

# Syndecans in skeletal muscle development, regeneration and homeostasis

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## Summary

**Skeletal muscle is a highly dynamic tissue that can change in size in response to physiological demands and undergo successful regeneration even upon extensive injury. A population of resident stem cells, termed satellite cells, accounts for skeletal muscle plasticity, maintenance and regeneration. Mammalian satellite cells, generated from muscle precursor cells during development, are maintained quiescent in the musculature throughout a lifespan, but ready to activate, proliferate and differentiate into myocytes upon demand. Syndecans are transmembrane heparan sulfate proteoglycans expressed in muscle precursors during embryonic development and in satellite cells during postnatal life. In the last decades a number of crucial functions for syndecans in myogenesis and muscle disease have been described. Here we review the current knowledge of the multiple roles played by syndecans in the skeletal muscle of several animal models and explore future perspectives for human muscle health, with a focus on muscle aging and muscular dystrophy.**

*Key words: syndecans, satellite cells, myogenesis, muscular dystrophy, aging, muscle regeneration.*

## Introduction

Skeletal muscle fibers (myofibers) are large syncytial cells

derived from the fusion of hundreds of progenitor cells during development (1). These muscle precursor cells (myoblasts) originate from the epaxial somite where, during mouse embryonic development, undifferentiated progenitors delaminate from the somite and migrate into the limb bud. Initially these progenitors proliferate and then terminally differentiate into myocytes prior to fusing with one another to form embryonic muscle fibers (2-5). A subset of these proliferating muscle progenitors are thought to be “set aside” during muscle development for the generation of satellite cells during the late stages of embryonic development (6).

Satellite cells, first described in frog muscle preparations (7), are the skeletal muscle stem cells (8,9) in all vertebrates, including humans (10). Satellite cells spend the vast majority of their lifespan mitotically quiescent, located within a specialized anatomic niche between the plasma membrane of the myofiber and the surrounding basal lamina (7). Each myofiber harbors 7-27 satellite cells, depending on the fiber type (11). In response to stimuli such as exercise or injury, satellite cells are activated, express the myogenic master gene MyoD and re-enter the cell cycle; activated and proliferating MyoD+ satellite cells are termed myoblasts. After one or more rounds of proliferation, myoblasts exit the cell cycle and terminally differentiate into myocytes, which express muscle contractile proteins and fuse either one to another to form new myofibers or to pre-existing damaged myofibers to repair them (12).

During embryonic development and in postnatal life, a family of transmembrane heparan sulfate proteoglycans (HSPGs) called syndecans have emerged as key regulators of skeletal muscle formation and maintenance. In this review we discuss the role played by syndecans in skeletal muscle development, maintenance and regeneration in healthy and diseased or aging organisms. We will then highlight future perspective for human muscle health that can be inferred based on studies carried out on animal models.

## Syndecan structure

Syndecans are transmembrane HSPGs, complex molecules comprising a core protein that covalently links one or more long, linear carbohydrate chains, the glycosaminoglycan (GAG) chains (13). Syndecans are conserved in all metazoans (14). The core protein structure is shared by all syndecans across large evolutionary distances, from the single syndecan expressed in invertebrates to the four different syndecans expressed in vertebrate organisms. However, the specific sequence can vary considerably across gene homologues and across species (14).

The ectodomain is the most variable region of the syndecan core protein containing a N-terminal signal peptide and several attachment sites for heparan sulfate chains. Additionally, syndecan-1 and syndecan-3 also contain attachment

sites for chondroitin sulfate chains (15). The syndecan ectodomain also contains at least one proteolytic cleavage site close to the transmembrane domain that is recognized by metalloproteinases (16). Syndecan shedding has an important regulatory function since shed, soluble ectodomains can function as paracrine or autocrine effectors or competitors (16). Moreover, ectodomain shedding is a way to quickly stop the processes that transmembrane syndecans take part in (16).

The transmembrane domain is a conserved single hydrophobic region, while the short intracellular domain contains three regions where a variable intermediate region (V) separates two highly conserved regions, C1 and C2, with C1 being essentially identical in all syndecans (17).

**Heparan sulfate** (HS) contains a linear backbone composed by repeating sequences of glucuronic acid and N-acetyl-glucosamine disaccharide units. In HSPGs, each HS chain is attached through a xylose-galactose-galactose-uronic acid tetrasaccharide linker to serine residues on the core protein (15). HS is synthesized in the Golgi where a complex set of enzymes catalyzes not only the addition of the linker and each alternating saccharide unit, but also subsequent sugar modifications, which include C-5 epimerization of glucuronic acid that yields iduronic acid, replacement of N-acetylation with N-sulfation at GlcNAc residues and three different O-sulfations: 2-O-sulfation, 3-O-sulfation and 6-O-sulfation (13). HS contains a variable number of disaccharide units (up to 200) with highly sulfated domains alternating with less sulfated domains. It appears that specificity of heparan sulfate for its interactors is determined mainly within the highly sulfated domains. Moreover, it has been shown that one single HS chain can bind multiple interactors simultaneously, thus yielding complex supramolecular structures such as in the case of FGF and FGF receptors (18). The highly variable number of repeating disaccharide units together with the large number and assortment of saccharide modifications yields an incredibly high number of possible "sequences" of functional units, which is why HS is considered the biomolecule with the highest degree of diversity (19).

**Chondroitin sulfate** (CS) chains have a backbone composed by repeating glucuronic acid and N-acetyl-galactosamine disaccharide units attached to the core protein through the same tetrasaccharide linker that connects HS to the core protein. As opposed to HS, CS chains contain a less diverse range of modifications and these are more equally distributed along the chain (13).

### Syndecans in skeletal muscle development

Syndecan involvement in skeletal muscle development has been investigated in flies, turkeys and mice (20-23).

During *Drosophila* development, the single syndecan is expressed in muscle fibers and appears to be involved in motor-axon guidance by acting as a receptor for the neural receptor tyrosine phosphatase (RPTP) LAR (22). Thus, *Drosophila* syndecan controls muscle innervation during development and therefore regulates the onset of muscle

functional maturation. Whether *Drosophila* syndecan is also involved directly in regulating embryonic myofiber formation, is unknown.

The role of syndecans in vertebrate muscle development has been studied in mice and birds (20,24). Developing mouse muscles express syndecan-1, syndecan-3 and syndecan-4 with similar topological distributions, but different temporal regulation (20,21). Northern and Western blot analyses of syndecan-1, syndecan-3 and syndecan-4 mRNA and protein, respectively, show that syndecan-1 protein peaks prior to other syndecans, around E12.5, then rapidly decreases and is completely absent by P2 (20). In contrast, syndecan-3 and syndecan-4 peak around E14.5 and E13.5 respectively, but then decrease much more slowly and are still expressed in newborn and adult mice (20,25). Expression of syndecan-1, syndecan-3 and syndecan-4 in embryonic muscle is localized to both myoblasts and myofibers. While syndecan-1 is not detected in postnatal muscle, syndecan-3 and syndecan-4 proteins are restricted to satellite cells and possibly vascular cells (21).

In embryonic turkey muscle, distribution of syndecan expression between E14 and E24 is regulated in a similar pattern as in mice, peaking between E14 (syndecan-3), E16 (syndecan-2) and E18 (syndecan-4), followed by a decline at later time points (E22-E24). Syndecan-2, 3 and 4 expression is presumably restricted to satellite cells in postnatal turkey muscle (23).

Important roles for syndecans in muscle development were confirmed in turkey embryonic pectoralis major muscle at different developmental stages (E14 - E24) derived from either a high body weight genetically selected line (F line) or a low body weight line (RBC2 line). In this study, Liu et al., found that the F line (high body weight) turkey muscle has higher levels of syndecan-2, syndecan-3 and syndecan-4 than the RBC2 line (low body weight) turkey muscle, supporting a key role for syndecans in the regulation of muscle development and size (23).

### Syndecans in skeletal muscle maintenance and regeneration

The hypothesis that HSPGs, such as syndecans, are involved in myogenesis could have been already inferred when a key role for HS in growth factor signaling in myoblasts was described (26,27). Subsequent studies from Brandan and colleagues showed a role for specific HSPGs in myogenic differentiation using the C2C12 myoblast cell line (28-33). Shortly after this group showed that gene expression levels and protein levels of a number of HSPGs were regulated *in vivo* during injury-induced regeneration in mouse limb muscles (34). The only two syndecans identified by Casar et al. that appeared to be expressed in regenerating muscle were syndecan-3 and syndecan-4 (34). Indeed, Cornelison et al. had previously shown that syndecan-3 and syndecan-4 are the only two syndecans detectable by immunofluorescence in postnatal mouse skeletal muscle, co-localizing with markers of satellite cells (21). The time course of syndecan-3 and syndecan-4 expression

during muscle regeneration together with the observation that their expression was restricted to satellite cells, led to the hypothesis that these two syndecans played a role in satellite cell-mediated muscle regeneration and prompted further analyses of the muscle phenotypes of *Sdc3*<sup>-/-</sup> and *Sdc4*<sup>-/-</sup> mice (35).

Though syndecan-3 and syndecan-4 are both expressed in quiescent satellite cells expression of these HSPGs in activated, proliferating and differentiating satellite cells during injury-induced regeneration is distinct (Tab. 1) and (34,36), in fact in 2004 Cornelison et al. described distinct roles for syndecan-3 and syndecan-4 in satellite cell-mediated muscle regeneration (35). *Sdc4*<sup>-/-</sup> satellite cells show impaired activation, leading to impaired regeneration upon BaCl<sub>2</sub>-induced injury (35). In contrast, *Sdc3*<sup>-/-</sup> satellite cells exhibit the opposite phenotype: impaired quiescence maintenance and renewal with a shift of the quiescent satellite cell pool toward a pool of transit-amplifying myoblasts (35,37).

A detailed inspection of the HSPG phenotype of quiescent and injury-activated satellite cells that our laboratory has recently performed, reveals that the most significant changes occurring in activated satellite cells *in vivo* is a general down-regulation of HSPGs (Tab. 1) accompanied by a decline in enzymes involved in GAG synthesis and modification (Tab. 2). Only Syndecan-4 and two enzymes involved

in HS sulfation are upregulated in response to injury (Tab. 1 and 2). Thus, these observations suggest that the heparanome of quiescent satellite cells may be responsible for coordinating signals in the satellite cell niche that are responsible for maintaining satellite cell quiescence. Interestingly, we recently showed that *Sdc3*<sup>-/-</sup> satellite cells fail to maintain quiescence and to renew (or re-acquire) a quiescent state following muscle injury (37). This observation, in addition to the observation that syndecan-3 is involved in the regulation of several signaling pathways (Pisconti et al., unpublished data) suggests that syndecan-3 may act as a master regulator of the satellite cell signaling network that is associated with quiescence. In contrast, syndecan-4 may be the only satellite cell-specific HSPG involved in satellite cell activation and cell cycle entry.

### Molecular mechanisms of syndecan function in skeletal muscle

Syndecans are complex molecules that have the potential to signal simultaneously through multiple pathways (38). Through both core protein and GAG chains each syndecan can interact with a large number of molecules, and the list of syndecan interactors is continuously increasing

Proteoglycan - gene name	Abbreviation	0h - 12h	0h - 24h	12h - 24h	24h - 48h	0h - 48h
CD44 antigen	CD44			7.85		
Syndecan 4	Sdc4			10.42		11.68
Chondroitin sulfate proteoglycan 4 (NG2)	Cspg4					-6.91
Glypican 4	Gpc4	-3.52	-3.56			-3.66
Glypican 6	Gpc6		-2.11			-2.20
Sparc/osteonectin, cwcv and kazal-like domains proteoglycan 2	Spock2			-2.26		
Syndecan 1	Sdc1	-4.00				-4.50
Syndecan 2	Sdc2	-9.97	-12.47			-10.58
Thrombomodulin	Thbd				-5.77	

Table 1. Many proteoglycan transcripts are downregulated upon satellite cell activation. Wild type satellite cells were isolated from uninjured and injured tibialis anterior muscles at 12, 24 and 48 h following BaCl<sub>2</sub>-induced injury, total mRNA extracted and hybridized on Affymetrix gene chips to perform global gene expression analysis. The Table shows all proteoglycans that changed 2-fold or greater with a p-value < 0.01 between time points as indicated. Green = downregulated transcripts. Red = upregulated transcripts.

Enzyme - gene name	Abbreviation	0h - 12h	0h - 24h	12h - 24h	24h - 48h	0h - 48h
Exostos (multiple)-like 2	Extl2				10.81	
Heparan sulfate (glucosamine) 3-O-sulfotransferase 3B1	Hs3st3b1	2.43	2.36			
N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 1	Ndst1	-3.01	-3.28			6.50
Exostos (multiple)-like 3	Extl3		-4.54		4.24	
Exostos (multiple) 1	Ext1	-4.63	-3.24			-4.55
Glucuronyl C5-epimerase	Glce	-12.00	-14.12			-8.94
Heparan sulfate 2-O-sulfotransferase 1	Hs2st1	-2.13				-2.22
3'-phosphoadenosine 5'-phosphosulfate synthase 2*	PAPSS2*	-6.87	-5.92			
Sulfatase 1	Sulf1	-5.10	-5.25			-12.11
Sulfatase 2	Sulf2	-29.94				

\*PAPSS2 is not directly in the biosynthesis pathway but is directly downstream as it controls how much active sulfate is available to the enzymes

Table 2. Proteoglycan biosynthesis and modifying enzymes are regulated upon satellite cell activation. Wild type satellite cells were isolated from uninjured and injured tibialis anterior muscles at 12, 24 and 48 h following BaCl<sub>2</sub>-induced injury, total mRNA extracted and hybridized on Affymetrix gene chips to perform global gene expression analysis. The Table shows all enzymes that changed 2-fold or greater with a p-value < 0.01 between time points as indicated. Green = downregulated transcripts. Yellow = transcripts variably regulated over time course. Red = upregulated transcripts.

(38). Decoration of HSPG core proteins with GAG chains and subsequent saccharide modifications may be more cell type-specific than core protein-specific. In other words, the same core protein tends to receive different GAG chains when expressed in different cell types, or under different physiological states, with this depending mainly on the set of GAG biosynthetic enzymes expressed in each cell type (39,40). The opposite case, that different core proteins receive similar GAG chains when expressed in the same cell type has been proposed in the context of fibroblast adhesion, where different membrane bound HSPGs bear GAG chains that are different in length but similar in sulfation pattern and capability to bind fibronectin (41). However, Tumova et al. did observe subtle differences in HS structure present on different core proteins expressed in the same cell type and the fact that the only function tested by Tumova et al., fibronectin binding, did not change significantly across the different core proteins examined, does not exclude that other functions that were not tested (e.g. growth factor binding) could change accordingly with the difference in HS structure observed. In support of this hypothesis it has been shown that even small differences in HS structure can dramatically affect FGF binding (42) and function (43). Thus, the possibility that different core proteins receive different GAG chains with different biological functions when expressed in the same cell type is still open.

In postnatal mammalian skeletal muscle the only two syndecan proteins detected are syndecan-3 and syndecan-4 (21), which are expressed in satellite cells and appear to control muscle homeostasis through distinct mechanisms (35,37).

**Syndecan-3** is the largest syndecan in mammals, harboring both HS and CS chains (17,44,45). In mouse satellite cells, syndecan-3 is found in a complex with Notch1 and promotes TACE-mediated cleavage of Notch, allowing for Notch signal transduction into satellite cells (37). In the absence of syndecan-3, Notch processing upon ligand binding is dramatically reduced as is generation of the Notch intracellular domain and subsequent induction of Notch target genes (37). As a consequence of reduced Notch signaling, *Sdc3<sup>-/-</sup>* satellite cells proliferate more slowly than wild type cells and fail to maintain or to return to a quiescent state (37). The resulting phenotype is intriguing: *Sdc3<sup>-/-</sup>* injured muscles retain full regenerative capacity and undergo progressive myofiber size increase over time despite showing a dramatic loss of satellite cells (37). A possible hypothesis to explain this paradoxical phenotype has been proposed (37): loss of syndecan-3 impairs satellite cell capacity to enter a quiescent state without affecting their ability to differentiate. Thus, a shift in the satellite cell population from a quiescent pool to an activated, proliferating and differentiating pool over time leads to myofiber hypertrophy and depletion of the quiescent satellite cell pool (37). Although loss of quiescence but not differentiation can be explained by loss of Notch signaling, this cannot explain how *Sdc3<sup>-/-</sup>* myoblasts remain proliferative for a long time. It is possible that other signaling pathways regulated by syndecan-3 in satellite cells compensate for loss of Notch signaling to maintain *Sdc3<sup>-/-</sup>* myoblasts in a proliferative cycle. Indeed,

syndecan-3 also regulates FGF and HGF signaling, though the molecular mechanisms involved are unknown (30,35). In *Sdc3<sup>-/-</sup>* satellite cells Notch signaling is decreased while FGF and HGF signaling are increased (35) and may account for the observed maintenance of a population of proliferating myoblasts. Whether the core protein or the GAG chains of syndecan-3 are the main mediators of syndecan-3 function in satellite cells and myoblasts is unknown, however both are required to rescue the Notch signaling phenotype in *Sdc3<sup>-/-</sup>* myoblasts (37).

**Syndecan-4** is the smallest syndecan, but the best studied in myoblasts as well as in other systems (46). In mouse muscle, syndecan-4 plays a key role in mediating satellite cell activation in response to injury (35). Though the molecular mechanisms underlying syndecan-4 function in mouse satellite cells are largely unknown, it has been hypothesized that syndecan-4 HS chains are involved in the regulation of FGF and HGF signaling in proliferating satellite cells (35). In the absence of syndecan-4, both FGF and HGF signaling are impaired, but can be rescued by heparin treatment (35). However, a direct mechanistic analysis of syndecan-4 function in mouse satellite cells is missing. In contrast, a significant effort has been made to understand whether a functional interaction between syndecan-4 and FGF2 exists in turkey satellite cells (47,48). This hypothesis is reasonable, since (a) syndecan-4 is involved in FGF2 signaling in other systems outside the musculature, (b) both FGF2 treatment and syndecan-4 ectopic expression in primary satellite cells lead to differentiation inhibition (47). However, genetic analysis revealed that in turkey satellite cells syndecan-4, with or without GAG chains, promotes proliferation and inhibits differentiation in an FGF2-independent manner (47-49).

Syndecan-4 is also expressed in young myotubes prior to myofiber growth and final maturation (21). An intriguing recent finding shows that syndecan-4 protein, along with  $\beta$ 1-integrin, localizes in costamers of cultured rat myotubes and is regulated by electrical activity (50). Denervation of rat tibialis anterior muscles or treatment of cultured myotubes with tetrodotoxin induce syndecan-4 and  $\beta$ 1-integrin downregulation and are associated with reduced myotube adhesion (50).

Lastly, a role for syndecan-4 in myoblast migration has been hypothesized, however a detailed analysis is missing.

**Heparan sulfate** and **chondroitin sulfate** are the two types of GAG chains covalently attached to syndecan core proteins (15). The role of HS and CS in muscle function and myogenesis has been studied irrespectively of which core proteins were attached to them (21,26,51-58). Understanding how GAGs function and signal is complicated and fascinating since: (1) the saccharide sequence of GAG chains is not template driven as is the amino acid sequence of proteins, but is the final result of multiple enzymes active simultaneously in a cell; (2) the signal mediated through GAG chains appears to be an "analog" signal where the entire pattern of saccharide and sulfated domains present at a given time in a given microdomain of the cell, can affect multiple functions simultaneously, as opposed to the "digital" type of signal driven by canonical protein-protein interactions such as ligand-receptor, kinase-substrate, etc;

and, (3) despite its generally “analog” nature, GAG interactors often exhibit high specificity for distinct oligosaccharide sequences.

Both HS and CS are involved in muscle precursor proliferation and differentiation, with highly sulfated HS generally promoting proliferation and CS generally promoting differentiation (26,34,51,52,55,57,59,60), though exceptions to this general trend have been described (58,61). Unexpectedly, we have recently determined that satellite cell activation induces downregulation of several proteoglycans (PGs) and GAG biosynthesis enzymes, suggesting a key role for PGs present in the satellite cell niche in maintaining satellite cells in a quiescent state (Tab. 1 and Tab. 2).

Interesting results have recently arisen from the study of knockout mice lacking expression of one or more enzymes involved in GAG biosynthesis. For example the satellite cell phenotype observed in *Sulf1<sup>-/-</sup>;Sulf2<sup>-/-</sup>* double knockout mice appears the opposite of the phenotype observed in *Sdc3<sup>-/-</sup>* mice (37,57). Sulfs are extracellular enzymes that remove sulfate groups from HSPGs (heparan sulfate endosulfatasases) (62). Based on these results it is plausible to hypothesize that loss of one HSPG (such as syndecan-3) leads to a general rearrangement of the satellite cell glycocalyx resulting in an overall reduction in HS on the satellite cell surface. Vice-versa, loss of two extracellular sulfatasases is expected to cause a general increase in sulfated HS on the satellite cell surface, which may explain why the *Sdc3<sup>-/-</sup>* muscle satellite cell phenotype appears opposite when compared to the *Sulf1<sup>-/-</sup>;Sulf2<sup>-/-</sup>* phenotype (37,57). Moreover, loss of one HSPG may also indirectly affect the level of decoration and amount of sulfation of other HSPGs by disrupting the normal distribution of GAG biosynthesis enzymes across several core proteins and by altering the normal balance between positive and negative feedback loops in each biosynthetic pathway.

### Syndecans in aged and diseased skeletal muscle

**Muscular dystrophy** is a family of genetic disorders characterized by muscle weakness, chronic inflammation, fibrosis and eventually muscle loss (63). Although mutations in more than 20 different genes have been found that cause a clinical phenotype classified as muscular dystrophy, some histopathological features are shared by the vast majority of muscular dystrophies, including alterations to the satellite cell niche that are associated with exhaustion of satellite cell regenerative capacity (63).

The level of HSPGs in muscles of dystrophic patients or animals is generally increased (33,54,64-68), suggesting a pathogenic role for HS and HSPGs in muscular dystrophy. In particular, syndecan-3 was augmented in Duchenne patients (33) and this finding, together with the finding that *mdx* satellite cells have increased levels of HS and CS and increased responsiveness to FGF (54), provided impetus to study the role of syndecan-3 in dystrophinopathies such as Duchenne muscular dystrophy (DMD). Indeed, our laboratory has recently observed a possible pathogenic role for syndecan-3 in a mouse model of DMD, although this work

is still in progress as we write.

**Aging** of human subjects is often associated with frailty, sarcopenia and impaired muscle regeneration, representing a major public health problem in modern societies where the average lifespan has increased (69). As in muscular dystrophy, also in aging the prevailing hypothesis to explain loss of regenerative capacity is the exhaustion of satellite cell numbers or function, although the underlying cellular and molecular mechanisms are a matter of debate (70-75). If progressive impairment of satellite cell regenerative capacity is a major cause of age-related muscle weakness and loss, it is reasonable to hypothesize a key pathogenic role for the aging satellite cell niche, which is characterized by reduced vascularization and increased fibrosis and adipogenesis (76,77). Several signaling pathways have been found altered in aging satellite cells *in vivo* (71,78,79). Moreover, it has been shown through parabiosis experiments that a “young environment” can rescue age-related muscle regeneration defect in mice (72). Our laboratory has recently shown that when young satellite cells are transplanted into young hosts together with their native niche (the myofiber), the transplanted muscle retains full regenerative capacity as the recipient animal ages as opposed to its non-transplanted contralateral, which undergoes the normal process of age-related loss of muscle mass and function (80). This prevention of muscle aging observed in transplanted muscles is entirely supported by donor-derived satellite cells, which remain viable in the host muscle throughout the mouse lifespan (80). When the donor cells (myofiber + associated satellite cells) were isolated from *Sdc4<sup>-/-</sup>* mice, this anti-aging effect was not observed, pointing out to syndecan-4 as a crucial component of the satellite cell niche (80).

### Perspective for human health

Only in the last decade have the HSPG and muscle biology communities begun to appreciate the importance of syndecans in skeletal muscle development and regeneration and therefore it is not surprising that only a few studies in humans are yet available. However, studies in mice and other model organisms show promising results that will certainly inspire more human research.

Of particular interest are the findings concerning muscle injury, muscular dystrophy and aging. While there is no information on the role of syndecans during muscle injury and aging in humans, it has been shown that expression levels of some proteoglycans, including syndecan-3, is augmented in Duchenne muscular dystrophy patients (33,64,67). This observation, in conjunction with our recent observations made in *Sdc3<sup>-/-</sup>* and dystrophic mice suggests that syndecans may be promising therapeutic targets.

The satellite cell niche is altered in dystrophic muscles, possibly due to continuous myofiber damage and leakage, myofiber necrosis and chronic inflammation, which in turn lead to extracellular matrix remodeling (81). In this context, targeting specific components of the niche, such as syndecans, may represent a potential therapeutic strategy for enhanc-

ing muscle regeneration and slowing disease progression. Although a therapy that enhances muscle regeneration is not expected to be curative for muscular dystrophy, it is reasonable to hypothesize that enhancing regeneration would greatly improve the lifestyle of dystrophic patients (82). Additionally, therapies aimed at improving muscle regeneration are also expected to increase the efficacy of stem cell and gene therapies, either by promoting exogenous stem cell contribution to host myofiber or by favoring contribution from transduced endogenous satellite cells.

Finally, a potential role for syndecans in human muscle health that has not been sufficiently explored is the use of syndecans as viral receptors for gene therapy. HS is involved in many viral infection processes acting as a receptor or co-receptor for viral particles (83). For example, infection of muscle fibers with herpes simplex virus type 1 (HSV-1) is mediated by HS, although inhibited by other unidentified ECM components (84). This is a field that has the potential to yield interesting results in the future, as the unique expression of syndecan-3 and syndecan-4 is satellite cells could be used, for example, to target viral vectors specifically to satellite cells.

In the last century the study of HSPGs in the musculoskeletal and other systems has produced a whole new level of understanding of cell adhesion, cell signaling and cell differentiation and provided essential tools for the protection of human health. For example, heparin, a highly sulfated heparan sulfate, is one of the most widely used therapeutic agents worldwide. Future studies aimed at identifying roles for syndecans in human healthy and diseased muscle in conjunction with a detailed characterization of the signaling pathways and molecular networks controlled by syndecans, are expected to contribute significantly to our understanding of muscle biology and our ability to treat muscle disorders.

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## References

- Schultz E, McCormick KM. Skeletal muscle satellite cells. *Rev Physiol Biochem Pharmacol* 1994;123:213-57.
- Ben-Yair R, Kalcheim C. Lineage analysis of the avian dermomyotome sheet reveals the existence of single cells with both dermal and muscle progenitor fates. *Development* 2005, Feb;132(4):689-701.
- Gros J, Manceau M, Thomé V, Marcelle C. A common somitic origin for embryonic muscle progenitors and satellite cells. *Nature* 2005, Jun 16;435(7044):954-958.
- Kassar-Duchossoy L, Giaccone E, Gayraud-Morel B, Jory A, Gomès D, Tajbakhsh S. Pax3/pax7 mark a novel population of primitive myogenic cells during development. *Genes Dev* 2005, Jun 15;19(12):1426-1431.
- Relaix F, Rocancourt D, Mansouri A, Buckingham M. A pax3/pax7-dependent population of skeletal muscle progenitor cells. *Nature* 2005, Jun 16;435(7044):948-953.
- Vasyutina E, Lenhard DC, Birchmeier C. Notch function in myogenesis. *Cell Cycle* 2007, Jun 15;6(12):1451-1454.
- Mauro A. Satellite cell of skeletal muscle fibers. *J Biophys Biochem Cytol* 1961, Feb;9:493-495.
- Collins CA, Olsen I, Zammit PS, Heslop L, Petrie A, Partridge TA, Morgan JE. Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. *Cell* 2005, Jul 29;122(2):289-301.
- Murphy MM, Lawson JA, Mathew SJ, Hutcheson DA, Kardon G. Satellite cells, connective tissue fibroblasts and their interactions are crucial for muscle regeneration. *Development* 2011, Sep;138(17):3625-3637.
- Péault B, Rudnicki M, Torrente Y, Cossu G, Tremblay JP, Partridge T, et al. Stem and progenitor cells in skeletal muscle development, maintenance, and therapy. *Mol Ther* 2007, May;15(5):867-877.
- Zammit PS, Heslop L, Hudon V, Rosenblatt JD, Tajbakhsh S, Buckingham ME, et al. Kinetics of myoblast proliferation show that resident satellite cells are competent to fully regenerate skeletal muscle fibers. *Exp Cell Res* 2002, Nov 15;281(1):39-49.
- Olguín HC, Pisconti A. Marking the tempo for myogenesis: Pax7 and the regulation of muscle stem cell fate decisions. *J Cell Mol Med* 2011, May 26.
- Bernfield M, Götte M, Park PW, Reizes O, Fitzgerald ML, Lincecum J, Zako M. Functions of cell surface heparan sulfate proteoglycans. *Annu Rev Biochem* 1999;68:729-777.
- Chakravarti R, Adams JC. Comparative genomics of the syndecans defines an ancestral genomic context associated with matrilins in vertebrates. *BMC Genomics* 2006;7:83.
- Carey DJ, Conner K, Asundi VK, O'Mahony DJ, Stahl RC, Showalter L, et al. Cdna cloning, genomic organization, and in vivo expression of rat n-syndecan. *J Biol Chem* 1997, Jan 31;272(5):2873-2879.
- Manon-Jensen T, Itoh Y, Couchman JR. Proteoglycans in health and disease: The multiple roles of syndecan shedding. *Febs J* 2010, Oct;277(19):3876-3889.
- Couchman JR. Transmembrane signaling proteoglycans. *Annu Rev Cell Dev Biol* 2010, Nov 10;26:89-114.
- Rapraeger AC. In the clutches of proteoglycans: How does heparan sulfate regulate FGF binding? *Chem Biol* 1995, Oct;2(10):645-649.
- Turnbull JE. Heparan sulfate glycomics: Towards systems biology strategies. *Biochem Soc Trans* 2010, Oct;38(5):1356-1360.
- Olguin H, Brandan E. Expression and localization of proteoglycans during limb myogenic activation. *Dev Dyn* 2001, May;221(1):106-115.
- Cornelison DD, Filla MS, Stanley HM, Rapraeger AC, Olwin BB. Syndecan-3 and syndecan-4 specifically mark skeletal muscle satellite cells and are implicated in satellite

- cell maintenance and muscle regeneration. *Dev Biol* 2001, Nov 1;239(1):79-94.
22. Fox AN, Zinn K. The heparan sulfate proteoglycan syndecan is an in vivo ligand for the drosophila LAR receptor tyrosine phosphatase. *Curr Biol* 2005, Oct 11;15(19):1701-1711.
  23. Liu C, McFarland DC, Nestor KE, Velleman SG. Differential expression of membrane-associated heparan sulfate proteoglycans in the skeletal muscle of turkeys with different growth rates. *Poult Sci* 2006, Mar;85(3):422-428.
  24. Cornelison DD, Filla MS, Stanley HM, Rapraeger AC, Olwin BB. Syndecan-3 and syndecan-4 specifically mark skeletal muscle satellite cells and are implicated in satellite cell maintenance and muscle regeneration. *Dev Biol* 2001, Nov 1;239(1):79-94.
  25. Cornelison DD, Filla MS, Stanley HM, Rapraeger AC, Olwin BB. Syndecan-3 and syndecan-4 specifically mark skeletal muscle satellite cells and are implicated in satellite cell maintenance and muscle regeneration. *Dev Biol* 2001, Nov 1;239(1):79-94.
  26. Rapraeger AC, Krufka A, Olwin BB. Requirement of heparan sulfate for bfgf-mediated fibroblast growth and myoblast differentiation. *Science* 1991, Jun 21;252(5013):1705-1708.
  27. Yayon A, Klagsbrun M, Esko JD, Leder P, Ornitz DM. Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. *Cell* 1991, Feb 22;64(4):841-848.
  28. Larraín J, Alvarez J, Hassell JR, Brandan E. Expression of perlecan, a proteoglycan that binds myogenic inhibitory basic fibroblast growth factor, is down regulated during skeletal muscle differentiation. *Exp Cell Res* 1997, Aug 1;234(2):405-412.
  29. Villar MJ, Hassell JR, Brandan E. Interaction of skeletal muscle cells with collagen type IV is mediated by perlecan associated with the cell surface. *J Cell Biochem* 1999, Dec 15;75(4):665-674.
  30. Fuentealba L, Carey DJ, Brandan E. Antisense inhibition of syndecan-3 expression during skeletal muscle differentiation accelerates myogenesis through a basic fibroblast growth factor-dependent mechanism. *J Biol Chem* 1999, Dec 31;274(53):37876-84.
  31. Riquelme C, Larrain J, Schonherr E, Henriquez JP, Kresse H, Brandan E. Antisense inhibition of decorin expression in myoblasts decreases cell responsiveness to transforming growth factor beta and accelerates skeletal muscle differentiation. *J Biol Chem* 2001, Feb 2;276(5):3589-3596.
  32. Henriquez JP, Casar JC, Fuentealba L, Carey DJ, Brandan E. Extracellular matrix histone H1 binds to perlecan, is present in regenerating skeletal muscle and stimulates myoblast proliferation. *J Cell Sci* 2002, May 15;115(Pt 10):2041-2051.
  33. Alvarez K, Fadic R, Brandan E. Augmented synthesis and differential localization of heparan sulfate proteoglycans in duchenne muscular dystrophy. *J Cell Biochem* 2002;85(4):703-713.
  34. Casar JC, Cabello-Verrugio C, Olguin H, Aldunate R, Inestrosa NC, Brandan E. Heparan sulfate proteoglycans are increased during skeletal muscle regeneration: Requirement of syndecan-3 for successful fiber formation. *J Cell Sci* 2004, Jan 1;117(Pt 1):73-84.
  35. Cornelison DD, Wilcox-Adelman SA, Goetinck PF, Rauvala H, Rapraeger AC, Olwin BB. Essential and separable roles for syndecan-3 and syndecan-4 in skeletal muscle development and regeneration. *Genes Dev* 2004, Sep 15;18(18):2231-2236.
  36. Tanaka KK, Hall JK, Troy AA, Cornelison DD, Majka SM, Olwin BB. Syndecan-4-Expressing muscle progenitor cells in the SP engraft as satellite cells during muscle regeneration. *Cell Stem Cell* 2009, Mar 6;4(3):217-225.
  37. Pisconti A, Cornelison DD, Olguin HC, Antwine TL, Olwin BB. Syndecan-3 and notch cooperate in regulating adult myogenesis. *J Cell Biol* 2010, Aug 9;190(3):427-441.
  38. Tkachenko E, Rhodes JM, Simons M. Syndecans: New kids on the signaling block. *Circ Res* 2005, Mar 18;96(5):488-500.
  39. Sanderson RD, Turnbull JE, Gallagher JT, Lander AD. Fine structure of heparan sulfate regulates syndecan-1 function and cell behavior. *J Biol Chem* 1994, May 6;269(18):13100-13106.
  40. Kato M, Wang H, Bernfield M, Gallagher JT, Turnbull JE. Cell surface syndecan-1 on distinct cell types differs in fine structure and ligand binding of its heparan sulfate chains. *J Biol Chem* 1994, Jul 22;269(29):18881-18890.
  41. Tumova S, Woods A, Couchman JR. Heparan sulfate chains from glypican and syndecans bind the hep II domain of fibronectin similarly despite minor structural differences. *J Biol Chem* 2000, Mar 31; 275 (13): 9410-9417.
  42. Brickman YG, Ford MD, Gallagher JT, Nurcombe V, Bartlett PF, Turnbull JE. Structural modification of fibroblast growth factor - binding heparan sulfate at a determinative stage of neural development. *J Biol Chem* 1998, Feb 20; 273(8): 4350-4359.
  43. Brickman YG, Nurcombe V, Ford MD, Gallagher JT, Bartlett PF, Turnbull JE. Structural comparison of fibroblast growth factor-specific heparan sulfates derived from a growing or differentiating neuroepithelial cell line. *Glycobiology* 1998, May;8(5):463-471.
  44. Gould SE, Upholt WB, Kosher RA. Syndecan 3: A member of the syndecan family of membrane-intercalated proteoglycans that is expressed in high amounts at the onset of chicken limb cartilage differentiation. *Proc Natl Acad Sci U S A* 1992, Apr 15;89(8):3271-3275.
  45. Berndt C, Casaroli-Marano RP, Vilaró S, Reina M. Cloning and characterization of human syndecan-3. *J Cell Biochem* 2001;82(2):246-259.
  46. Mulhaupt HA, Yoneda A, Whiteford JR, Oh ES, Lee W, Couchman JR. Syndecan signaling: When, where and why? *J Physiol Pharmacol* 2009, Oct;60 Suppl 4:31-38.
  47. Velleman SG, Coy CS, McFarland DC. Effect of syndecan-1, syndecan-4, and glypican-1 on turkey muscle satellite cell proliferation, differentiation, and responsiveness to fibroblast growth factor 2. *Poult Sci* 2007, Jul;86(7):1406-1413.
  48. Zhang X, Nestor KE, McFarland DC, Velleman SG. The role of syndecan-4 and attached glycosaminoglycan chains on myogenic satellite cell growth. *Matrix Biol* 2008,

- Sep; 27(7): 619-630.
49. Song Y, McFarland DC, Velleman SG. Role of syndecan-4 side chains in turkey satellite cell growth and development. *Dev Growth Differ* 2011, Jan; 53(1): 97-109.
  50. Ugarte G, Santander C, Brandan E. Syndecan-4 and beta1 integrin are regulated by electrical activity in skeletal muscle: Implications for cell adhesion. *Matrix Biol* 2010, Jun;29(5):383-392.
  51. Kardami E, Spector D, Strohman RC. Heparin inhibits skeletal muscle growth in vitro. *Dev Biol* 1988, Mar;126(1):19-28.
  52. Olwin BB, Rapraeger A. Repression of myogenic differentiation by atgf, bfgf, and K-FGF is dependent on cellular heparan sulfate. *J Cell Biol* 1992, Aug; 118(3): 631-639.
  53. Guimond S, Maccarana M, Olwin BB, Lindahl U, Rapraeger AC. Activating and inhibitory heparin sequences for FGF-2 (basic FGF). Distinct requirements for FGF-1, FGF-2, and FGF-4. *J Biol Chem* 1993, Nov 15; 268(32): 23906-23914.
  54. Crisona NJ, Allen KD, Strohman RC. Muscle satellite cells from dystrophic (mdx) mice have elevated levels of heparan sulphate proteoglycan receptors for fibroblast growth factor. *J Muscle Res Cell Motil* 1998, Jan; 19(1): 43-51.
  55. Bink RJ, Habuchi H, Lele Z, Dolk E, Joore J, Rauch GJ, et al. Heparan sulfate 6-o-sulfotransferase is essential for muscle development in zebrafish. *J Biol Chem* 2003, Aug 15; 278(33): 31118-31127.
  56. Jenniskens GJ, Veerkamp JH, van Kuppevelt TH. Heparan sulfates in skeletal muscle development and physiology. *J Cell Physiol* 2006, Feb; 206 (2): 283 - 294.
  57. Langsdorf A, Do AT, Kusche-Gullberg M, Emerson CP, Ai X. Sulfs are regulators of growth factor signaling for satellite cell differentiation and muscle regeneration. *Dev Biol* 2007, Nov 15;311(2):464-477.
  58. Sangaj N, Kyriakakis P, Yang D, Chang CW, Arya G, Varghese S. Heparin mimicking polymer promotes myogenic differentiation of muscle progenitor cells. *Biomacromolecules* 2010, Dec 13; 11(12): 3294-3300.
  59. Hutchison CJ, Yasin R. Developmental changes in sulphation of chondroitin sulphate proteoglycan during myogenesis of human muscle cultures. *Dev Biol* 1986, May; 115(1): 78-83.
  60. Gill R, Hitchins L, Fletcher F, Dhoot GK. Sulf1A and HGF regulate satellite - cell growth. *J Cell Sci* 2010, Jun 1; 123 (Pt 11): 1873-1883.
  61. Carrino DA, Sorrell JM, Caplan AI. Dynamic expression of proteoglycans during chicken skeletal muscle development and maturation. *Poult Sci* 1999, May; 78(5): 769-777.
  62. Dhoot GK, Gustafsson MK, Ai X, Sun W, Standiford DM, Emerson CP. Regulation of wnt signaling and embryo patterning by an extracellular sulfatase. *Science* 2001, Aug 31;293(5535):1663-1666.
  63. Cohn RD, Campbell KP. Molecular basis of muscular dystrophies. *Muscle Nerve* 2000, Oct;23(10):1456-1471.
  64. Hutchison CJ, Yasin R. Altered secretion of chondroitin sulfate proteoglycan in duchenne muscular dystrophy cultures. *J Neurol Sci* 1987, Jun;79(1-2):77-81.
  65. Bowe MA, Mendis DB, Fallon JR. The small leucine-rich repeat proteoglycan biglycan binds to alpha-dystroglycan and is upregulated in dystrophic muscle. *J Cell Biol* 2000, Feb 21;148(4):801-810.
  66. Abe S, Hirose D, Kado S, Iwanuma O, Saka H, Yanagisawa N, Ide Y. Increased expression of decorin during the regeneration stage of mdx mouse. *Anat Sci Int* 2009, Apr 1.
  67. Fadic R, Mezzano V, Alvarez K, Cabrera D, Holmgren J, Brandan E. Increase in decorin and biglycan in duchenne muscular dystrophy: Role of fibroblasts as cell source of these proteoglycans in the disease. *J Cell Mol Med* 2006;10(3):758-769.
  68. Zanotti S, Saredi S, Ruggieri A, Fabbri M, Blasevich F, Romaggi S, et al. Altered extracellular matrix transcript expression and protein modulation in primary duchenne muscular dystrophy myotubes. *Matrix Biol* 2007, Oct;26(8):615-624.
  69. Janssen I, Shepard DS, Katzmarzyk PT, Roubenoff R. The healthcare costs of sarcopenia in the united states. *J Am Geriatr Soc* 2004, Jan;52(1):80-85.
  70. Bockhold KJ, Rosenblatt JD, Partridge TA. Aging normal and dystrophic mouse muscle: Analysis of myogenicity in cultures of living single fibers. *Muscle Nerve* 1998, Feb;21(2):173-183.
  71. Conboy IM, Conboy MJ, Smythe GM, Rando TA. Notch-Mediated restoration of regenerative potential to aged muscle. *Science* 2003, Nov 28;302(5650):1575-1577.
  72. Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature* 2005, Feb 17;433(7027):760-764.
  73. Shefer G, Van de Mark DP, Richardson JB, Yablonka-Reuveni Z. Satellite-Cell pool size does matter: Defining the myogenic potency of aging skeletal muscle. *Dev Biol* 2006, Jun 1; 294(1): 50-66.
  74. Collins CA, Zammit PS, Ruiz AP, Morgan JE, Partridge TA. A population of myogenic stem cells that survives skeletal muscle aging. *Stem Cells* 2007, Apr; 25(4): 885-894.
  75. Day K, Shefer G, Shearer A, Yablonka-Reuveni Z. The depletion of skeletal muscle satellite cells with age is concomitant with reduced capacity of single progenitors to produce reserve progeny. *Dev Biol* 2010, Apr 15; 340(2): 330-343.
  76. Rogers MA, Evans WJ. Changes in skeletal muscle with aging: Effects of exercise training. *Exerc Sport Sci Rev* 1993; 21: 65-102.
  77. Cree MG, Newcomer BR, Katsanos CS, Sheffield-Moore M, Chinkes D, Aarsland A, et al. Intramuscular and liver triglycerides are increased in the elderly. *J Clin Endocrinol Metab* 2004, Aug; 89(8): 3864-3871.
  78. Brack AS, Conboy MJ, Roy S, Lee M, Kuo CJ, Keller C, Rando TA. Increased wnt signaling during aging alters muscle stem cell fate and increases fibrosis. *Science* 2007, Aug 10;317(5839):807-810.
  79. Carlson ME, Hsu M, Conboy IM. Imbalance between psmd3 and notch induces CDK inhibitors in old muscle stem cells. *Nature* 2008, Jun 15.
  80. Hall JK, Banks GB, Chamberlain JS, Olwin BB. Prevention of muscle aging by myofiber-associated satellite cell transplantation. *Sci Transl Med* 2010, Nov 10; 2(57): 57ra83.

81. Serrano AL, Muñoz-Cánoves P. Regulation and dysregulation of fibrosis in skeletal muscle. *Exp Cell Res* 2010, Nov 1; 316(18): 3050-3058.
82. Oexle K, Kohlschütter A. Cause of progression in duchenne muscular dystrophy: Impaired differentiation more probable than replicative aging. *Neuropediatrics* 2001, Jun;32(3):123-129.
83. Vivès RR, Lortat-Jacob H, Fender P. Heparan sulphate proteoglycans and viral vectors : Ally or foe? *Curr Gene Ther* 2006, Feb;6(1):35-44.
84. Huard J, Feero WG, Watkins SC, Hoffman EP, Rosenblatt DJ, Glorioso JC. The basal lamina is a physical barrier to herpes simplex virus-mediated gene delivery to mature muscle fibers. *J Virol* 1996, Nov;70(11):8117-8123.