

Contribution of skeletal muscle defects in spinal muscular atrophy

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ABSTRACT

Spinal muscular atrophy (SMA) is an autosomal recessively inherited motor neuron disease that causes alpha motor neuron degeneration in the spinal cord, symmetrical muscle weakness, and atrophy. SMA is caused by mutations in the survival of motor neuron 1 (SMN1) gene, which results in a reduced amount of survival motor neuron (SMN) protein synthesis. Although there have been many studies investigating SMA pathogenesis, the mechanism by which the reduced SMN protein levels cause motor neuron degeneration and muscle atrophy is unclear. Generally, muscle weakness is considered a secondary outcome of motor neuron degeneration in SMA. However, recent studies have shown that intrinsic skeletal muscle defects contribute to SMA pathogenesis and targeting skeletal muscle might be beneficial as a potential therapy approach. This paper aims to review the recent findings on the role of skeletal muscle in SMA pathogenesis..

Key words: Spinal muscular atrophy, SMN, muscle atrophy, skeletal muscle

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessively inherited motor neuron disease that causes alpha motor neuron degeneration in the spinal cord and muscle atrophy. SMA is one of the leading genetic disorders causing infant death with an incidence of 1 in 6000–10,000 live births and a carrier frequency of 1 in 50 [1]. Ranging from very severe to mild, based on the age of onset and the achieved motor function, 4 clinical phenotypes (Types I–IV) are defined in SMA [2].

SMA is caused by mutations in the *survival of motor neuron 1 (SMN1)* gene [3–6]. *SMN1* encodes survival motor neuron (SMN) protein and its absence results in embryonic lethality [7]. Human *SMN1* has a homologue copy called *SMN2*, formed as a result of an intrachromosomal duplication, which also encodes SMN protein [8]. The *SMN1* and *SMN2* gene sequences differ from each other by only 5 nucleotides. The C840T transition in the exon 7 of *SMN2* causes an important functional difference. As a result of this transition, *SMN2* produces exon 7-skipped transcripts, which results in truncated and unstable SMN protein [9]. Only a small amount (10%) of full-length SMN protein is synthesized due to alternative splicing.

However, depending on the number of *SMN2* gene copies, the functional protein level is increased and this correlates with a milder clinical phenotype [10–13].

SMN is an ubiquitous 38-kDa protein expressed in a developmental and tissue-specific manner [14,15]. It is localized in the cytoplasm and nucleus as a SMN complex, and plays a role in small nuclear ribonucleoprotein (snRNP) biogenesis, mRNA splicing, and RNA transport [16–21]. Since SMN has various interaction partners, it is hypothesized that SMN might have additional functions aside from its housekeeping roles. As reported by Boyer *et al.*, SMN plays differing roles in motor neurons, neuromuscular junctions, and skeletal muscle, which are all components of the motor unit (Figure 1) [22]. In motor neurons, SMN plays a role in neurite outgrowth, neuronal differentiation, axonal pathfinding, and the regulation of actin dynamics. Several defects have been reported related to these roles in the case of low SMN levels [22–24]. A decrease in SMN levels is associated with abnormal endplate morphology, endplate denervation, neurofilament accumulation, and perturbed malfunction at the neuromuscular junctions [22].

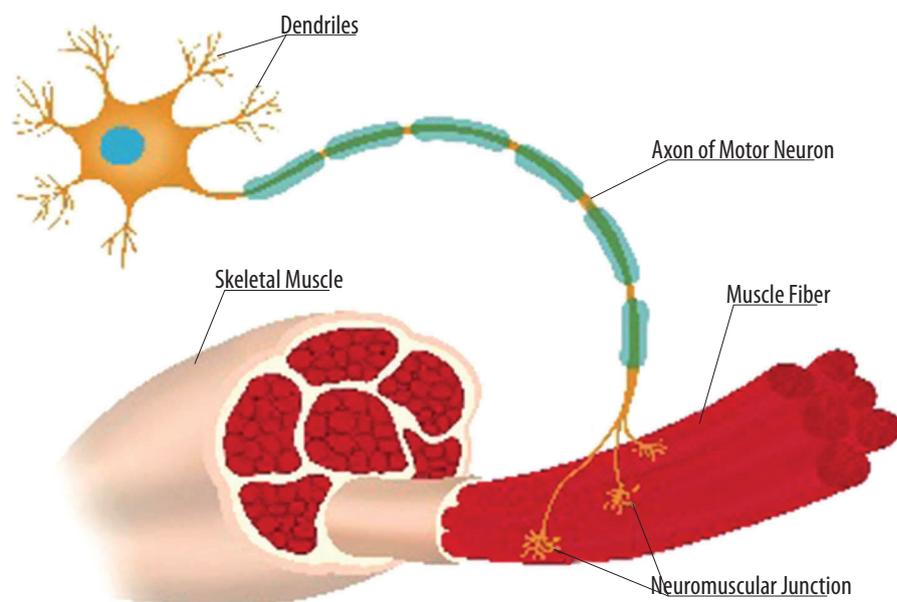


Figure 1. The figurative image of the motor unit.

Although there are many studies investigating SMA pathogenesis, the relationship between SMN protein levels and motor neuron degeneration or muscle atrophy is unclear. Muscle weakness has been considered a secondary outcome of motor neuron degeneration in motor neuron diseases; thus, most studies have delved into motor neuron pathology. However, as 2 important components of the motor unit, motor neurons and muscle cells are dependent on each other in development and contractile function [25,26]. It is difficult to independently study motor neuron and skeletal muscle involvement in the disease pathogenesis without the use of cell culture and animal models [26].

Recent studies have shown that SMN may have muscle-specific functions and intrinsic muscle defects may contribute to SMA pathogenesis [26–37]. It has also been reported that muscle weakness was observed in the early phase of the disease and could be the result of a delay in muscle development [38–46]. It is therefore crucial to understand the skeletal muscle pathology of SMA aside from than that of motor neurons. Moreover, the regenerative capacity of the muscle is an important therapeutic target for SMA and would eliminate the necessity for the development of a blood-brain-barrier permeable drug [40]. This paper aims to review the recent findings on the role of skeletal muscle in SMA pathogenesis.

Muscle-specific Functions of SMN

Recent studies have shown that SMN may have muscle-specific functions in addition to its housekeeping

functions. SMN was identified as a sarcomeric Z-disc protein (Figure 2a) in both *Drosophila* and mice [28]. Further studies on SMA performed in animal and cell culture models showed that SMN colocalizes with Z-disc proteins alpha-actinin and alpha B crystallin in skeletal and cardiac muscle, and its decrease causes sarcomeric defects such as altered Z-disc spacing (Figure 2b) [28–30]. The absence of snRNPs in Z-discs suggests that SMN could contribute to the maintenance of Z-disc homeostasis, acting as a Z-disc signaling factor and ensuring the

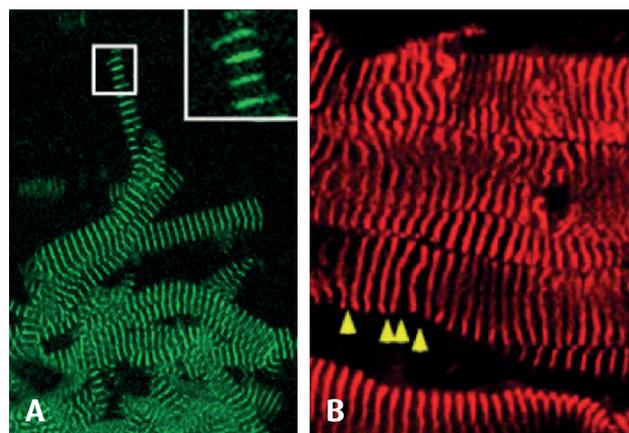


Figure 2. Representative images of the sarcomere structure and SMN localization in purified mouse myofibrils (adapted from [29] with the permission of the author). **a.** Sarcomeric localization of SMN in wild-type myofibrils revealed by immunofluorescent staining via anti-SMN antibody. **b.** Altered Z-disc spacing (arrowheads) of myofibrils in the case of SMN reduction revealed by immunofluorescent staining via alpha-actinin antibody.

local translation of proteins by mRNA transport. Additionally, analysis of the interaction partners of SMN in different developmental stages of myoblast cell lines showed that SMN interacting proteins were variable during myogenesis. This implies that in addition to being a structural muscle protein, SMN has unique functions in muscle growth and differentiation [26,31].

Intrinsic Muscle Defects in SMA

Low levels of SMN protein expression is accompanied by skeletal muscle defects such as reduced myoblast proliferation rate, myotube fusion defects, and consequent abnormal myotube formation [27,32]. The following reports indicate an intrinsic skeletal muscle pathology in SMA and a requirement for SMN to maintain the motor neuron-muscle connection: a) the presence of skeletal muscle defects in severe SMA mice at the presymptomatic stage, b) denervated muscle in mice displayed a significantly different proteomic profile compared to SMA mice, c) motor neuron degeneration occurred when the skeletal muscle cells of SMA patients were cocultured with healthy motor neurons [33–37,39]. When considered together, these studies suggest that the skeletal muscle pathology in SMA might be intrinsic and could arise independently of motor neuron degeneration.

Developmental Delay in Skeletal Muscle in SMA

Skeletal muscle defects are observed earlier than spinal cord defects during SMA pathogenesis [39,40]. In mouse models of SMA, the early defects are associated with a failure in muscle growth and a delay in

the maturation of muscle proteins involved in muscle contraction [39–42]. In accordance with this, small and disorganized myotubes and myotube-like fibers were detected in the prenatal and postnatal stages of SMA patients, respectively [38,43].

Satellite cells were investigated for their function in SMA, since early neonatal muscle growth is supported by satellite cell activity. Although there was no decrease in the number of satellite cells per myofiber, and the proliferation rate was not affected by low levels of SMN, in a severe SMA mouse model, satellite cells failed to form multinucleated myotubes. This indicates a need for SMN function for normal satellite cell differentiation [44].

The expression of structural and functional muscle proteins takes place during early postnatal skeletal muscle development. The development process is regulated by myogenic regulatory factors (MRF) such as myoblast determination 1 (MyoD), myogenin, muscle-specific regulatory factor 4 (Mrf4), and myosin heavy chain (MHC). (Figure 3) [45]. Under normal circumstances, MRFs have a high expression profile at the beginning of the early postnatal stage and show a rapid decrease over time. The impairment in the myogenic program may cause a delay in skeletal muscle development independently from motor neuron loss [39,45]. Accordingly, in SMA mice, myoblast fusion defects and cytoskeletal abnormalities are caused by the lower expression of the MRFs at the early presymptomatic stage, followed by a delayed increased expression at the symptomatic stage. SMN potentially corrects the mentioned defects [46]. Additionally, adult isoforms of other functional muscle proteins must be expressed subsequent to the expression of embryonic isoforms

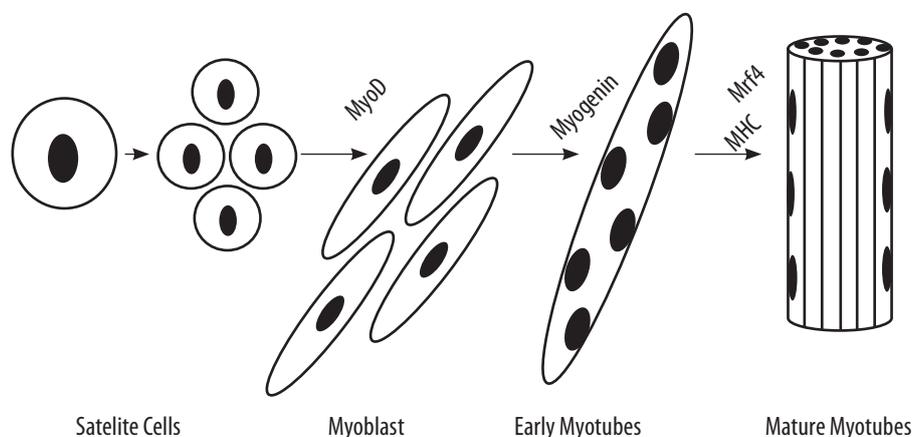


Figure 3. Figurative image of muscle development. (MyoD: myoblast determination 1, Mrf4: the muscle-specific regulatory factor 4, and MHC: myosin heavy chain).

for regular postnatal development [45]. A failure in this shift from embryonic to adult isoforms can cause a delay in skeletal muscle maturation in SMA. The expression of MHC and acetylcholine receptors are important for muscle fiber typing and synapse formation, respectively, are well known examples in SMA [32,41,42,47–49].

In conclusion, these studies indicate a delay in skeletal muscle development that contributes to SMA pathogenesis. Hence, skeletal muscle could be a target for early treatment in SMA.

Targeting Skeletal Muscle in SMA

Skeletal muscle is a dynamic tissue that can change size as a response to environmental and molecular signaling factors [50,51]. Insulin-like growth factor 1 (IGF1) and myostatin are the main signaling pathways (Figure 4) that regulate skeletal muscle size. These pathways were investigated in SMA to understand their effects on skeletal muscle pathology.

As a member of the transforming growth factor beta super-family, myostatin has a skeletal muscle-specific expression, which results in negative regulation of the skeletal muscle mass. Myostatin stimulates the signaling cascades related to skeletal muscle atrophy by binding to the activin receptor IIB (Figure 4) [52]. Myostatin knockout mouse models or

loss of function mutations in humans lead to muscle fiber hypertrophy. This supports the idea that the modulation of myostatin might promote muscle growth [53–55]. In this sense, research groups have investigated the effects of a potent myostatin inhibitor, follistatin, in SMA [56–58]. Although the results are controversial, the administration of recombinant follistatin in SMA mice showed a positive effect on muscle growth, body weight, and median survival [58].

Contrary to myostatin, IGF1 is known to be a positive regulator of skeletal muscle size. IGF1 induces satellite cell proliferation, myoblast differentiation, and myotube formation, not only during muscle development but also during muscle regeneration after damage or denervation [59,60]. Muscle-specific over-expression of IGF1 increases muscle mass and causes hypertrophy, and IGF1-null animal models show growth deficiencies with a reduced muscle mass [60–65]. Studies on SMA mouse models report low IGF1 levels in the blood; however, currently, there is no research on SMA patients [66,67]. It was also shown that in gene therapy approaches, the peripheral administration or muscle-specific overexpression of IGF1 increased muscle mass, body weight, and survival in SMA models [59,67,68]. The majority of studies on SMA models

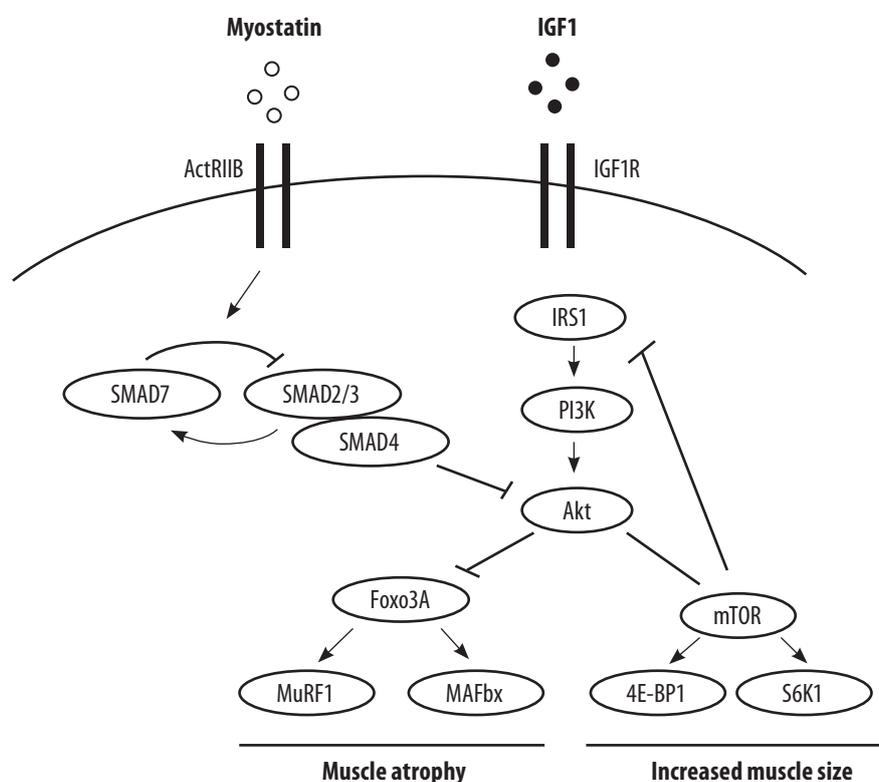


Figure 4. IGF1 and myostatin signaling pathways.

have been focused on IGF1, since rodents have no postnatal expression of IGF2. However, IGF2 has a postnatal expression in humans and plays a role in skeletal muscle differentiation [69–71]. Thus, IGF2 functions, as well as those of IGF1, should be emphasized in SMA patients.

IGF1 positively affects muscle growth by inducing the PI3K/Akt/mTOR pathway, while myostatin induces muscle atrophy by regulating Akt, Foxo, MuRF1 and MAFbx expressions (Figure 4) [50,72]. Moreover, myostatin may act as an antagonist to IGF1 over the PI3K/Akt/mTOR pathway [73]. Hence, both myostatin- and IGF1-related signaling cascades might be probable candidates of future investigation in SMA.

Conclusion

SMA research has previously been focused on motor neuron degeneration, since it was considered the primary cause of the disease [74–77]. Hence, muscle

weakness and atrophy in SMA patients were considered as a secondary outcome of alpha motor neuron degeneration in the spinal cord. However, the studies presented here collectively indicate that skeletal muscle defects are prominent contributors to SMA pathogenesis and could arise independently from motor neuron degeneration. Therefore, skeletal muscle defects in SMA pathology should not be disregarded and the molecules/signaling pathways could contribute to the therapeutic repair of muscle defects in SMA. The intrinsic regenerative capacity of skeletal muscle and the fact that muscle tissue is more easily accessible than motor neurons draws attention to muscle defects as promising therapy targets.

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