weight of 52 kDa. LAP provides the correct folding and dimerization of the mature domain by means of disulfide bonds. LAP and C-terminal myostatin form a latent myostatin complex consisting of 375 amino acid residues [5, 6].

Previously it was assumed that the secretion of myostatin by a cell occurs constitutively in the form of a latent complex. However, it has been shown that skeletal muscle myocytes secrete promyostatin [5]. After skeletal muscle fibers secrete promyostatin into the extracellular space, latent transforming growth factor-β-binding protein-3 (LTBP-3) captures it. The LTBP-3 protein forms a disulfide bond with cysteine-33 of LAP, and then covalently binds to extracellular matrix proteins such as fibromodulin, fibronectin, and laminin, thus "sewing" myostatin to them [7]. Further, furin protein convertase cuts promyostatin secreted into the extracellular space [5].

Unlike skeletal muscles in the blood plasma, myostatin is in the form of a latent complex or in a combination with the follistatin-related gene (FLRG) protein. Both LAP and FLRG protein negatively regulate myostatin activity, preventing it from binding to its receptor [8]. Myostatin can be secreted into plasma from adipose tissue, myocardium, or skeletal muscle [9, 10].

As a result, there are two sources of potentially active myostatin: a local pool of myostatin (in the form of promyostatin) in skeletal muscles and a system pool in plasma (in the form of a latent complex) (Fig. 3).

Biologically active myostatin is released from the latent complex after proteolytic cleavage with a metalloproteinase of the family of bone morphogenic protein-1/tolloid (BMP-1/TLL-1 and TLL-2) in the region between Arg-75 and Asp-76 with the cleavage of LAP [11]. Expression of BMP-1 and TLL-1 was found in both skeletal musculature and in the heart. In addition, during differentiation and development of myoblasts, the level BMP-1 and TLL-1 expression is reduced in adult animals, which reduces the amount of active myostatin, thus allowing muscle development [12].

At a mutation in the RSRR site, in which LAP is cleaved from the latent complex, muscle hypertrophy occurs in mice, similar to hypertrophy in mice that are knocked out for the myostatin gene [13]. The LAP overproduction results in severe muscle hypertrophy, as in mice knocked out for the myostatin gene [6]. Thus, the LAP level is an important regulator of the myostatin functioning.

MYOSTATIN-BINDING PROTEINS

In addition to LAP, there are other proteins that inhibit myostatin activity: follistatin, FLRG, and GASP-1. Follistatin binds to the C-terminal fragment of myostatin and counteracts its binding to the receptor. The result of follistatin overexpression, is a more severe form of muscle hypertrophy than that which occurs in the myostatin-knockout mice [6]. FLRG and GASP-1 bind in the blood plasma with the C-terminal fragment of myostatin with high affinity and inhibit its activity. GASP-1 was discovered relatively recently; it contains a follistatin-related domain, as well as many domains similar to domains of proteinase inhibitors. GASP-1 is also able to bind to LAP in the absence of mature myostatin. FLRG, in addition to myostatin, blocks the activity of other members of the TGF-β family: activin and BMP-2 [14].

FLRG and GASP-1 together with LAP can form triple or more complicated complexes with the C-terminal dimer of myostatin (active myostatin); in this case, each of these proteins in such complex has a certain regulatory role in maintaining the latent state of myostatin. GASP-1 can interact simultaneously with LAP and the C-terminal dimer of myostatin, forming a triple complex. The presence of many proteinase inhibitor domains in the GASP-1 molecule provides regulation of the cleavage of prepromyostatin to promyostatin [14]. In addition, GASP-1 can regulate the activity of metalloproteinases that break down promyostatin in the RSRR site.

The circulating myostatin is a mixture of various complexes in which it is bound to LAP, GASP-1, or FLRG. Cleavage of LAP from the C-terminal dimer of myostatin is a necessary factor for association with

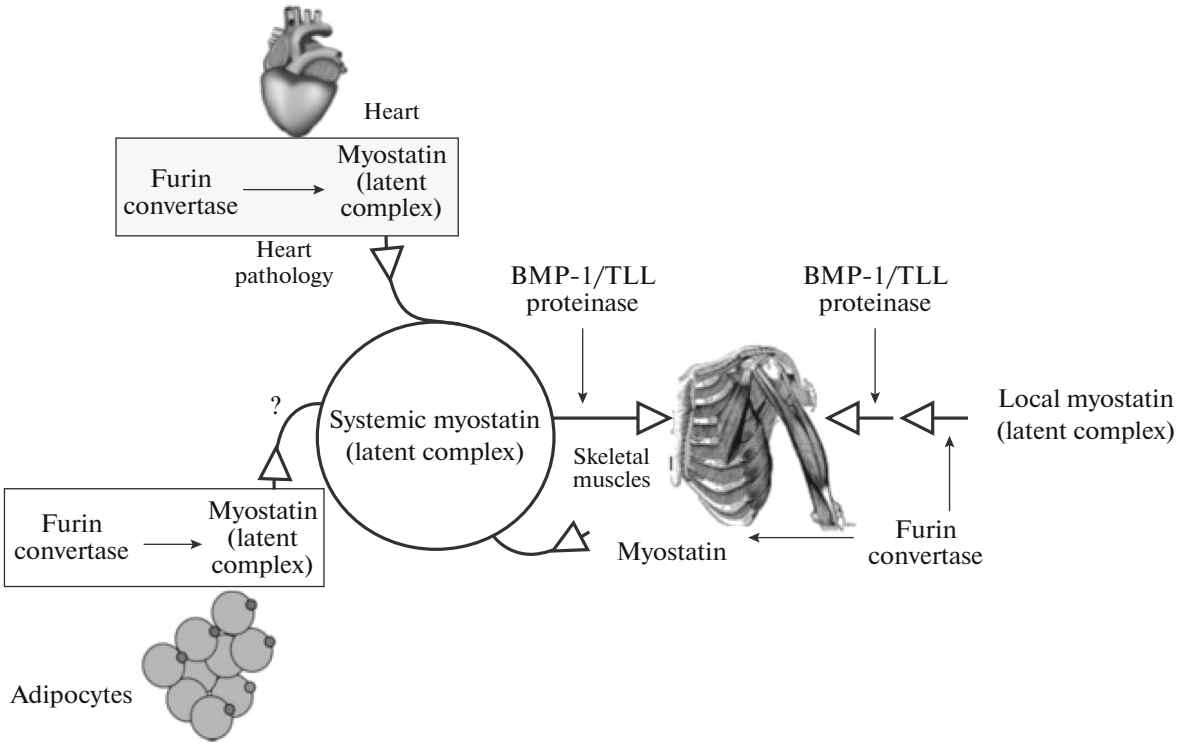

Fig. 3. Local and systemic pools of myostatin regulate the growth of skeletal muscles. Systemic myostatin is secreted into plasma from skeletal muscles, myocardium, and adipose tissue in the form of a latent complex. Local myostatin in the form of promyostatin is in skeletal muscles [64].

GASP-1 or FLRG proteins; i.e., the association of myostatin with different proteins is mutually exclusive. Thus, due to the presence of many different myostatin complexes, specific activation and regulation of the hormone level are possible, depending on the specific physiological stimulus [15].

FLRG and GASP-1 can also be directly involved in the termination of the signal after myostatin has bound to its receptor and activated it [8]. According to this hypothesis, FLRG and GASP-1 bind to the myostatin–receptor complex and then dissociate together with myostatin, remaining in the complex with it in the bloodstream. Thus, the FLRG– or GASP-1–myostatin complexes may represent the final products of the myostatin signaling pathway.

LOCALIZATION AND EXPRESSION OF MYOSTATIN

Myostatin is present in skeletal muscles in high concentration. During embryogenesis, the amount of matrix ribonucleic acid (mRNA) of myostatin is low, which allows the development of skeletal muscles [1]. Analysis of the amount of myostatin mRNA in mouse embryos, showed that the myostatin transcription gradually increased during embryonic development. The first transcripts of myostatin were detected in about one third of somites on day 9.5 of prenatal development of mouse, myostatin mRNA was detected in most rostral somites of the embryo by day 10.5. In the late stages of embryogenesis, myostatin mRNA is found in almost all developing muscles. The myostatin expression is also present in the postnatal period. The myostatin mRNA is detected in almost all skeletal muscles in adult animals [1]. In a much lesser amount, myostatin was found in the heart and adipose tissue [9, 10].

SIGNAL RECEPTION AND TRANSMISSION

Myostatin like as activin and GDF-11 binds to the activin receptor, a heterotetrameric complex of type I and type II receptors belonging to the class of receptor serine-threonine kinases [16]. Seven kinds of type I receptors and five kinds of type II receptors are encoded in the human genome [17]. Both types of receptors consist of an N-terminal extracellular binding domain, a single transmembrane domain, and a C-terminal cytoplasmic domain with the serine-threonine kinase activity. Myostatin has the greatest affinity for the following receptor subtypes: ALK4, ALK5 of type I, and ACVR2A, ACVR2B of type II [18]. The type I receptors are not active in the absence of a ligand, while type II receptors are constitutively active. The ligand binding to the type II receptor leads to heterodimerization of the latter with the type I receptor,

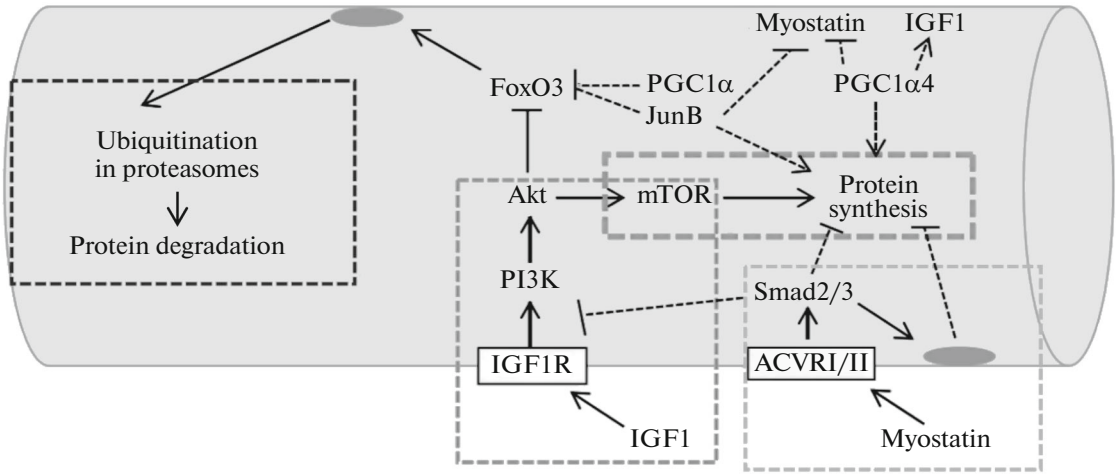

Fig. 4. Intracellular signaling pathways activated by myostatin and IGF1 ([22] with changes). The myostatin signal pathway, the IGF1 signaling pathway, the ubiquitin-proteasome pathway for protein degradation, and the mTOR activation pathway for protein synthesis are shown by dashed line. ACVRI/II, activin receptor; Smad2/3, the Smad 2 and 3 transcription factors; IGF1 and IGF1R, insulin-like growth factor 1 and its receptor; PI3K, phosphoinositide-3-kinase, Akt, B/Akt protein kinase; mTOR, kinase, mammalian target of rapamycin; FoxO3 and JunB, transcription factors; PGC1α-1α4, co-activators of 1α-1α4 receptors activated by the peroxisome proliferator γ.

which allows the type II receptor to phosphorylate the type I receptor. This leads to activation of the type I receptor kinase domain, which transmits further signaling.

The main signaling pathway activated by myostatin is carried out through the Smad transcription factors [17]. Eight Smad proteins have been described in humans, they can be divided into three groups: receptor-regulated Smads (R-Smads), inhibitory Smads (I-Smads), and comediate Smads (co-Smads) [16]. Each Smad protein consists of an N-terminal Mad-homologous 1 (MH1) domain and a C-terminal MH2 domain, with the exception of I-Smads that do not have MH1 domain. The MH1 domain contains a signal sequence for moving into the nucleus and DNA-binding motifs; the MH2 domain interacts with the type I receptor and leads to homo- and hetero-oligomerization of the Smad protein complexes. The activated type I receptor phosphorylates two R-Smads: Smad2 and Smad3. Then Smad4 being a co-Smad protein binds to Smad2 and Smad3; as a result, a trimeric complex is formed and moves into the nucleus to regulate the transcription of certain genes. Moreover, the signal from the myostatin receptor leads to the induction of expression of Smad7 that is I-Smad and participates in negative feedback, regulating the strength and duration of the transmitted signal. Smad7 competes with Smad2 and Smad3 for binding sites on Smad4 and type I receptors and also can induce degradation of this receptor. The Smad transcription factors have their own DNA-binding activity; however, they can work in combination with cofactors such as p300, TGIF, c-Ski, and Evi-1 [19].

The Smad transcription factors enhance the expression of "atrogenes", genes that encode proteins ensuring the degradation of muscle proteins by the ubiquitin-proteasome proteolytic pathway. Two key atrogenes encode E3 ubiquitin ligases for muscle atrophy MAFbx (muscle atrophy F-box or atrogin-1) and MuRF1 (muscle RING-finger 1) [20]. The function of these ligases is the degradation of proteins of the damaged sarcomere in myofibrils by the ubiquitin-proteasome pathway.

The signaling pathway through Smad proteins is considered to be canonical; however, there are also non-canonical pathways that may be run together or separately from the Smad-pathway [16]. For example, the interaction of a ligand with the activin and TGF-β receptors activates the TAK1 kinase, which is a member of the family of mitogen-activated kinases (MAPKKK); this leads to the activation of the p38 MAPK kinase through kinase 3/6 (MKK 3/6) [17]. The p38 MAPK regulates the transcription of genes via the activation of other factors.

Activation of the Smad transcription factors also leads to inhibition of protein kinase B/Akt and, after it, mammalian target of rapamycin (mTOR) kinase (Fig. 4) [21, 22]. It has been previously found that the Akt kinase activated by insulin-like growth factor 1 (IGF-1) via phosphoinositide-3-kinase (PI3K) triggers an intracellular pathway leading to protein overexpression and muscle hypertrophy. The Akt kinase triggers protein synthesis via the Akt/mTOR/p70S6K pathway. In addition, it blocks the expression of MAFbx and MuRF1 atrogenes. The Akt kinase transmits the signal through two signaling complexes, TORC1 and TORC2.

The mTOR kinase is responsible for the regulation of protein synthesis and cell size; however, only about

40% hypertrophy effect caused by myostatin blocking is compensated by mTOR inhibition. In addition, inhibition of mTOR under control conditions does not result in muscle reduction. Probably, a decrease in mTOR activity is necessary, but not sufficient to provide the effect of atrophy, and myostatin also has other intracellular targets that provide it [23].

Another target of the Akt kinase is class O type forkhead transcription factors FoxO1 and FoxO3. It has been shown that these factors are key inducers of the MuRF1 ubiquitin ligase gene in muscular dystrophy [24–26]. Activated Akt kinase leads to an increase in protein synthesis and the phosphorylation of FoxO1 and FoxO3, which is a signal for transporting these factors from the nucleus. Thus, blocking the activity of Akt, myostatin reduces the level of protein synthesis and increases its degradation.

The skeletal muscle cells express the isoform of the PGC-1α, PGC-1α4 transcription factor (co-activators of 1α-1α4 receptors activated by the peroxisome proliferator γ). It has been found that with increased physical activity, there is the PGC-1α4 overexpression that stimulates IGF-1 and represses myostatin, which leads to a decrease in muscle atrophy and even to muscle hypertrophy [26].

In addition, it has been shown that the JunB transcription factor that belongs to the family of activating protein 1 (AP-1) induces muscle hypertrophy regardless of the Akt kinase signaling pathway. JunB is a necessary transcription factor that promotes muscle growth in adult organisms. The JunB overexpression leads to suppression of myostatin transcription and Smad3 dephosphorylation, which provides inhibition of the myostatin signaling pathway and muscle degradation [27]. It has been shown that for different types of muscular dystrophy and cachexia caused by cancer, the mRNA of this transcription factor is at a low level, which apparently leads to the facilitation of the myostatin signal in the cell [28, 29].

REGULATION OF MYOSTATIN PRODUCTION

1. Glucocorticoids

Glucocorticoids stimulate the production of myostatin in muscles [30]. Stimulation of myostatin expression appears not only as a result of increased transcription of its gene, but also due to posttranscriptional processes. The myostatin gene promoter contains the *GRE* motif; therefore, glucocorticoids stimulate the transcription of the myostatin gene [3]. Regulation of myostatin expression at the posttranscriptional level can be due to a decrease in the expression of *miR*-27*a* microRNA, which leads to the stabilization of the hormone mRNA [31]. For these reasons, it has been suggested that an increase in muscle myostatin plays a key role in the muscle glucocorticoid-induced atrophy. This hypothesis was confirmed in vivo using a model of the myostatin gene knockout "mighty mice" [32]. In contrast to wild-type mice, in knockout mice, a decrease in the muscle mass was not developed and the muscle fiber cross-sectional area was not reduced after treatment with glucocorticoids. This observation proves that myostatin is an essential component of the atrophic effects of glucocorticoids on muscles.

2. Sex Steroids

Androgens. When myostatin gene was knocked out, a more pronounced muscular hypertrophy was observed in male mice than in females [1], and, conversely, at myostatin overexpression, the weight loss was significantly greater in males compared to female mice [33]. Hence, one of the mediators of the androgen effects on muscle is myostatin; however, exact mechanisms are not completely known. It has been shown in silico that the androgen-sensitive element (ARE) is located in the myostatin gene promoter [3]; however, another mechanism is probably most functionally significant. From several studies, it is known that androgens stimulate the signaling of β-catenin that increases the expression of different genes, including the follistatin gene which suppresses myostatin [34]. This is due to the fact that androgen receptors (ARs) interact with β-catenin, which can prevent its degradation and direct the AR/β-catenin complex to specific genes. It has been also shown that, in castrated male rats, testosterone induced a decrease in mRNA and the axin protein that is a negative regulator of β-catenin [35].

It is interesting that androgens have not only an anabolic effect on muscles. In mice with AR specifically knocked out, the level of myostatin expression in satellite cells (and their descendants) decreased by more than 6 times in the groin muscle [36]. The authors conclude that androgens that have an anabolic effect on the muscles also stimulate the expression of myostatin, which restrains the anabolic effect, preventing excessive muscle growth.

Progesterone and estradiol. When analyzing the effect of myostatin on human myometrium, it was shown that estradiol reduced the level of myostatin expression [37]. Conversely, an increased level of progesterone in pregnant women increases the expression of myostatin in the uterus endometrium [38].

3. Erythropoietin

It was discovered that erythropoietin inhibits the expression of myostatin. It has been shown that a significant reduction in the myostatin gene expression occurs during prolonged therapy of the degenerative muscle process in mice using recombinant erythropoietin [39].

MYOSTATIN AND SKELETAL MUSCULATURE

Myostatin and muscle cells. It is known that physical activity leads to muscle hypertrophy. In particular, this occurs with an eccentric type of contraction, when the muscle fibers elongate at the generation of force. This type of contraction causes mechanical damage to sarcomeres and sarcolemma, which results in the activation of signaling cascades leading to the degradation and renewal of damaged proteins [40]. With repeated small muscular injuries that occur with an eccentric type of contraction, the balance shifts toward protein synthesis. As a result, muscle volume and muscle strength are increased. In addition, under such loads, a change in the size and mechanical properties of the extracellular matrix surrounding the muscle fibers occurs. Myostatin plays the primary role in the above processes, since it is involved in the activation of proteolytic systems and regulation of the extracellular matrix restructuring.

The eccentric contraction causes damage to the sarcolemma, as a result of which Ca^{2+} ions activating the m-calpain protease enter the muscle fiber [41]. M-Calpain causes the titin degradation, thereby destroying the sarcomere and facilitating further degradation of other proteins. In the sarcomer, there is a local stock of myostatin that is released during the sarcomer degradation and acts either autocrinically or paracrinically on the satellite cells and fibroblasts located near the affected zone [42]. In addition, damage to myofibrils can lead to the formation of reactive oxygen species that activate proteases responsible for the proteolysis of *LAP* followed by the release of active myostatin.

Myostatin can increase the expression of atrogin-1 and MuRF-1 ubiquitin ligase, and thereby cause protein degradation in myofibrils [20]. It has been shown that the level of atrogin-1 is lowered and the muscles are hypertrophied in the myostatin knockout mice; however, the maximum muscle contraction strength is not increased in comparison with the control group [43]. Consequently, myostatin is important for the normal functioning of muscle fibers, because it "frees" the cell from unnecessary, used proteins and forms a place for newly synthesized proteins.

Myostatin is one of the main factors of muscular atrophy occurring in the conditions of space flight. In direct experiments in rats, it has been found that the loss of muscle mass that occur during space flight is associated with an increase in the level of myostatin in skeletal muscles (two- to fivefold in different muscles by the 17th day of flight) [43]. In terrestial studies with participation of humans, it has been found that the level of myostatin is increased by 12% by the 25th day of the immobility regime (as a model of space flight) [44].

Myostatin and satellite cells. Myostatin can regulate the functioning of not only muscle fibers, but also nearby cells, which include fibroblasts and satellite cells.

Mature muscle fibers are the final differentiation product; i.e., neither they themselves, as a whole structure, nor cell nuclei inside fibers can be divided; and growth and regeneration of muscles are realized due to the proliferation of satellite cells. Satellite cells have dimensions close to the dimensions of the cellular nuclei of muscle fibers, and, like these nuclei, are located on the periphery of the muscle fibers. Only electronic microscopy allowed us to establish that they are physically separated from mature muscle fibers and are located between the sarcolemma and the basal membrane. An increase in the fiber size is achieved through the fusion of the proliferating satellite cells with the fiber. First of all, a trauma, including that at the level of a single muscle fiber, is a stimulus for the proliferation of satellite cells in adult organisms. Part of the cells after division is returned to a state of rest (to restore the pool of satellite cells). During activation of the satellite cells and leaving a state of rest, expression of genes that are characteristic of myoblasts begin in them, and the satellite cells become myoblasts. In the case of chemotaxis, they migrate to damaged areas and, depending on the degree of damage, either merge with damaged muscle fibers (hypertrophy) or between themselves, creating new fibers (hyperplasia). In the process of birth, the nuclei of the satellite cells constitute about 30% of the total number of nuclei in the muscles of the lower limbs. These neonatal satellite cells proliferate and merge with the growing muscle fibers, introducing additional nuclei during the postnatal growth of skeletal muscles. In the adult organism, the nuclei of the satellite cells constitute 2–7% of the total number of nuclei in different muscles.

Thus, satellite cells provide the maintenance of the functional state of the skeletal muscles of an adult organism. They are necessary for the restoration of damaged muscle fibers and are a source of additional nuclei in the muscle hypertrophy as a resullt of sports training. Hyperortrophy and/or hyperplasia of the skeletal muscles in animals without functionally active myostatin proves that myostatin adversely affects the proliferation of satellite cells.

The formation of reactive oxygen species and mechanical stretching of the muscle occur during muscle load, which leads to releasing the hepatic growth factor (HGF) from the extracellular matrix. HGF activates satellite cells with their subsequent migration to the site of injury, proliferation, and fusion with myofibril [46]. There is an evidence that myostatin inhibits the proliferation of satellite cells by enhancing the expression of genes involved in the regulation of the cell cycle. Myostatin acts on the *p21* (upregulation), *CDK2*, and *Wnt4* (downregulation) protein genes [47–49]. This shows that myostatin negatively regulates the transition from G1 to S phase of the cell cycle in postnatal development. Thus, myosta-

tin negatively regulates the activation of resting satellite cells that are in the G1 and G0 phase, preventing the myotubules from developing (Fig. 5) [48]. It has been also found that myostatin reduces the expression of myogenic regulators, such as MyoD, myogenin, and Myf-5 [49–51].

This inhibitory action is necessary for the normal process of muscle regeneration, since the premature fusion of satellite cells with myofibrils can lead to a disruption in the functioning of the muscle fiber.

Myostatin and fibroblasts. Another target of myostatin in the muscles is fibroblasts located in the extracellular matrix near the satellite cells. They are responsible for the synthesis, degradation, and remodeling of the extracellular matrix. In physical training, myostatin can stimulate both proliferation and degradation of fibroblasts, thereby regulating the properties of the extracellular matrix. During physical exertion, fibroblasts synthesize type I and III collagen into the extracellular matrix, which leads to an increase in its rigidity; this reduces the damage to myofibrils [52].

Myostatin and tendons. An important component for the operation of skeletal muscles is tendons. It is the tendons that attach the muscles to the bones, which allows the force of muscle contraction to move parts of the body in space. Previously it was assumed that tendons are metabolically inert; however, at present it is known that physical training changes their structure and functioning [16]. Tendons consist of collagen types I and III, elastin, proteoglycans, and mucopolysaccharides that produce fibroblasts located along the main direction of tension [53]. Proliferation of fibroblasts, an increase in collagen synthesis and, correspondingly, an increase in the cross-sectional area of the tendon accompanied by an increase in its rigidity take place during eccentric training. This allows the tendons to withstand a heavy load and reduces the risk of damage to the tendons.

Interesting results were obtained in myostatin-deficient mice: their muscle mass was increased, as expected; however, their tendons were smaller in size than the control [54]. In this study on tendon fibroblasts, the ACTR2B receptors were detected, as well as the fact that their activation leads to phosphorylation of both p38 MAPK and Smad2/3. However, only the MAPK pathway leads to the proliferation of fibroblasts, since it results in an increase in the expression of scleraxis and tenomodulin genes (the expression of these genes is necessary for the proliferation of fibroblasts). Thus, myostatin can regulate the structure of tendons. In this study it was also shown that the absence of myostatin leads to a change in the mechanical properties of the tendons, namely, to deterioration in their ability to stretch, which increases the risk of their damage. Such data call into question the usefulness of suppressing the work of myostatin for therapeutic and sports purposes.

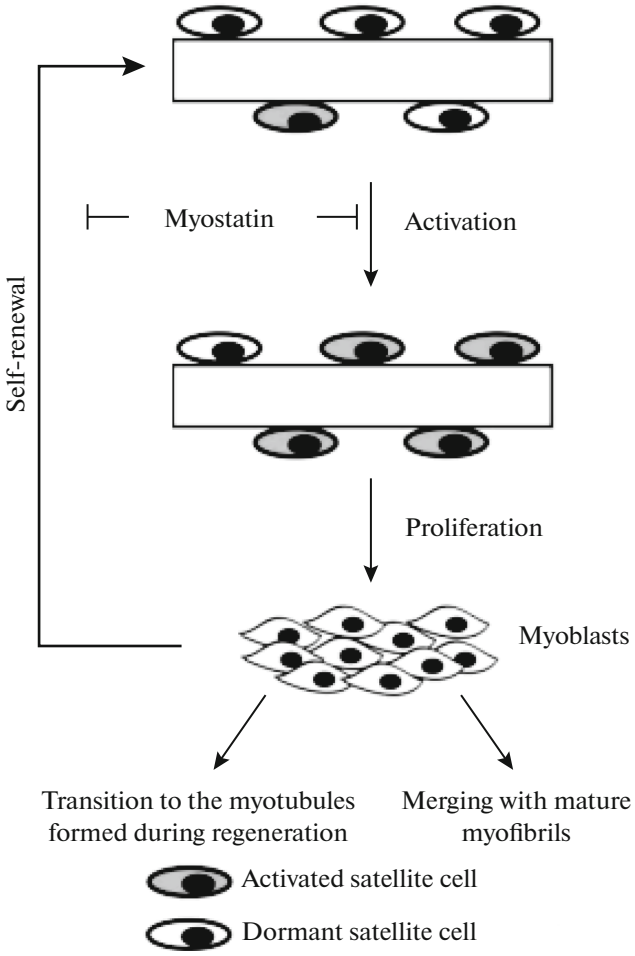

Fig. 5. The role of myostatin in the growth of muscle fibers during postnatal development. Activated satellite cells (S phase) are designated in gray color; the dormant satellite cells (G1 and G0 phases), in white. Dormant satellite cells are activated in response to muscle damage. Proliferating myoblasts can differentiate into a new myotubule, or merge with mature myofibrils. Myostatin suppresses the self-renewal stage (transition of myoblasts to dormant state) (Modified from [48]).

MYOSTATIN AND CARDIAC MUSCLE

Myostatin was detected on a much lower level in the heart and adipose tissue compared to skeletal muscle. “Cardiac” myostatin was found in Purkinje fibers and in working cardiomyocytes in both fetal and adult animals. The levels of mRNA and myostatin protein were low in fetal cardiomyocytes of rats on day 18 of embryogenesis, which provides proliferation and development of the myocardium. A sixfold increase in the level of myostatin expression occurs in adult rats by day 10 of postnatal development, and then the expression level gradually decreases as it reaches adulthood [55]. When comparing ventricular myocardial samples in twenty-day-old piglets, the highest expression of myostatin was detected in the left ventricle (it is six

times greater than the expression level in the right ventricle) [56].

A number of studies have shown that the level of cardiac myostatin increases in adult organisms with increased cardiac loading and in a number of other forms of HF, which will be discussed below.

Myocardial infarction. During myocardial infarction, the myostatin transcription increases in cardiomyocytes located around the affected area during the period from 12 h to 30 days of myocardial ischemia, which indicates the involvement of myostatin in the regulation of myocardial work in pathological conditions [10]. Other researchers observed a fourfold increase in myostatin and a threefold increase in mRNA in the myocardium of rats after eight weeks from the time of myocardial infarction [57]. In the same study, it was shown that under experimental conditions, there was the myostatin overexpression in skeletal muscles when applying the pro-inflammatory tumor necrosis factor α (TNF-α). It is known that in chronic HF, the level of TNF-α increases in blood plasma and tissues. From this, *TNF*-α can be a possible activator of the myostatin overexpression during myocardial infarction.

Hypervolemia. An increase in the myostatin level was also observed in other pathological conditions. For example, the mRNA overexpression and threefold increase in the myostatin amount were observed in the model of heart failure in hypervolemia in rats [58]. In transgenic mice with cardiac hypertrophy caused by the Akt kinase overexpression, an increase in myostatin expression by 18.4 times was observed [59]. Experiments in vitro showed that the amount of mRNA and myostatin protein increases in cardiomyocytes under mechanical action or under the influence of humoral factors, such as insulin-like growth factor 1 (IGF1), phenylephrine, and angiotensin II, which triggered the activation of MAPK (p38 and/or ERK) and the MEF2 transcription factor binding to the site of the myostatin gene promoter [60, 61]. Thus, myostatin expression was increased in response to an increased content of factors leading to myocardial hypertrophy in chronic HF, which in theory should restrain the development of hypertrophy.

The secretion of active myostatin by myocardium to the bloodstream during hypervolemia was demonstrated by Heineke et al. in a study in mice with a selective inhibition of the cardiac myostatin expression [62]. The myostatin mRNA was not detected in the heart of such mice, while mRNA and the myostatin precursor were detected in normal amounts in skeletal muscles. It was shown that after a two-week heart overload with hypervolemia, the myostatin level in plasma increased threefold in wild mice, and no increase in myostatin level was found in the knockout mice. This shows that in the case of pathology, the myocardium secretes myostatin into the bloodstream. The increased level of myostatin in wild mice subsequently led to skeletal muscle atrophy, which was not observed in the knockout mice and during blockade of myostatin by antibodies in wild mice. Thus, the myocardium secretes myostatin into the blood plasma during hypervolemia accompanied by a significant increase in the hormone level compared with the normal state, which leads to the induction of muscle atrophy [62].

Cardiomyopathy. In clinical trials, George et al. found that myostatin levels were increased in the left ventricular myocardial samples of patients suffering from ischemic (ICM) or dilated (DCM) cardiomyopathies [63]. An increased amount of *LAP* (twofold in ICM, sixfold in DCM) was recorded by Western blotting, and the level of promyostatin was unchanged. This suggests that the cleavage of the latent myostatin complex with furin convertases is enhanced in the heart of patients with cardiomyopathy. Despite this, the level of the latent myostatin complex did not change, which may have been provided by reverse regulation of LAP or an active ligand. An increased level of cardiac myostatin can lead to both local and systemic effects due to the secretion of myostatin from the myocardium into the bloodstream. Local effects of myostatin in HF may be associated with BMP-1 proteinase overexpression in the myocardium, with increased expression of ACVR2B myostatin receptor, and increased activation of SMAD2/3 transcription factors [63].

To confirm the role of cardiac myostatin in cardiac pathology, the researchers produced transgenic mice with the myostatin overexpression in cardiomyocytes [62]. In these animals, an increase in the myostatin level in the plasma by a factor of three to four and the reduction of muscle mass to 30% as compared to the wild-type mice were observed.

POSSIBLE MECHANISMS OF MYOSTATIN ACTION ON THE HEART IN HEART FAILURE

The main source of myostatin in the systemic bloodstream is the skeletal musculature; however, cardiac myostatin also contributes to this indicator in cardiac pathology. This was shown in the knockout of skeletal muscle myostatin in mice, which resulted in a decrease in the amount of myostatin in the blood by 60–70% [64].

The effect of muscular atrophy in HF can occur due to the existence of two different pools of myostatin in mammals. The main difference between the two pools of myostatin, systemic (plasma) and local (muscular), is the mechanism of myostatin activation. Most of myostatin in skeletal muscles is in the form of promyostatin that is constitutively inhibited by the LTBP-3 protein and "bound" by the same protein to the extracellular matrix [5]. Activation of this local muscular pool requires two consecutive cleavages (Fig. 2). In contrast to the local pool, the plasma myostatin is

already in the form of a latent complex. Therefore, when the latent complex of myostatin reaches the target organ (skeletal musculature), it requires only one cleavage by metalloproteinases for activation. In this regard, the systemic myostatin is able to carry out its effects faster than the locally located myostatin of the skeletal muscle.

The results obtained by the research team of Heineke in knockout mice are consistent with the data obtained by George et al. in the study of myostatin in patients with cardiomyopathy [63]. In the by George et al., an elevated level of the latent myostatin complex in plasma in patients suffering from cardiomyopathy was also detected. A significantly higher content of the myostatin LAP was found in myocardial tissue samples in patients with both DCM and ICM compared to the healthy people samples, which supports the hypothesis that the myocardium is the source of systemic myostatin in the form of a latent complex [63].

Thus, in the pathology of cardiac activity, an increase in the secretion of the latent myostatin complex from the myocardium to the plasma results in a significant increase in the level of active ligand in the skeletal musculature, which causes muscle atrophy. It should also be noted that the contribution of adipose tissue to the level of systemic myostatin is not known at the moment. However, an increase in the production of myostatin by adipocytes in obesity was also shown, which led to a reduction of skeletal musculature [65].

POSSIBLE VARIANTS OF THERAPEUTIC APPLICATION OF MYOSTATIN

Increased myostatin level and enhancement of its signaling pathways occur under certain specific conditions of the body and lead to a decrease in muscle mass. Such conditions include: aging [66], prolonged stay in a stationary state [45], and being in space [44]. An increase in the myostatin level was also observed in such diseases as cardiac [64] and renal failure [67], AIDS [68], and cachexia in oncology [69]. Suppression of the myostatin work could potentially prevent the loss of muscle mass under the above conditions and also lead to an increase in it, in which athletes and bodybuilders are also interested. In a professional sports environment, the myostatin inhibition has been already prohibited by the decision of the World Anti-Doping Agency (https://www.wada-ama.org/en/prohibited-list/prohibited-at-all-times/hormone-and-metabolic-modulators).

In 2004, a paper was published in which the case of the myostatin gene mutation in humans was described [70]. In both copies of the myostatin gene of the patient, a newborn baby boy, there were mutations that suppress the synthesis of functioning protein. This child had enlarged muscles of the tights and upper limbs already at birth. All the patient's reflexes were normal, except for those associated with tendons. It is interesting that this mutation was present in other members of this family. One of the relatives possessed extraordinary strength, and the 24-year-old mother of the child was a professional athlete and had developed musculature, though to a lesser extent than her son. This study showed for the first time on a human that the absence of myostatin leads to an increase in muscle mass and strength.

Unfortunately, there are no works that would show what happened to the boy in adulthood. Now there is a set of methods and substances that can suppress the work of myostatin in the human body for therapeutic and sports purposes. Many of these substances are of great interest for study; clinical trials were even conducted with some of them. However, none of the potential myostatin inhibitors has currently been clinically approved [71].

At present, attention is drawn to the study of antibodies to myostatin, e.g., MYO-029; however, the efficiency of these antibodies is still low [72]. Despite the fact that administration of MYO-029 caused muscle growth by 20–30% in mice, there was no a statistically significant effect in humans, which may be due to a small sample in the study.

In another study, J. Heineke et al. inhibited myostatin in mice using monoclonal antibodies to myostatin, which interfere with the binding of myostatin to its receptor ACVR2B [62]. Treatment was started after the development of HF caused by aortic clamping. As a result, the skeletal musculature atrophy was stopped and the weight of the heart returned to the control values after six weeks of therapy [62]. It is important to note that the side effects of JA-16 monoclonal antibodies on cardiac function or on the survival of mice with HF were not recorded [64].

In addition, there are analogues of myostatin receptors (ACVR2B) that circulate in the blood and compete with endogenous receptors for binding to the agonist. They are a soluble ligand-binding domain attached to the Fc region of immunoglobulin G (IgG) (sACVR2B-Fc). The therapy with the false receptor sACVR2B-Fc blocks the pathway of the normal ACVR2B receptor: there are the decreased activation of the proteasomal ubiquitylation system; the underexpression of atrogin-1, MuRF1, and ubiquitin; and the increased level of myosin. In order to reduce the loss of muscle mass in cancer and chemotherapy, studies of such agents were conducted in mice. It was shown that, for example, the administration of sACVR2B-Fc in combination with doxorubicin neutralizes the side effects of the latter that were associated with the loss of muscle mass and strength, without reducing the efficiency of suppressing tumor growth [73].

In another study, it was shown that in mice with transplanted cells of intestinal carcinoma, melanoma, or ovarian carcinoma, as well as in the inhibin knockout mice, therapy with the sACVR2B-Fc receptor not

only stops the loss of muscle and cardiac mass, but also restores it to the initial level [74]. With systemic blockade of ACVR2B, myocardial hypertrophy also occurs during cancer-induced cachexia in mice [75]. These data allow treating the therapy with the sACVR2B-Fc false receptors as a potential treatment of cachexia in cancer patients.

A decrease in the level of myostatin in HF was observed at regular physical exercise in rats after myocardial infarction [57]. Perhaps, moderate physical exercises for patients with HF can also be an efficient remedy against muscle atrophy. However, considering that activin A activates the same ACVR2B receptor as myostatin, it is reasonable to assume that blockade of the receptor may be a more efficient method for treating muscle atrophy in HF.

Moreover, antibodies interacting with the ACVR2B receptor (bymagrumab or BYM338) and thereby blocking its binding to myostatin were been synthesized [76]. The authors indicate that the high efficiency of *BYM*338 (up to 50% increase in muscle mass) is due to the fact that these antibodies completely block the signaling caused by the activation of the ACVR2B receptors, i.e., suppress the action of not only myostatin, but also GDF-11 and activin capable of interacting with the same receptor. To confirm their hypothesis, the authors performed a comparison of the efficiency of BYM338 and D76A, the artificially synthesized latent complex of myostatin. In the first case, the increase in body weight was twice as much (up to 36% versus 15%). D76A interacts with myostatin; in this case, the further release and activation of myostatin does not occur, since the proteolytic cleavage of BMP1/TLL by metalloproteinases is impossible in this molecule [11]. The introduction of the latent complex of myostatin in its natural form also leads to inhibition of the myostatin action, since the competition of proteases for the substrate takes place [77].

Another method of inhibiting the activity of myostatin is RNA interference. For example, it was shown that the introduction of miRNA binding to myostatin RNA into the large tibia muscle of rats suppresses myostatin expression by 48% and leads to an increase in the mass of this muscle by 10% [78]. Currently, active research in the field of the myostatin inhibition is under way; however, the creation of an efficient agent is hardly possible in the near future. As often happens, interference with the body's natural processes can lead to undesirable results. For example, when trying to grow a large muscle mass, local problems with blood circulation in the muscles can arise. Thus, when studying the myostatin gene-knockout mice, a decrease in capillary density in the rectus femoris and longissimus dorsi muscles with increasing their mass was observed, as well as the muscle metabolism was shifted towards glycolysis [79]. In another study, it was shown that although the increased extensor digitorum longus muscle mass was observed in knockout mice, the contraction force per muscle mass was lower than that in the wild type [80]. The authors of this study found a decrease in the number of mitochondria in the muscle fibers of knockout mice and concluded that myostatin is necessary for normal aerobic metabolism in skeletal muscles. As mentioned above, myostatin is also required for the correct degradation of the "spent" muscle proteins and the formation of the correct structure of tendons. Still, the benefits of studying this protein and its inhibitors are incomparably greater.

CONCLUSIONS

Thus, myostatin is an autocrine, paracrine, and systemic factor that acts as a negative regulator of skeletal muscle growth. Synthesis and secretion of myostatin is the most present in skeletal muscles, but can also occur in cardiomyocytes and adipocytes. During embryogenesis, myostatin regulates a finite number of the formed muscle fibers. In the adult organisms, myostatin acts as a limiter of the muscle mass growth. It is important that the targets of myostatin in skeletal musculature are not only the muscle fibers, but also the cells surrounding them, namely the satellite cells and fibroblasts. Thus, during physical exercise, myostatin promotes normal muscle regeneration and the correct remodeling of muscle tendons. The myostatin signaling pathways in the cell are complex, include multi-level cascades and are not fully understood.

In addition, many studies have shown that the myostatin level increases under various pathological conditions, for example, in heart failure. Thus, myostatin is an important mediator in the processes of muscular atrophy and cardiac cachexia. In this case, the myocardium increases the pool of both local and systemic myostatin. In the bloodstream, myostatin acts as an endocrine factor interacting remotely with skeletal muscles; in the heart it acts as a paracrine factor acting on the myocardial tissue itself. The secretion of myostatin in the heart is activated only during increased cardiac stress, possibly in order to maintain unbalanced myocardial growth. Thus, the secretion of cardiac myostatin may act in order to maintain normal heart function.

Inhibition of myostatin is a promising therapeutic method for the treatment of pathologies associated with atrophy of skeletal and cardiac musculature. There is a variety of mechanisms and targets of inhibitors of myostatin functioning. However, at the moment none of the substances in this series were approved for clinical application.

Myostatin is an interesting object of research, as to today only a small part of the information about it is reliably known. At the moment, researchers are particularly interested in its role in the development of many pathologies, especially taking into account the

fact that it is directly associated with a large number of diseases of high social significance. To summarize, it should be noted that scientists face serious challenges in the study of this hormone, its functions, and methods for its inhibition, and even 20 years after its discovery, we are only at the very beginning of the journey.

REFERENCES

1. McPherron, A.C., Lawler, A.M., and Lee, S.-J., Regulation of skeletal muscle mass in mice by a new TGF-β superfamily member, *Nature*, 1997, vol. 387, no. 6628, p. 83.
2. Lessard, S.J., Rivas, D.A., Alves-Wagner, A.B., et al., Resistance to aerobic exercise training causes metabolic dysfunction and reveals novel exercise-regulated signaling networks, *Diabetes*, 2013, vol. 62, no. 8, p. 2717.
3. Ma, K., Mallidis, C., Artaza, J., et al., Characterization of 5'-regulatory region of human myostatin gene: regulation by dexamethasone in vitro, *Am. J. Physiol. Endocrinol. Metab*., 2001, vol. 281, no. 6, p. E1128.
4. Du, R., Chen, Y.-F., An, X.-R., et al., Cloning and sequence analysis of myostatin promoter in sheep, *DNA Sequencing*, 2005, vol. 16, no. 6, p. 412.
5. Anderson, S.B., Goldberg, A.L., and Whitman, M., Identification of a novel pool of extracellular pro-myostatin in skeletal muscle, *J. Biol. Chem*., 2008, vol. 283, no. 11, p. 7027.
6. Lee, S.J. and McPherron, A.C., Regulation of myostatin activity and muscle growth, *Proc. Natl. Acad. Sci. U.S.A*., 2001, vol. 98, no. 16, p. 9306.
7. Miura, T., Kishioka, Y., Wakamatsu, J.I., et al., Interaction between myostatin and extracellular matrix components, *Anim. Sci. J*., 2010, vol. 81, no. 1, p. 102.
8. Hill, J.J., Davies, M.V., Pearson, A.A., et al., The myostatin propeptide and the follistatin-related gene are inhibitory binding proteins of myostatin in normal serum, *J. Biol. Chem*., 2002, vol. 277, no. 43, p. 40735.
9. Allen, D.L., Cleary, A.S., Speaker, K.J., et al., Myostatin, activin receptor IIb, and follistatin-like-3 gene expression are altered in adipose tissue and skeletal muscle of obese mice, *Am. J. Physiol. Endocrinol. Metab*., 2008, vol. 294, no. 5, p. E918.
10. Sharma, M., Kambadur, R., Matthews, K.G., et al., Myostatin, a transforming growth factor-β superfamily member is expressed in heart muscle and is upregulated in cardiomyocytes after infarct, *J. Cell. Physiol*., 1999, vol. 180, no. 1, p. 1.
11. Wolfman, N.M., McPherron, A.C., Pappano, W.N., et al., Activation of latent myostatin by the BMP-1/tolloid family of metalloproteinases, *Proc. Natl. Acad. Sci. U.S.A*., 2003, vol. 100, no. 26, p. 15842.
12. Allen, D.L., Greyback, B.J., Hanson, A.M., et al., Skeletal muscle expression of bone morphogenetic protein-1 and tolloid-like-1 extracellular proteases in different fiber types and in response to unloading, food deprivation and differentiation, *J. Physiol. Sci*., 2010, vol. 60, no. 5, p. 343.
13. Lee, S.J., Genetic analysis of the role of proteolysis in the activation of latent myostatin, *PLoS One*, 2008, vol. 3, no. 2, p. e1628.
14. Hill, J.J., Qiu, Y., Hewick, R.M., and Wolfman, N.M., Regulation of myostatin in vivo by growth and differentiation factor-associated serum protein-1: a novel protein with protease inhibitor and follistatin domains, *Mol. Endocrinol*., 2003, vol. 17, no. 6, p. 1144.
15. Lee, S.-J., Regulation of muscle mass by myostatin, *Annu. Rev. Cell Dev. Biol*., 2004, vol. 20, no. 1, p. 61.
16. Massagué, J., TGFβ signaling in context, *Nat. Rev. Mol. Cell Biol*., 2012, vol. 13, no. 10, p. 616.
17. Gumucio, J.P., Sugg, C.B., and Mendias, C.L., TGF-β superfamily signaling in muscle and tendon adaptation to resistance exercise, *IUBMB Life*, 2015, vol. 67, no. 8, p. 14.
18. Gumucio, J.P. and Mendias, C.L., Atrogin-1, MuRF-1, and sarcopenia, *Endocrine*, 2013, vol. 43, no. 1, p. 12.
19. Tsuchida, K., Nakatani, M., Hitachi, K., et al. Activin signaling as an emerging target for therapeutic interventions, *Cell Commun. Signal*., 2009, vol. 7, no. 1, p. 15.
20. Sandri, M., Signaling in muscle atrophy and hypertrophy, *Physiology* (Bethesda), 2008, vol. 23, no. 3, p. 160.
21. Trendelenburg, A.U., Meyer, A., Rohner, D., et al., Myostatin reduces Akt/TORC1/p70S6K signaling, inhibiting myoblast differentiation and myotube size, *Am. J. Physiol. Cell Physiol*., 2009, vol. 296, no. 6, p. C1258.
22. Sandri, M., Protein breakdown in muscle wasting: role of autophagy-lysosome and ubiquitin-proteasome, *Int. J. Biochem. Cell Biol*., 2013, vol. 45, no. 10, p. 2121.
23. Welle, S.L., Myostatin and muscle fiber size. Focus on "Smad2 and 3 transcription factors control muscle mass in adulthood" and "Myostatin reduces Akt/TORC1/p70S6K signaling, inhibiting myoblast differentiation and myotube size", *Am. J. Physiol.: Cell Physiol*., 2009, vol. 1, no. 6, p. 1245.
24. Sandri, M., Sandri, C., Gilbert, A., et al., Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy, *Cell*, 2004, vol. 117, no. 3, p. 399.
25. Stitt, T.N., Drujan, D., Clarke, B.A., et al., The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors, *Mol. Cell*, 2004, vol. 14, no. 3, p. 395.
26. Ruas, J.L., White, J.P., Rao, R.R., et al., A PGC-1α isoform induced by resistance training regulates skeletal muscle hypertrophy, *Cell*, 2012, vol. 151, no. 6, p. 1319.
27. Raffaello, A., Milan, G., Masiero, E., et al., JunB transcription factor maintains skeletal muscle mass and promotes hypertrophy, *J. Cell Biol*., 2010, vol. 191, no. 1, p. 101.
28. Sacheck, J.M., IGF-I stimulates muscle growth by suppressing protein breakdown and expression of atrophy-related ubiquitin ligases, atrogin-1 and MuRF1, *Am. J. Physiol.: Endocrinol. Metab*., 2004, vol. 287, no. 4, p. E591.
29. Lecker, S.H., Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression, *FASEB J*., 2004, vol. 18, no. 1, p. 39.
30. Artaza, J.N., Bhasin, S., Mallidis, C., et al., Endogenous expression and localization of myostatin and its relation to myosin heavy chain distribution in C2C12

skeletal muscle cells, *J. Cell. Physiol.*, 2002, vol. 190, no. 2, p. 170.

31. Allen, D.L. and Loh, A.S., Posttranscriptional mechanisms involving microRNA-27a and b contribute to fast-specific and glucocorticoid-mediated myostatin expression in skeletal muscle, *Am. J. Physiol.: Cell Physiol.*, 2011, vol. 300, no. 1, p. C124.
32. Gilson, H., Schakman, O., Combaret, L., et al., Myostatin gene deletion prevents glucocorticoid-induced muscle atrophy, *Endocrinology*, 2007, vol. 148, no. 1, p. 452.
33. Reisz-Porszasz, S., Bhasin, S., Artaza, J.N., et al., Lower skeletal muscle mass in male transgenic mice with muscle-specific overexpression of myostatin, *Am. J. Physiol.: Endocrinol. Metab.*, 2003, vol. 4, no. 285, p. E876.
34. Singh, R., Bhasin, S., Braga, M., et al., Regulation of myogenic differentiation by androgens: cross talk between androgen receptor/β-catenin and follistatin/transforming growth factor-β signaling pathways, *Endocrinology*, 2009, vol. 150, no. 3, p. 1259.
35. Gentile, M.A., Nantermet, P.V., Vogel, R.L., et al., Androgen-mediated improvement of body composition and muscle function involves a novel early transcriptional program including IGF1, mechano growth factor, and induction of β-catenin, *J. Mol. Endocrinol.*, 2010, vol. 44, no. 1, p. 55.
36. Dubois, V., Laurent, M.R., Sinnesael, M., et al., A satellite cell-specific knockout of the androgen receptor reveals myostatin as a direct androgen target in skeletal muscle, *FASEB J.*, 2014, vol. 28, no. 7, p. 2979.
37. Pasquapina, C., Bloise, E., Gray, P.C., et al., Activin-A and myostatin response and steroid regulation in human myometrium: disruption of their signaling in uterine fibroid, *Gynecol. Surg.*, 2010, vol. 7, no. 3, p. 307.
38. Forde, N., Carter, F., Fair, T., et al., Progesterone-regulated changes in endometrial gene expression contribute to advanced concept development in cattle, *Biol. Reprod.*, 2009, vol. 81, no. 4, p. 784.
39. Feder, D., Rugollini, M., Santomauro, A., et al., Erythropoietin reduces the expression of myostatin in mdx dystrophic mice, *Braz. J. Med. Biol. Res.*, 2014, vol. 47, no. 11, p. 966.
40. Schoenfeld, B.J., Does exercise-induced muscle damage play a role in skeletal muscle hypertrophy? *J. Strength*, 2012, vol. 26, no. 5, p. 1441.
41. Jackman, R.W. and Kandarian, S.C., The molecular basis of skeletal muscle atrophy, *Am. J. Physiol.: Cell Physiol.*, 2004, vol. 287, no. 4, p. C834.
42. Nicholas, G., Thomas, M., Langley, B., et al., Titincap associates with, and regulates secretion of, myostatin, *J. Cell. Physiol.*, 2002, vol. 193, no. 1, p. 120.
43. Mendias, C.L., Kayupov, E., Bradley, J.R., et al., Decreased specific force and power production of muscle fibers from myostatin-deficient mice are associated with a suppression of protein degradation, *J. Appl. Physiol.*, 2011, vol. 111, no. 1985, p. 185.
44. Lalani, R., Bhasin, S., Byhower, F., et al., Myostatin and insulin-like growth factor-I and -II expression in the muscle of rats exposed to the microgravity environment of the NeuroLab space shuttle flight, *J. Endocrinol.*, 2000, vol. 3, no. 167, p. 417.
45. Zachwieja, J.J., Smith, S.R., Sinha-Hikim, I., et al., Plasma myostatin-immunoreactive protein is increased after prolonged bed rest with low-dose T3 administration, *J. Gravitational Physiol.*, 1999, vol. 6, no. 2, p. 11.
46. Tatsumi, R., Liu, X., Pulido, A., et al. Satellite cell activation in stretched skeletal muscle and the role of nitric oxide and hepatocyte growth factor, *Am. J. Physiol.: Cell Physiol.*, 2006, vol. 6, no. 290, p. 1487.
47. Steelman, C.A., Recknor, J.C., Nettleton, D., and Reecy, J.M., Transcriptional profiling of myostatin-knockout mice implicates Wnt signaling in postnatal skeletal muscle growth and hypertrophy, *FASEB J.*, 2006, vol. 20, no. 2, p. 580.
48. McCroskery, S., Thomas, M., Maxwell, L., et al., Myostatin negatively regulates satellite cell activation and self-renewal, *J. Cell Biol.*, 2003, vol. 162, no. 6, p. 1135.
49. Joulia, D., Bernardi, H., Garandel, V., et al., Mechanisms involved in the inhibition of myoblast proliferation and differentiation by myostatin, *Exp. Cell Res.*, 2003, vol. 286, no. 2, p. 263.
50. Langley, B., Thomas, M., Bishop, A., et al., Myostatin inhibits myoblast differentiation by down-regulating MyoD expression, *J. Biol. Chem.*, 2002, vol. 277, no. 51, p. 49831.
51. Ríos, R., Carneiro, I., Arce, V.M., and Devesa, J., Myostatin is an inhibitor of myogenic differentiation, *Am. J. Physiol.: Cell Physiol.*, 2002, vol. 282, no. 5, p. C993.
52. Mendias, C.L., Gumucio, J.P., Bakhurin, K.I., et al., Physiological loading of tendons induces scleraxis expression in epitenon fibroblasts, *J. Orthop. Res.*, 2012, vol. 30, no. 4, p. 606.
53. Kjær, M., Langberg, H., Heinemeier, K., et al., From mechanical loading to collagen synthesis, structural changes and function in human tendon, *Scand. J. Med. Sci. Sport*, 2009, vol. 19, no. 4, p. 500.
54. Mendias, C.L., Bakhurin, K.I., and Faulkner, J.A., Tendons of myostatin-deficient mice are small, brittle, and hypocellular, *Proc. Natl. Acad. Sci. U.S.A.*, 2008, vol. 105, no. 1, p. 388.
55. McKoy, G., Bicknell, K.A., Patel, K., and Brooks, G., Developmental expression of myostatin in cardiomyocytes and its effect on foetal and neonatal rat cardiomyocyte proliferation, *Cardiovasc. Res.*, 2007, vol. 74, no. 2, p. 304.
56. Torrado, M., Iglesias, R., Nespereira, B., and Mikhailov, A.T., Identification of candidate genes potentially relevant to chamber-specific remodeling in postnatal ventricular myocardium, *J. Biomed. Biotechnol.*, 2010, vol. 2010, no. 603159, p. 10.
57. Lenk, K., Schur, R., Linke, A., et al., Impact of exercise training on myostatin expression in the myocardium and skeletal muscle in a chronic heart failure model, *Eur. J. Heart Failure*, 2009, vol. 11, no. 4, p. 342.
58. Shyu, K.G., Lu, M.J., Wang, B.W., et al., Myostatin expression in ventricular myocardium in a rat model of volume-overload heart failure, *Eur. J. Clin. Invest.*, 2006, vol. 36, no. 10, p. 713.

59. Cook, S.A., Matsui, T., Li, N., and Rosenzweig, A., Transcriptional effects of chronic Akt activation in the heart, *J. Biol. Chem.*, 2002, vol. 277, no. 25, p. 22528.

60. Shyu, K.G., Ko, W.H., Yang, W.S., et al., Insulin-like growth factor-1 mediates stretch-induced upregulation of myostatin expression in neonatal rat cardiomyocytes, *Cardiovasc. Res.*, 2005, vol. 68, no. 3, p. 405.

61. Wang, B.W., Chang, H., Kuan, P., and Shyu, K.G., Angiotensin II activates myostatin expression in cultured rat neonatal cardiomyocytes via p38 MAP kinase and myocyte enhance factor 2 pathway, *J. Endocrinol.*, 2008, vol. 197, no. 1, p. 85.

62. Heineke, J., Auger-Messier, M., Xu, J., et al., Genetic deletion of myostatin from the heart prevents skeletal muscle atrophy in heart failure, *Circulation*, 2010, vol. 121, no. 3, p. 419.

63. George, I., Bish, L.T., Kamalakkannan, G., et al. Myostatin activation in patients with advanced heart failure and after mechanical unloading, *Eur. J. Heart Failure*, 2010, vol. 12, no. 5, p. 444.

64. Breitbart, A., Auger-Messier, M., Molkentin, J.D., and Heineke, J., Myostatin from the heart: local and systemic actions in cardiac failure and muscle wasting, *Am. J. Physiol. Heart Circ. Physiol.*, 2011, vol. 300, no. 6, p. H1973.

65. McPherron, A.C., Metabolic functions of myostatin and Gdf11, *Immunol. Endocrinol. Metab. Agents Med. Chem.*, 2010, vol. 10, no. 4, p. 217.

66. Yarasheski, K.E., Bhasin, S., Sinha-Hikim, I., et al., Serum myostatin-immunoreactive protein is increased in 60-92 year old women and men with muscle wasting, *J. Nutr., Health Aging*, 2002, vol. 6, no. 5, p. 343.

67. Sun, D.F., Chen, Y., and Rabkin, R., Work-induced changes in skeletal muscle IGF-1 and myostatin gene expression in uremia, *Kidney Int.*, 2006, vol. 70, no. 3, p. 453.

68. Gonzalez-Cadavid, N.F., Taylor, W.E., Yarasheski, K., et al., Organization of the human myostatin gene and expression in healthy men and HIV-infected men with muscle wasting, *Proc. Natl. Acad. Sci. U.S.A.*, 1998, vol. 95, no. 25, p. 14938.

69. Costelli, P., Muscaritoli, M., Bonetto, A., et al., Muscle myostatin signaling is enhanced in experimental cancer cachexia, *Eur. J. Clin. Invest.*, 2008, vol. 38, no. 7, p. 531.

70. Schuelke, M., Wagner, K.R., Stolz, L.E., et al., Myostatin mutation associated with gross muscle hypertrophy in a child, *N. Engl. J. Med.*, 2004, vol. 350, no. 26, p. 2682.

71. Thevis, M. and Schänzer, W., Emerging drugs affecting skeletal muscle function and mitochondrial biogenesis—potential implications for sports drug testing programs, *Rapid Commun. Mass Spectrom.*, 2016, vol. 30, no. 5, p. 635.

72. Singh, P., Rong, H., Gordi, T., et al., Translational pharmacokinetic/pharmacodynamic analysis of MYO-029 antibody for muscular dystrophy, *Clin. Transl. Sci.*, 2016, vol. 9, no. 6, p. 302.

73. Nissinen, T.A., Degerman, J., Räsänen, M., et al., Systemic blockade of ACVR2B ligands prevents chemotherapy-induced muscle wasting by restoring muscle protein synthesis without affecting oxidative capacity or atrogenes, *Sci. Rep.*, 2016, vol. 26, no. 6, p. 32695.

74. Zhou, X., Wang, J.L., Lu, J., et al., Reversal of cancer cachexia and muscle wasting by ActRIIB antagonism leads to prolonged survival, *Cell*, 2010, vol. 142, no. 4, p. 531.

75. Tisdale, M.J., Reversing cachexia, *Cell*, 2010, vol. 142, no. 4, p. 511.

76. Lach-Trifilieff, E., Minetti, G.C., Sheppard, K., et al., An antibody blocking activin type II receptors induces strong skeletal muscle hypertrophy and protects from atrophy, *Mol. Cell. Biol.*, 2014, vol. 34, no. 4, p. 606.

77. Fedoruk, M.N. and Rupert, J.L., Myostatin inhibition: a potential performance enhancement strategy? *Scand. J. Med. Sci. Sport.*, 2008, vol. 18, no. 2, p. 123.

78. Konishi, M., Kawamoto, K., Izumikawa, M., et al., Myostatin short interfering hairpin RNA gene transfer increases skeletal muscle mass, *J. Gene Med.*, 2006, vol. 8, p. 1171.

79. Rehfeldt, C., Ott, G., Gerrard, D.E., et al., Effects of the *Compact* mutant myostatin allele MstnCmpt-dl1Abc introgressed into a high growth mouse line on skeletal muscle cellularity, *J. Muscle Res. Cell Motil.*, 2005, vol. 26, nos. 2–3, p. 103.

80. Amthor, H., Macharia, R., Navarrete, R., et al., Lack of myostatin results in excessive muscle growth but impaired force generation, *Proc. Natl. Acad. Sci. U.S.A.*, 2007, vol. 104, no. 6, p. 1835.

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