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Wnt Signaling in Myogenesis

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Abstract

The formation of skeletal muscle is a tightly regulated process that is critically modulated by Wnt signaling. Myogenesis is dependent on the precise and dynamic integration of multiple Wnt signals allowing for self-renewal and progression of muscle precursors in the myogenic lineage. Dis-regulation of Wnt signaling can lead to severe developmental defects and perturbation of muscle homeostasis. Recent work has revealed novel roles of the non-canonical planar-cell-polarity (PCP) and AKT/mTOR pathways in mediating the effects of Wnt on skeletal muscle. In this review, we discuss the role of Wnt signaling in myogenesis and in regulating the homeostasis of adult muscle.

Keywords

Wnt; Frizzled; myogenesis; skeletal muscle; satellite cell; myofiber

A role for Wnt in muscle

Wnt signaling plays an essential role during embryonic muscle development and in the maintenance of skeletal muscle homeostasis in the adult. During embryonic development, Wnt signals control the expression of myogenic regulatory factors (MRFs), which are essential for myogenic lineage progression. In adult skeletal muscle, canonical Wnt signaling regulates the differentiation of muscle stem cells (satellite cells), whereas non-canonical signals mediate the self-renewal of satellite stem cells and the growth of muscle fibers. In the following sections, we provide a comprehensive overview of canonical and non-canonical Wnt signaling in myogenesis during development and in the adult.

Wnt signaling

Wnt proteins constitute a large family of secreted glycoproteins which are related to the *Drosophila* wingless gene [1](Glossary). In mammals, the Wnt family comprises 19 members that share homologies in their amino acid sequence but often have fundamentally distinct signaling properties [2]. All Wnt proteins share a signal sequence for secretion, several glycosylation sites and a characteristic distribution of 22 cysteine residues [2].

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Wnt proteins typically bind to Frizzled receptors (Fzd) located in the plasma membrane of target cells [1, 3](Glossary). Fzd receptors are seven-transmembrane proteins containing a large extracellular cystein-rich domain that is involved in Wnt binding. They are known to interact with Dishevelled (Dsh) and heterotrimeric G-proteins, which are required for downstream signaling [4].

Wnt-receptor interactions can elicit a variety of intracellular responses[5]; the best understood and most widely studied being the activation of β -catenin/TCF transcriptional complexes. This process is known as canonical Wnt signaling (Figure 1, in pink). A key component of the canonical Wnt signaling pathway, also referred to as the classical Wnt-signaling pathway, is β -catenin. β -catenin is associated with its own degradation complex, which consists of Axin, APC (adenomatous polyposis coli) and the serine-threonine kinase GSK-3- β (glycogen synthase kinase-3). In the absence of Wnt ligands, β -catenin is phosphorylated within the complex, leading to its ubiquitin-dependent degradation (Figure 1, [4]). When canonical Wnts bind to their respective Fzd receptors, heterotrimeric G-proteins and Dsh get activated and lead to the recruitment of Axin to the Fzd co-receptor LRP (low density lipoprotein receptor-related protein[6](Glossary). Subsequently, the degradation complex is inactivated and β -catenin accumulates in the cytoplasm. Upon its release, β -catenin translocates into the nucleus and binds members of the TCF and LEF family of transcription factors. β -catenin functions as a transcriptional co-activator to induce context-dependent Wnt/ β -catenin target genes whose transcription controls several biological processes such as early myogenesis in the somite [7].

In contrast to canonical Wnt signaling, non-canonical Wnt signaling does not require the transcriptional activity of β -catenin. Non-canonical Wnt signaling pathways are less well characterized and understood. Non-canonical Wnt pathways signal independently of β -catenin through Fzd receptors either in concert or independent of LRP (low density lipoprotein receptor-related protein). Additionally, Fzd-independent non-canonical Wnt signaling pathways have been proposed. Examples for non-canonical Wnt signaling pathways include the PCP (planar-cell-polarity), the Wnt/ Ca^{2+} and PI3K/AKT/mTOR signaling cascades (Figure 1, in green)[8–10]. The PCP signaling pathway was first discovered in *Drosophila* and has been shown to be critical for epithelial and mesenchymal cell polarity in various organisms [11, 12]. Wnt/PCP signaling mediates changes in cytoskeletal organization, which are a prerequisite for migration and cell polarization, for instance controlling the orientation of hair cells of the inner ear [13, 14]. Core components of the Wnt/PCP pathway include Fzd, Vangl, Dsh and Prickle[15]. The interplay between these factors upon Wnt signaling can result in the activation of the small GTPases, Rac or Rho, leading to cytoskeletal remodeling and/or induction of Jun target genes ([16], Figure 1, in blue). The non-canonical Wnt/ Ca^{2+} pathway has also been implicated in multiple functions including cell adhesion and cell movements during gastrulation. In this signaling cascade, binding of Wnt to the Fzd receptor leads to the release of intracellular Ca^{2+} , a process which is mediated through heterotrimeric G proteins, PLC (phospholipase C) and CamKII (calcium-calmodulin-dependent kinase II) as well as PKC (protein kinase C) ([9, 17], Figure 1, in yellow). The increased intracellular Ca^{2+} concentration also activates the calcineurin phosphatase, leading to activation of the transcription factor NFAT (nuclear factor of activated T cell)[18]. It was recently discovered that a non-canonical pathway activates the AKT/mTOR pathway resulting in myofiber hypertrophy ([10], Figure 1, Glossary).

Wnt signaling during muscle development

The process of embryonic myogenesis is orchestrated via a complex signaling network of temporally regulated morphogenetic cues from adjacent structures surrounding the

developing muscle tissue (Box 1). These extrinsic signaling molecules include Wnts, Shh (Sonic hedgehog), and BMPs (bone morphogenetic proteins). In the scope of this review, we focus on the role of Wnts in regulating embryonic muscle development, particularly in mice (Figure 2).

Box 1

Skeletal muscle development

Vertebrate skeletal muscle development originates from the mesoderm primary germ layer [55]. Cells of the paraxial mesoderm mature and shape the somites (Glossary), segmented structures that form pair-wise along the anterior/posterior axis of the developing embryo. The majority of skeletal muscles in vertebrates, with the exception of certain head muscles, develop from the somites [56, 57]. Maturing somites develop the dorsally located epithelial dermomyotome and the ventrally located mesenchymal sclerotome (Figure 2). The sclerotome forms cartilage and bone, tendons arise from the syndetome, while the dermomyotome develops into the dermis and the skeletal muscles of the trunk and limbs [58]. The myogenic precursor cells (MPCs), which arise in the dermomyotome, are specified by the expression of the paired-box transcription factors Pax3 and Pax7 [59]. Delaminating cells from the dermomyotome express myogenic regulatory factors and eventually downregulate Pax3/Pax7 to generate the first skeletal muscle tissue, the myotome. At the level of the limbs, myogenic progenitors with long-range migratory capacity delaminate from the somite. These cells will later on form muscles in the extremities. In mice at embryonic day 15.5, the first satellite cells arise, when Pax3/Pax7 positive cells align with nascent myotubes and take up a sublaminar position [60].

Wnt1, Wnt3a and Wnt4 are expressed in the dorsal regions of the neural tube and induce somitic myogenesis in cooperation with Shh signaling from the notochord [19]. As well, Wnt signaling has been demonstrated to influence expression of MRFs (myogenic regulatory factors), which are key transcriptional regulators of myogenic lineage progression and differentiation. In explant cultures of mouse paraxial mesoderm, Wnt1 induced expression of the MRF Myf5, while Wnt7a or Wnt6 preferentially activated the MRF MyoD [20]. Wnt7a, which is expressed in the dorsal ectoderm activates MyoD in presomitic mesoderm through a PKC-dependent β -catenin-independent non-canonical pathway [21]. Distinctly, Wnt1 signals through Fzd receptors 1 and 6 in the epaxial domain of the somite, to regulate Myf5 expression via the canonical β -catenin pathway [22].

Analysis of the expression of the Fzd receptors during somitogenesis demonstrated that Fzd7 is expressed in the hypaxial region of the somite suggesting an interaction with Wnt7a [23]. Accordingly, Fzd1 and Fzd6 are expressed in the epaxial somite, which correlates with Myf5 expression. Additionally, Wnt signaling was shown to be indispensable during embryonic myogenic development as transplacental delivery of sFRP3, a soluble Wnt antagonist, reduces skeletal myogenesis in a dose-dependent fashion [24].

Within the somite, Wnt1, 3a, 4 and 6 signal from the surface ectoderm and neural tube to maintain Pax3 and Pax7 expression of premyogenic cells [25]. Wnt-induced expression of the myogenic genes Myf5, MyoD and Pax3 in the somite is mediated by PKA and CREB (cAMP response element binding protein) [26]. More recently, Wnt signals directly affecting Lef1 transcriptional activator and Pitx2 transcription factor activity were found to determine the number of premyogenic Pax3/Pax7 cells [7].

The importance of Wnt signaling in formation of the dermomyotome has been demonstrated. Mouse embryos that lack both Wnt1 and Wnt3a do not form the medial compartment of the dermomyotome concomitant with a reduction in Myf5 expression [27]. In addition, Wnt6 β -catenin-dependent signaling from the dorsal ectoderm is required for the maintenance of the epithelial organization in somites and formation of the dermomyotome [28]. Furthermore, conditional deletion of β -catenin driven by Pax3-Cre or Pax7-Cre showed that β -catenin is necessary within the somite for dermomyotome and myotome formation and for the determination of the number of fetal progenitors and myofibers in the limb [29] (Glossary).

Studies in chick embryo revealed that expression of Wnt11 in the epaxial dermomyotome acts as a local cue to direct and organize the elongation of primitive myofibers in the myotome [30]. This effect of Wnt11 is mediated through the PCP pathway. Wnt11 itself is induced through a β -catenin-dependent mechanism involving Wnt1 and Wnt3a from the dorsal neural tube [27, 31].

Genetic knockout of many Wnt's and Fzd's in the mouse results in early embryonic lethality often affecting multiple tissues. Many questions on the precise role of these molecules, however, remain unresolved [32]. Moreover, Wnt molecules are secreted and have the ability to affect surrounding developing tissues, thus hindering the conclusive characterization of mutant phenotypes. Generation of conditional alleles for Fzd receptors will aid in further advancing our understanding of the role of Wnt signaling during embryonic myogenesis.

Wnt signaling during adult skeletal muscle regeneration

In resting skeletal muscle several Wnt's -- including Wnt5a, Wnt5b, Wnt7a and Wnt4 -- are expressed [33]. During regeneration of adult skeletal muscle following injury, satellite cells become activated and fuse to damaged myofibers or themselves thereby generating new myofibers (Box 2). The fine regulation of satellite cell differentiation and their self-renewal is essential during this process in order to prevent the depletion of the satellite cell pool and facilitate enough myoblasts for generating new fibers. Wnt signaling is involved in the regulation of satellite cell differentiation as well as in satellite cell self-renewal. In the early phase of muscle regeneration, Wnt5a, Wnt5b and Wnt7a become upregulated while Wnt4 expression is downregulated. In later stages after injury two additional Wnt ligands, Wnt7b and Wnt3a, are expressed [33, 34].

Box 2

Regeneration of adult skeletal muscle

Skeletal muscle comprises a remarkable ability to regenerate after injury as well as the capability to adapt to physiological demands such as growth or training. In the adult the ability to regenerate is attributed to satellite cells, a small population of cells residing beneath the basal lamina of muscle fibers [61]. Under resting conditions these cells are mitotically quiescent. Upon injury they get activated, enter the cell cycle and either fuse to each other generating newly formed fibers or fuse to damaged fibers for tissue repair. Recent studies demonstrate that satellite cells are indispensable for regeneration of skeletal muscle [62, 63].

Satellite cells undergo a highly proliferative phase during early muscle regeneration [35]. This is followed by a phase of differentiation leading to the generation of newly formed myofibers. Wnt7a has been shown to induce the division of a sub-population of satellite cells with stem cell characteristics termed 'satellite stem cells' through the PCP pathway [8].

Satellite stem cells can also give rise to committed progenitor cells through asymmetric cell division and by this means control the overall satellite cell pool ([36], Figure 3). Wnt7a binds to its receptor Fzd7 in satellite stem cells thereby stimulating their symmetric expansion. The binding of Wnt7a to Fzd7 also leads to a polarized distribution of the PCP effector Vangl2. Overexpression of Wnt7a during regeneration of skeletal muscle results in enhanced regeneration and increased numbers of satellite cells. The importance of Wnt7a signaling in regenerating skeletal muscle is emphasized by experiments performed on Wnt7a deficient mice. These animals exhibit reduced numbers of satellite cells following regeneration [8].

In contrast, the differentiation of satellite cells is controlled mostly through canonical Wnt signaling. It was demonstrated that a switch from Notch to canonical Wnt signaling is necessary for the onset of satellite cell differentiation [34]. Exogenous induction of canonical Wnt signaling through Wnt3a during the early phase of regeneration resulted in premature differentiation of progenitor cells thereby leading to a depletion of the satellite cell pool (Figure 3). Further evidence for the importance of canonical Wnt signaling in the differentiation of myoblasts arises from cell culture studies demonstrating that inhibition of GSK3 β leads to enhanced differentiation of C2 myogenic cells [37]. Studies using pharmacological activators of canonical Wnt signaling also support a role for canonical Wnt signaling in facilitating the differentiation of satellite cells and myoblasts [33, 34, 37, 38]. Moreover, R-spondins, a family of secreted proteins known to activate canonical Wnt signaling, have also been shown to promote myogenic differentiation in cell culture [39]. Another recent study revealed that *BCL9*, the mammalian ortholog of *Legless* in *Drosophila*, and its homolog *BCL9-2*, are required for the activation of canonical Wnt signaling and normal muscle regeneration in mice [40].

Interestingly, a role for canonical Wnt signaling in the development of muscle fibrosis in the elderly has been proposed. Exogenous addition of Wnt3a protein leads to increased deposition of connective tissue resembling aged regenerating muscle ([41], Figure 3). In muscles of aged mice, canonical Wnt signaling demonstrates higher activity compared to that of young mice. This has been suggested to be due to myogenic-fibrogenic conversion of proliferating satellite cells [41].

The studies suggest that canonical and non-canonical Wnt signaling antagonize each other in adult muscle [8, 34, 41]. It remains to be determined whether or not there is direct cross-talk between these pathways. Moreover, the exact nature of the canonical signaling mechanisms that lead to transdifferentiation rather than to normal differentiation are not well understood.

Wnt signaling in myofibers

Training or mechanical overload can lead to hypertrophy of the muscle, a condition that is characterized by the increase in muscle mass as a result of an increase in myofiber size. Multiple studies investigating the role of canonical Wnt signaling describe the induction of muscle fiber hypertrophy following activation of the canonical Wnt signaling pathway [37, 39, 42]. A gain of function mutant for Wnt/ β -catenin signaling in zebrafish results in hypertrophy due to unscheduled muscle progenitor proliferation [43]. The authors suggest a cross-talk between canonical Wnt/ β -catenin signaling and myostatin, a known inhibitor of muscle differentiation and growth. A synergist ablation model was used to induce hypertrophy and increased levels of active β -catenin were observed concomitant with increased levels of Dsh1 and Fzd1 in the overloaded muscle [44]. It was further suggested that β -catenin expression is necessary for overload-induced muscle hypertrophy [45]. However, it has not been addressed whether β -catenin is also required for the post-natal physiological growth of adult skeletal muscle. A separate study demonstrated that Wnt4

induces hypertrophy through the canonical pathway in tissue culture models of myogenesis concomitant with increased differentiation marked by the expression of Myf5, myogenin and MRF4 as well as increased proliferation [42]. The authors suggest that a downregulation of myostatin underlies this phenotype. In agreement with this, overexpression of Wnt4 in chicken embryos lead to enhanced differentiation (increased Pax7 and MyoD expression) resulting in increased muscle mass. In contrast, it was suggested that Wnt4 counteracts canonical Wnt signaling in C2C12 cells [46]. Whether Wnt4 is acting canonically or non-canonically in the context of hypertrophy is still unclear. Recent work has described a novel non-canonical Wnt signaling pathway involved in the induction of muscle hypertrophy [10]. Wnt7a expression/application leads to hypertrophy in resting adult skeletal muscle and in muscle cell culture. Wnt7a binding to Fzd7 activates PI3K through a G protein alpha S-dependent mechanism. This results in the activation of the AKT/mTOR anabolic pathway independent of IGF receptor activity (Figure 1, in green). Interestingly, inhibition of GSK3 β in this study did not lead to hypertrophy in a cell culture model [10]. Future work is required in order to clarify whether canonical Wnt signaling (e.g. through Wnt4) is indeed inducing hypertrophy or if increased myotube diameters are due to accelerated differentiation.

In addition to determining the fate of myogenic precursor cells and regulating the orientation of newly formed myofibers, Wnt signaling plays a role in the determination of fiber types. A role was described for Wnt5a in the developing avian wing to induce the numbers of slow MHC positive myofibers, while Wnt11 resulted in increased numbers of fast MHC positive myofibers [47]. It was demonstrated that overexpression of Wnt4 in chicken embryos not only leads to enlarged muscle mass but also to a shift in fiber types [46]. Forced Wnt4 expression increased the amount of fast contracting fibers and decreased numbers of slow contracting fibers compared to control conditions. Moreover, β -catenin is critical for the determination of muscle fiber type and myofiber number in the vertebrate embryo [29]. Active β -catenin expression results in a higher amount of slow myosin positive fibers during development.

Therapeutic implications

Wnts are promising candidates for therapeutic intervention since they are secreted factors and are naturally occurring in the body thereby avoiding an immune response. However, Wnt proteins are highly hydrophobic due to palmitoylation, which is thought to be essential for their function [48, 49]. Their hydrophobicity makes the production of recombinant Wnt proteins notoriously difficult [50]. Alternative approaches in manipulating Wnt signaling pathways through known inhibitors such as sFRPs (secreted frizzled related proteins) or Dkk (Dkkopf) may circumvent these problems.

Aged skeletal muscle display increased canonical Wnt signaling activity, resulting in myogenic-fibrogenic conversion of proliferating satellite cells, which can be suppressed by canonical Wnt inhibitors [41]. Fibrosis is often associated with muscular dystrophies, which is thought to contribute to disease pathology [51]. Inhibition of canonical Wnt signaling by Dkk in *mdx* mice, a mouse model for Duchenne muscular dystrophy (DMD), was shown to reduce fibrosis [52]. Muscular dystrophy and age-related muscle loss are accompanied by fatty infiltration of the muscle thereby negatively affecting its function [53]. Interestingly, overexpression of Wnt10b or inhibition of GSK3 in aged myoblasts can prevent adipogenic conversion [54]. These results suggest that inhibition of canonical Wnt signaling in dystrophic or aged skeletal muscle has the potential to reduce development of fibrosis and adipogenic infiltrations.

Degenerative diseases of the muscular system are thought to lead to impaired regenerative capacity due to decreased satellite cell function as well as to muscle atrophy from disuse or

instability of fibers. Wnt7a harbors the dual ability to drive symmetric satellite stem cell expansion through the PCP pathway and induce hypertrophy in muscle fibers by activating the AKT/mTOR pathway [8, 10]. This unprecedented dual function makes Wnt7a-derived biological compounds promising candidates for treatment of muscular wasting diseases such as muscular dystrophies or sarcopenia. Another advantage in using Wnts (e.g. Wnt7a) for the treatment of muscle wasting diseases is due to the fact that Wnts are secreted molecules, thus allowing Wnt proteins to be injected directly into the affected muscle. Because Wnt proteins are palmitoylated, dispersion may be limited. This would be beneficial for the treatment of single muscles, especially because Wnt signaling is also implicated in cancer. For treatment of larger muscle groups one would have to generate Wnt variants that are more dispersible.

Concluding remarks

It is evident that Wnt molecules play critical roles in various aspects of developmental and regenerative myogenesis. While Wnts certainly have indispensable functions, it is clear that they are part of a highly complex and elaborate network involving the coordination and cross-talk of a plethora of pathways that include both extrinsic and intrinsic signaling events. Many outstanding questions remain to be addressed by future research. The role of many relevant Wnt family members in regeneration of adult skeletal muscle has yet to be studied and complex crosstalk between Wnt signaling and other pathways during myogenesis and regeneration of skeletal muscle is only beginning to emerge. Furthermore, Wnt target genes involved in regeneration of skeletal muscle need to be characterized. Lastly, the biological functions of the different Wnt ligands in adult skeletal muscle regeneration should be investigated.

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Glossary

Wnt	family of highly conserved secreted signaling molecules, which typically bind to Frizzled receptors
Frizzled (Fzd) receptors	family of seven-pass transmembrane receptors, receptors for Wnt ligands
LRP	LDL (low-density-lipoprotein) receptor-related protein, co-receptor of Fzd in canonical Wnt signaling
Myogenesis	process of functional muscle formation
Dermomyotome	dorsal-lateral sheet during embryogenesis which gives rise to skeletal muscle and the dermis
Somite	bilaterally paired structure during embryogenesis which gives rise to adult skeletal muscle and skeleton
Myoblasts	skeletal muscle precursor cells, which can differentiate into myocytes and further into myotubes
Satellite cells	adult stem cells of skeletal muscle

Self-renewal	process by which a stem cell gives rise to an identical daughter cell, either through symmetric or asymmetric division
Myofiber	multinucleated muscle cell that contracts upon stimulation
Muscle hypertrophy	an increase in myofiber size, either with or without addition of new myonuclei

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Figure 1.

Overview of Wnt signaling cascades

Wnt signals can be transduced either through the canonical pathway (colored in pink) or different non-canonical pathways. Canonical Wnt signals are mediated by Frizzled (Fzds) receptors and their LRP co-receptors. In the absence of Wnt stimulation, β -catenin forms a degradation complex with APC (adenomatous polyposis coli), Axin and GSK-3 (glycogen synthase kinase-3) (dashed pink line). Phosphorylation of β -catenin by CK1 (casein kinase I) primes β -catenin and GSK-3 for proteasome-mediated degradation. The presence of Wnt ligand results in the activation of Dsh (dishevelled), which leads to a phosphorylation-dependent recruitment of Axin to the LRP co-receptor and disassembly of the β -catenin degradation complex. This leads to an accumulation and stabilization of β -catenin in the cytoplasm and the nuclear translocation of β -catenin. β -catenin complexes with TCF/LEF (T-cell factor/lymphoid enhancer factor) transcription factors and acts as a transcriptional co-activator to induce context-dependent Wnt/ β -catenin target genes. Non-canonical Wnt signals are mostly transduced through Fzd receptors without involvement of LRPs. Stimulation of Fzd through Wnt can lead to the activation of PI3K (Phosphatidylinositol 3-kinase), which then activates the AKT/mTOR pathway resulting in increased protein synthesis (shown in green). Other G-protein mediated pathways are the PCP (planar-cell polarity) pathway (shown in blue) leading to the activation of Rac/Rho, JNK (c-Jun N-terminal kinase) and/or ROCK (Rho associated kinase). JNK can induce Jun, which together with Fos, forms the AP-1 early response transcription factor. Both PCP pathways have been

implicated in cytoskeletal rearrangements. The Wnt/ Ca^{2+} signaling pathway (colored in yellow) is defined by the activation of PLC (phospholipase C) through Wnt/Fzd resulting in an increase in intracellular Ca^{2+} levels, which activate PKCs (protein kinase C) and CamKII (calcium-calmodulin-dependent kinase II) or CN (calcineurin), a phosphatase that activates the transcription factor NFAT (nuclear factor of activated T cell).

Figure 2.

Wnt signaling and the embryonic origin of limb and trunk skeletal muscle Developmental myogenesis is influenced by Wnt signaling from tissues surrounding the developing muscle. Wnt1, Wnt3a and Wnt4 are expressed in the dorsal regions of the neural tube. The dorsal ectoderm expresses Wnt4, Wnt6 and Wnt7a. Wnt11 is expressed in the epaxial dermomyotome. These Wnts regulate embryonic muscle development in a spatiotemporal manner.

Figure 3.

Effects of exogenous Wnt7a and Wnt3a on regenerating adult skeletal muscle (a) Upon muscle injury satellite cells become activated and fuse with existing fibers or with each other to repair the damaged tissue. This results in newly repaired fibers with centralized nuclei. (b) Wnt7a application to regenerating muscle expands the satellite cell pool through the PCP pathway and induces hypertrophy through the AKT/mTOR signaling cascade. (c) Application of Wnt3a following muscle injury induces the differentiation of satellite cells thereby leading to a depletion of the progenitor cell pool and increased generation of new muscle fibers (hyperplasia). Furthermore, exogenous Wnt3a leads to increased connective tissue deposition and increased myogenic-fibrogenic conversion of satellite cells, and impairs muscle regeneration.