The role of TGF-β1 during skeletal muscle regeneration
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Abstract
The injury of adult skeletal muscle initiates series of well-coordinated events that lead to the efficient repair of the damaged tissue. Any disturbances during muscle myolysis or reconstruction may result in the unsuccessful regeneration, characterised by strong inflammatory response and formation of connective tissue, that is, fibrosis. The switch between proper regeneration of skeletal muscle and development of fibrosis is controlled by various factors. Amongst them are those belonging to the transforming growth factor β family. One of the TGF-β family members is TGF-β1, a multifunctional cytokine involved in the regulation of muscle repair via satellite cells activation, connective tissue formation, as well as regulation of the immune response intensity. Here, we present the role of TGF-β1 in myogenic differentiation and muscle repair. The understanding of the mechanisms controlling these processes can contribute to the better understanding of skeletal muscle atrophy and diseases which consequence is fibrosis disrupting muscle function.

Keywords: fibrosis; myogenic differentiation; skeletal muscle regeneration; TGF-β1

Introduction
Regeneration is one of the most crucial events that occurs in living organism, including vertebrates. Since tissues and organs could be exposed to injuries, the mechanisms of their repair need to be properly operating in order to maintain correct physiology and function of the organism. However, the regeneration potential of specific tissues differs. For example, kidneys and heart muscle are not capable of effective regeneration (Benigni et al., 2010; Laflamme and Murry, 2011), whilst skin epithelium and connective tissue are amongst the examples of tissues characterised by very high ability to restore themselves (Driskell et al., 2013). Similarly, skeletal muscles are able, at least in part, to reconstruct in the response to injury or disease, such as Duchenne Muscular Dystrophy (DMD) (Jarvinen et al., 2005). However, even the regeneration of such tissue as muscle might be affected, for example, by the excessive development of fibrosis, that is, connective tissue, which can affect the regeneration and result in tissue malfunctioning. Importantly, skeletal muscles differ in their ability to regenerate and some of them are more prone to fibrosis than the others (Bassaglia and Gautron, 1995). Unfortunately, the molecular mechanisms differing poorly from properly regenerating muscles are not fully characterised. Many lines of evidence suggest the involvement of such factors as transforming growth factor type beta 1 (TGF-β1) in the control of muscle regeneration. This cytokine—belonging to TGF-β family—is widely known to promote connective tissue formation within the regenerating area. It can modulate muscle repair not only by impacting at fibroblasts responsible for excessive extracellular matrix (ECM) deposition, but also by influencing the stem cells and inflammatory cells present, and active within the injured area (Li et al., 2006; Kollias and McDermott, 2008; Jackson et al., 2011; Cisternas et al., 2014).

Skeletal muscles regeneration
Skeletal muscle regeneration consists of two phases: myolysis and reconstruction. Myolysis is related to rapid interruption of muscle fibers integrity followed by necrosis and infiltration of the injury site by inflammatory cells (neutrophils, macrophages and lymphocytes). After removal of damaged fibers reconstruction begins. Formation of new myofibers relies at the activation of muscle specific precursor cells, that is, satellite cells, localised between sarcolemma and basal lamina surrounding single muscle fiber. These cells remain quiescent until their activation caused by muscle damage. Once activated they start to proliferate, differentiate into myoblasts, fuse to create myotubes and finally to reconstruct multinuclear myofibers (Jarvinen et al., 2005; Tidball, 2005; Karalaki et al., 2009; Murphy et al., 2011). Satellite cells activation and differentiation of myoblasts...
derived from them is precisely controlled by the sequential expression of muscle-specific transcription factors (MRFs), such as Myf5, MyoD and myogenin, as well as structural proteins, such as myosin heavy chains (Rudnicki et al., 1993; Kablar et al., 1998; Cooper et al., 1999; Buckingham, 2007; Ciemerych et al., 2011; Mok and Sweetman, 2011). Simultaneously, differentiating myoblasts and also fibroblast synthesize ECM components allowing reconstruction of proper muscle architecture. Amongst the ECM proteins characteristic for skeletal muscle are collagens (i.e., collagens I and III, IV), proteoglycans (i.e., syndecans and decorin), fibronectin and laminin (Stupka et al., 2001; Casar et al., 2004; Tidball, 2005; Wynn, 2008; Van Ry et al., 2014). Next, development of inflammation and its timely and efficient silencing, associated with the restoration of the proper vascularisation and innervation, as well as correct modification of ECM, are the events crucial for effective regeneration (Jarvinen et al., 2005; Tidball, 2005; Mourkioti and Rosenthal, 2005; Karalaki et al., 2009). Under normal conditions regenerated muscle is morphologically and functionally indistinguishable from undamaged one. However, as mentioned above, muscle regeneration is often inefficient and hindered by the development of fibrosis, that is, accumulation of ECM components, such as collagen and fibronectin (Jarvinen et al., 2005; Wynn, 2008; Karalaki et al., 2009; Murphy et al., 2011).

Mammalian skeletal muscles are heterogeneous in nature. Based on their different morphological and biochemical characteristics muscle fibers can be divided into two main types, that is, so called slow twitch or red muscle fibers and fast twitch or white muscle fibers (Schiaffino and Reggiani, 2011). The domination of one of these fiber types determines the type of the muscle, that is, the properties of contraction and also characteristics of its regeneration (Soukup et al., 2002; Zierath and Hawley, 2004; Westerblad et al., 2010). It is well known that repair efficiency of muscles containing mostly slow or mostly fast twitch fibers differs. Two limb muscles—Soleus and Extensor digitorum longus (Kubiczkova et al., 2012)—are often used as models of slow and fast twitch muscles. EDL that contains 95% of fast fibers regenerate efficiently and show normal structure at day 14 after injury. In contrast, slow twitch muscle, for example, Soleus that contains 80–100% of slow fibers, fails to properly reconstruct and develops fibrosis. Moreover, regeneration of Soleus muscle is accompanied by prolonged inflammation, as compared to the fast twitch EDL muscle (Bassaglia and Gautron, 1995; Zimowska et al., 2016).

**TGF-β family**

The skeletal muscle repair is controlled by various extracellular factors, for example, HGF, FGFs, IGFs, PDGF and TGF-β (Brzoska et al., 2011; Ciemerych et al., 2011; Philippou et al., 2012). Amongst them hepatocyte growth factor (HGF) triggers the activation of quiescent satellite cells as well as promotes angiogenesis (Miller et al., 2000; Karalaki et al., 2009). Fibroblast growth factor (FGF) family members (i.e., FGF-1, -2, -4, -6 and -9) are shown to induce proliferation of already activated myoblast and fibroblasts and also to promote angiogenesis (Floss et al., 1997; Kastner et al., 2000). Insulin-like growth factors I and II (IGFs I and II) stimulate both myoblasts proliferation and differentiation (Adams and McCue, 1998; Mourkioti and Rosenthal, 2005; Ciemerych et al., 2011). TGF-β family includes several factors that control broad spectrum of cellular events—from cell divisions to differentiation and tissue maturation. Some of TGF-β, such as TGF-β1, are known to promote development of fibrosis associated with wound healing and to participate in inflammatory response (McLennan and Koishi, 2002; Mauviel, 2005; Li et al., 2006; Kollias and McDermott, 2008; MacDonald and Cohn, 2012).

First TGF-β family members were discovered in early 80s as a result of a cancer studies during which Roberts et al. (1980) were trying to isolate polypeptides responsible for cell transformation. As a result new polypeptide, initially called sarcoma growth factor (SGF) that was able to induce changes in the morphology of rat fibroblasts colonies—they were becoming significantly bigger, was identified. Further investigation led to the conclusion that SGF activity can be attributed to at least two factors characterised by different functions. As a reference to their transforming properties they were called transforming growth factor α and β (TGF-α and TGF-β) (Anzano et al., 1982; Roberts et al., 1983). TGF-α was initially recognised as a potent hepatocyte mitogen (Mead and Fausto, 1989), whilst TGF-β as being able to induce fibroblasts growth and collagen production (Roberts et al., 1986; Sporn et al., 1986).

Currently, TGF-β family is divided into two subgroups—BMP/GDF (bone morphogenetic protein/growth and differentiation factor) and TGF-β/activin. Such division is based on the differences in their activity and expression patterns. BMP/GDF subgroup includes such factors as BMP2, BMP4, osteogenin and myostatin, described as factors inducing not only cartilage and bone development, as suggested by their names, but also other processes, such as neuronal differentiation and organogenesis of heart and kidney. TGF-β/activin subgroup contains three isoforms of TGF-β (TGF-β1, TGF-β2 and TGF-β3) and activins, that is, activin A and inhibin (Mauviel, 2005; Stern, 2005; Tsukumi and Yoshikawa, 2005; Wan and Cao, 2005; Zimowska, 2006; Makanji et al., 2014). These factors were shown to control many processes, including for example, myotube formation, development and maintenance of motor neurons or fibrosis development.
TGF-β synthesis

TGF-β family members are synthesised as large precursor molecules. Pre-TGF-βs are not able to bind to any of its receptors, that is, TβRI, TβRII or TβRIII, and subsequently have to be proteolytically processed. They are cut by furin-type enzymes and as a result mature TGF-β and latency-associated peptide, that is, LAP, necessary for proper folding of mature protein, are created. Mature TGF-β remains associated with LAP via noncovalent interactions, what prevents its spontaneous binding to the TGF-β receptors. Another mechanism preventing TGF-β from unjustified binding to its receptor is association with latent TGF-β binding protein (LTBP). LTBP interacts with LAP to stabilize the complex formed between TGF-β and LAP. It also regulates TGF-β bioavailability by directing it to ECM and allowing its interaction with ECM proteins. The activation of TGF-β is initiated by releasing the TGF-β-LAP-LTBP complex from ECM. Next, thrombospondin cleaves the complex to free TGF-β from LAP and LTBP. As a result TGF-β is activated and free to initiate signal transduction (Cheifetz et al., 1987; Schultz-Cherry and Murphy-Ullrich, 1993; Saharinen et al., 1999; Blobe et al., 2001; Hyytiainen et al., 2004; Kubiczkova et al., 2012).

TGF-β signalling pathway

Members of TGF-β family elicit their cellular responses via receptors that form two subfamilies, that is, one consisting seven members TβRI (type I) and second consisting five members TβRII (type II) (Figure 1). Depending on subfamily TGF-β binds to one of type II (i.e., TGF-β1, TGF-β2, TGF-β3, Nodal and Activin) or type I receptors (i.e., BMP). Such binding triggers the assembly of a complex including two TβRI and two TβRII subunits. Once complexed, TβRII phosphorylate TβRI at multiple serine and threonine residues located N-terminally within receptor kinase domain. Signal binding and receptor assembly can either activate canonical SMAD pathway involving SMAD1, 2, 3, 4, 5, 6, 7 or 8, or non-canonical TGF-β signalling pathways (Derynck et al., 1996; Massague and Weis-Garcia, 1996; Zimowska, 2006; Massague, 2012). For example, TGF-β1 dimer binds to TβRII and TβRI. Upon receptors heterodimerisation and activation SMADs become phosphorylated what leads to the formation of so called Receptor-phosphorylated SMAD proteins (R-SMADs). TβRII activated by TGF-β, activin or Nodal phosphorylate mainly SMAD2 (at Ser465 and Ser467) and SMAD3 (at Ser423 and Ser425). Signalling pathway initialised by that is, BMP phosphorylate SMAD1, SMAD5 and SMAD8. (Abdollah et al., 1997; Nakao et al., 1997; Kretzschmar and Massague, 1998; Massague, 2012; Macias et al., 2015). SMADs are unique proteins responsible for transducing the signal from activated receptor into nucleus. They act by associating with transcription factors, what directly leads to the expression of the target genes. SMAD proteins are characterised by the presence of two domains—MH1 which contains DNA-binding sequence and MH2 capable to interact with activated TGF-β receptors, as well as with co-activators and co-repressors. R-SMADs interact via their MH2 with MH2 of SMAD4, which act as shared partners of all R-SMADs (Nakao et al., 1997; Kretzschmar and Massague, 1998; Shi et al., 1998). In the nucleus, R-SMAD-SMAD4 complexes bind transcription factors and regulate transcription. On the other hand, SMAD6 and SMAD7 operate like SMADs inhibitors, that is, blocking R-SMAD phosphorylation by TβRI. SMAD6 may also prevent the interaction between R-SMAD and SMAD4, that is, SMAD6 competes with the R-SMAD1 for binding to SMAD4, acting as an inhibitor of BMP signalling. Furthermore, SMAD7 has been shown to inactivate TβRI (Nakao et al., 1997; Kavsak et al., 2000; Wu et al., 2000; Suzuki et al., 2002; Liu et al., 2004b; Wan et al., 2004; Cohen et al., 2015; Cordova et al., 2015; Macias et al., 2015).

Although, TGF-β signals mainly via SMAD proteins it was also demonstrated that TGF-β can act via SMAD-independent pathway, that is, involving mitogen-activated protein kinases (MAPKs) such as ERK1/ERK2 or p38 MAPK (Sporn et al., 1986; Zimowska, 2006; Kubiczkova et al., 2012; Massague, 2012; Weiss and Attisano, 2013).

TGF-β1 impact on myoblasts proliferation and differentiation

The TGF-β family members are implicated in the regulation of myogenic differentiation as they were shown to be potent inhibitors of myoblasts differentiation. The role of TGF-β and other factors in myogenic differentiation was and still is extensively tested using in vitro cultured primary myoblasts or myoblasts cell lines, such as C2C12. The latter cell line serves as a good model in such studies. Standard protocols allow, by manipulating culture conditions, either to sustain myoblast proliferation or to induce their differentiation, fusion, and finally myotube formation. Using such in vitro culture systems TGF-β1 role in skeletal myoblasts differentiation was tested for the first time. Exogenous TGF-β1 was shown to prevent differentiation, that is, fusion and myotube formation, of rat primary myoblasts and other in vitro cultured cell lines (Massague et al., 1986; Yablonka-Reuveni and Rivera, 1997). Surprisingly, TGF-β1 increased proliferation of C2C12 myoblasts. Although TGF-β1 activates distinct signalling pathways, it stimulates myoblasts proliferation primarily via Smad 2. It was also shown that TGF-β increased proliferation of C2C12 myoblasts by changing the cellular localisation of PCNA resulting in the increased cell
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showed that embryonic myoblasts, that is, present within
developing mouse embryos, for example, at approximately
12 day of development, are relatively insensitive to TGF-
1. On contrary, fetal myoblasts, for example, isolated at 17
day of development were inhibited by this factor (Cusella-De
et al., 1994; Messina and Cossu, 2009).

TGF-

b

negatively impacts at myoblasts differentiation by inhibiting expression of two myogenic regulatory factors
(MRF), that is, MyoD and myogenin (Brennan et al., 1991;
Goulding et al., 1994). C2C12 myoblasts expressing
truncated version of T\(\beta\)RII, that is, deficient in the ability
to correctly respond to TGF-

b

1, were characterised by
decreased expression of MyoD and as a result were unable to
complete the differentiation, that is, fuse and form myotubes
(Filvaroff et al., 1994). The signalling events that lead to the
decrease in MRF expression and inhibition of differentiation
are at least in part related to SMAD3-mediated transcriptional repression. SMAD3 is known to interfere with the
bHLH domain of MyoD preventing MyoD/E protein
dimerisation and subsequent binding to E-box (Liu et al.,
2001). SMAD3 has also the ability to interact with MEF2,
and this interaction prevents the association of this factor
with the MyoD/E-47 dimer. As a result myogenin expression
is repressed (Liu et al., 2004a). Importantly, TGF-

b

and SMAD3 mediated signalling pathway is involved in the
regulation of expression of ECM components, for example, vimentin (Wu et al., 2007). Besides the inhibition of
myoblast differentiation, TGF-

b

1 is also known as a factor elicitng apoptosis of satellite cells (Li et al., 2009; Cencetti et al., 2013). Thus, TGF-

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1 impacts at myoblasts differentiation via different routes, including regulation of prolifera-
tion, differentiation and apoptosis.

**TGF-

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1 impact on fibrosis and inflammation during muscle regeneration**

The injury of adult skeletal muscle initiates a series of well-coordinated events that lead to the repair of the damaged
tissue. However, as already mentioned above, failure at any
stage of this process may lead to the unsuccessful muscle
regeneration characterised by degradation of newly formed
fibers, strong inflammatory response and/or fibrosis.
Fibrosis, that is, excessive deposition of ECM components,
leads to the loss of proper muscle architecture and function
(Mauviel, 2005; Verrecchia and Mauviel, 2007; Wynn, 2008).
In skeletal muscles TGF-

b

1 is known as one of the most potent profibrogenic factors and regulators of fibrosis
development, as it controls ECM synthesis, remodelling
and degradation. During muscle repair it is able to activate
the expression of genes that code ECM proteins, such as
fibronectin or collagen. On the other hand, TGF-

b

1 impacts the expression of ECM degrading proteases, for example,
matrix metalloproteinases (MMPs) and their specific
inhibitors (tissue inhibitors of MMPs, TIMPs) (Zanotti
et al., 2007). Interestingly, it was shown that at the early
stages of muscle regeneration TGF-

b

1 co-localises with
myostatin—other member of TGF-

b

family. In the response
to the damage and denervation rat skeletal muscles show
strong upregulation of TGF-

b

1 (Zimowska et al., 2009). The
injection of myostatin into regenerating rat skeletal muscle
stimulates TGF-

b

1 synthesis and causes the connective
tissue formation within the injected area, suggesting
cor- regulatory relationships of different members of TGF-

b

family (Zhu et al., 2007). On the other hand, the inhibition of
TGF-

b

1 using neutralising antibodies against TGF-

b

1 and T\(\beta\)RI causes significant improvement in muscle
regeneration accompanied by reduction of fibrosis development
(Zimowska et al., 2009). Such fibrosis reduction is also
detected in decorin (small leucine-rich proteoglycan that
binds and inhibits TGF-

b

1 activity), suramin (competitively
binds TGF-

b

1) or angiotensin receptor blocker (blocks
TGF-

b

1) treated mouse muscles (Chan et al., 2003; Bedair et al.,
2008). Next, systemic attenuation of TGF-

b

1 achieved by inhibition of T\(\beta\)RI significantly improves the repair of
skeletal muscle of aged mice (Yousef et al., 2015).

Improvement of muscle repair could be achieved not only
by reduction of fibrosis development but also by modifica-
tion of inflammatory response. Inhibition of TGF-

b

1 by

Figure 1 TGF-

b

1/SMAD signalling pathway (1) TGF-

b

1 dimer ligand binds to T\(\beta\)RII what leads to the formation of the complex (2) composed
of: two ligands after dimerisation, two type I receptor components (T\(\beta\)RI), and two type II receptor components (T\(\beta\)RII). T\(\beta\)RII phosphorylate the T\(\beta\)RI (3). Next, T\(\beta\)RII phosphorylates SMAD2 and SMAD3 (4). Activated SMAD2 and SMAD3 create the complex with SMAD4—what allows SMADs to be transported to the nucleus (5) to interact with appropriate transcription factors (TF) and induce expression of the target gene (TG). SMAD7 inhibits SMAD2 and SMAD3 phosphorylation.
platelet-rich plasma in combination with decorin alters myoblast proliferation and expression of MRFs (Kelc et al., 2015). The inhibition of TGF-β1 may result not only from direct block of growth factor-receptor binding but can also result from block of another signalling pathways, for example, insulin-like growth factor-1 (IGF-1) treatment prevents skeletal muscle fibrosis by inhibition of TGF-β1-induced SMAD phosphorylation, resulting in decrease of ECM components accumulation (Dong et al., 2013).

TGF-β1 also acts at fibroblasts, attracted to the site of injury, increasing their synthesis of ECM proteins (Ihn et al., 2001). Importantly, fibroblasts are amongst the cells that produce TGF-β1 promoting synthesis and accumulation of ECM components and contributing to fibrosis development. Improvement in muscle repair resulting from suramin treatment is, at least in part, related to the decrease in fibroblast proliferation (Chan et al., 2003). Similarly, treatment of injured muscle with gamma interferon, known to inhibit TGF-β1 signalling, reduces fibrosis—probably by reducing the proliferation of fibroblasts (Foster et al., 2003). Thus, decrease in fibroblast migration to the site of injury as well as drop of ECM components synthesis appears to be responsible for more efficient muscle repair achieved via improvement of basal membrane and ECM remodelling.

Except fibroblasts also other cells are present in or attracted to the site of injury. Skeletal muscle repair is accompanied by the infiltration of injured region with inflammatory cells, such as neutrophils, macrophages and lymphocytes. These cells remove damaged fibers, as well as produce signals facilitating satellite cells activation, and as a result muscle regeneration (Prisk and Huard, 2003; Tidball, 2005; Yoshimura et al., 2010; Pillow et al., 2013). TGF-β1 chemoattracts macrophages and lymphocytes, induces maturation of monocytes into macrophages and has negative effect on activation, and proliferation of lymphocytes (Wahl, 1992). Interestingly, it was also shown that the neutralisation of TGF-β1 promotes macrophage infiltration and increases the number of mast cells or neutrophils (Lefaucheur et al., 1995; Lefaucheur et al., 1996a,b). Thus, TGF-β1 appears to be involved in inflammatory oedema development although it has both pro- and anti-inflammatory effects, depending on the stage of regeneration during which it is present and active. Upregulation of TGF-β1 activity accompanies inflammation and fibrosis, and abolishes homeostasis required for proper and efficient muscle regeneration. Thus, inhibition of TGF-β1 modifies proliferation of myoblasts, fibroblasts or inflammatory cells found within injured muscle. It also prevents accumulation of ECM components restoring balance between deposition and degradation of ECM proteins and proteoglycans and allowing the efficient tissue repair.

TGF-β1 affects the force generating capacity of skeletal muscle

Muscle fibers, fibroblasts, and other cells found within the skeletal muscle, when TGF-β level increases, start to extensively synthetize extracellular matrix components. As a result they contribute to the development of fibrosis followed by muscle fibers atrophy. Even when it does not change muscle mass, consistent with accumulation of ECM, the contractile forces of TGF-β treated muscles are also reduced. Importantly, as TGF-β treatment can directly induce muscle fiber atrophy in the absence of physical injury, reduction in the force generating capacity also affects treated with TGF-β intact muscle (Mendias et al., 2012). Surprisingly, inhibition of TGF-β results in an initial improvement but long-term deficit in the force generation after contraction-induced skeletal muscle injury. It also impair or at least delay muscle repair (Gumucio et al., 2013).

TGF-β1 in muscle disorders

The excessive connective tissue deposition within skeletal muscle and other organs is observed under various pathological conditions. Thus, if development of fibrosis could be abrogated or shifted towards repair mechanisms this would considerably improve tissue repair. This might be the case in muscular diseases, for example, muscular dystrophies which are heterogeneous group of degenerative disorders characterised by repeating rounds of muscle degeneration and regeneration. Several subgroups of dystrophies have been described, including Duchenne (DMD)/Becker (BMD), fascioscapulohumeral, limb-girdle, oculopharyngeal and congenital muscular dystrophy (Emery, 2002; Zhou and Lu, 2010; Pines and Halevy, 2011; MacDonald and Cohn, 2012). The progressive muscle wasting, weakness and loss of muscle mass observed in patients suffering from these diseases were shown to be accompanied by increased TGF-β1 at mRNA (Gosselin et al., 2004) and protein levels (Bernasconi et al., 1999). Interestingly, the plasma level of TGF-β1 is also significantly elevated in patients suffering from Duchenne muscular dystrophy and congenital muscular dystrophy, but not in those once developing Becker muscular dystrophy (Ishitobi et al., 2000).

Many lines of evidence for the participation of TGF-β1 in muscular dystrophies come from the animal studies, for example, involving mdx mouse which serve as DMD model. The elevated level of TGF-β1, accompanied by fibrosis development and muscle wasting, was found in diaphragm (Hartel et al., 2001; Andreetta et al., 2006) and gastrocnemius muscle (Zhou et al., 2006) of mdx mice. In addition, upregulation of TGF-β1 increases progressively during the life of mdx mouse. Reduction in the level of TGF-β1,
achieved by use of anti-TGF-β1 antibody, resulted in the inhibition of fibrosis development and prevention of further muscle loss during advanced stages of the disease (Andreetta et al., 2006). Similarly, suramin that blocks TGF-β receptors (McGeary et al., 2008), reduces fibrosis in diaphragm and limb muscles of mdx mice. As a result functional loss of muscle strength is attenuated (Taniguti et al., 2011). Next, myofiber-specific inhibition of TGF-β signalling in transgenic mice expressing truncated and inactive form of TβRII resulted in the improvement of structure and function of skeletal muscles and protected this tissue from the consequences of dystrophy progression (Accornero et al., 2014).

As already mentioned, function of TGF-β1 can also be modified by its binding to small proteoglycans, that is, decorin and biglycan (Blobe et al., 2001; Brandan and Gutierrez, 2013). Interestingly, the high level of TGF-β1, characteristic for many muscular dystrophies, is often accompanied by increased level of these proteoglycans. Muscles of patients suffering from DMD as well as mdx mice exhibit elevated levels of both decorin and biglycan (Bowd et al., 2000; Caceres et al., 2000; Fadic et al., 2006). Muscles of paediatric BMD patients show normal levels of decorin mRNA, whereas this mRNA, as well as biglycan mRNA, are upregulated in adult patients suffering from BMD, sarcoglycanopathies or dysferlinopathy (Zanotti et al., 2005). However, decorin mRNA is significantly downregulated in muscles of DMD patients, whereas TGF-β1 is significantly upregulated. Similarly, myotubes derived from myoblasts of DMD patients exhibit significantly lower levels of decorin mRNA and significantly higher levels of TGF-β1 (Zanotti et al., 2007). As both proteoglycans are able to interfere with TGF-β1 in a dose dependent manner differences in their expression may be responsible for the modification of TGF-β1 bioactivity.

**Summary**

Regeneration is a complex phenomenon needed to recover the proper architecture and function of skeletal muscles. Even though many attempts were made to improve this process, especially in context of chronic muscle disorders, there are no satisfactory therapies available yet. Elements of TGF-β1 signalling pathway could be potential targets of the therapeutic strategies aiming to improve muscle regeneration and function. As described above, TGF-β1 is able not only to inhibit myoblasts proliferation, but also to promote fibrosis formation. Thus, specific inhibition of TGF-β1 signalling pathway can significantly improve ability of skeletal muscle to repair. Many lines of evidence document the function and targets of this cytokine. However, precise mechanisms of signal transduction characteristic for TGF-β1 still remain elusive. Thus, more insight in its specific interaction with other factors would be necessary to bring us closer to understand real capabilities of TGF-β1 modulation in regenerating muscle. It seems obvious that in such complicated process as skeletal muscle regeneration one protein cannot be the only player responsible for the successful muscle restoration. Thus, deeper understanding of TGF-β1 role combined with more insight into other aspects of regeneration can bring promising perspectives for strategies that may allow us to significantly and maybe even permanently improve skeletal muscles regeneration.

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**Conflicts of interest**

None.

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