Notch signaling in the regulation of skeletal muscle stem cells

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Abstract
Resident muscle stem cells are satellite cells that are responsible for the postnatal maintenance, growth, repair, and regeneration of skeletal muscle. In healthy adult muscle, satellite cells are mitotically quiescent, but are activated in response to stimulation such as muscle injury. Once activated, these cells then proliferate, with the majority of satellite cell progeny undergoing myogenic differentiation while the other cells return to a quiescent state and self-renew. Notch signaling is a highly conserved pathway that controls stem cell function in a variety of tissues including skeletal muscle. In this review, we discuss how Notch signaling acts as a regulator of the satellite cell pool and their fate decisions. Recent mouse genetic studies revealed that Notch signaling is essential for maintaining the satellite cell quiescent state in uninjured muscle, while it also allows for population expansion and promotes self-renewal when satellite cells are activated. Notably, diminished Notch activity in satellite cells is associated with muscle disorders such as age-related sarcopenia and muscular dystrophy. This review provides an overview of the multiple aspects of Notch signaling in muscle development and regeneration, and highlights recent studies that address its role in physiological and pathological conditions within muscle.

Keywords: satellite cells, skeletal muscle, Notch1, Notch2

Introduction
Skeletal muscle satellite cells are the residential stem cells that play pivotal roles in the postnatal maintenance, growth, repair, and regeneration of skeletal muscle. Normally, satellite cells are mitotically quiescent in healthy adult muscle. However, these cells are activated in response to stimulation such as exercise-induced muscle injury. Activated satellite cells become myoblasts and then proliferate extensively. The majority of proliferated myoblasts then undergo myogenic differentiation to form myofibers, while the other cells return to a quiescent state to self-renew and maintain the stem cell pool. The mechanism by which satellite cells self-renew is carefully controlled since the total number of satellite cells is relatively constant even after repeated muscle damage and regeneration.

Notch signaling is highly conserved and plays important roles in many biological events including survival, proliferation, and differentiation in various tissues in both development and regeneration. Four Notch receptors (Notch1, Notch2, Notch3, and Notch4) and five Notch ligands (Jagged1, Jagged2, delta-like 1 [Dll1], Dll3, and Dll4) have been identified in mammals. Notch receptors are transmembrane proteins comprised of an extracellular domain, a transmembrane domain, and an intracellular domain (NICD). Notch signaling is activated when Notch receptors bind to their ligands, which facilitates the subsequent proteolytic cleavage of the Notch receptors. The first cleavage is mediated by a disintegrin and metalloproteinase (ADAM) and the second cleavage is mediated by γ-secretase, resulting in the release of NICD. The NICD then translocates into the nucleus and interacts with the recombining binding protein-J (RBP-J), a DNA-binding protein that acts as a key mediator of Notch signaling. In the absence of Notch signals, RBP-J is associated with corepressors and inhibits the transcription of target genes including Hairy/enhancer of split (Hes) and Hes-related with YRPW motif protein (Hey) family genes, whereas NICD displaces the corepressors from RBP-J, leading to transcriptional activation. This review focuses on the diverse roles of Notch signaling in muscle stem cell function in development as well as regeneration in adults, and describes the relationship between Notch dysfunction and muscle disorders.

Notch signaling in muscle development
Notch signaling during muscle development has been studied extensively. The mutation of Notch signaling...
components leads to perturbed somitogenesis in mice and humans. In the absence of RBP-J, muscle progenitor cells in the limbs and branchial arches undergo precocious myogenic differentiation, resulting in a depletion of the progenitor pool and failed muscle growth during development. RBP-J null muscle progenitor cells are also mislocalized outside their niche in developing muscle. Constitutive activation of Notch signaling is sufficient to autonomously maintain muscle progenitor cells, but is abrogated to produce differentiating cells during muscle development. The Notch ligand Dll1 is required for maintaining the muscle progenitor pool during the development of muscles in the head and trunk. Intriguingly, during muscle development in chick embryos, Dll1 is expressed in a subpopulation of neural crest cells that transiently activates NOTCH signaling in muscle stem cells in somites to establish a balance between undergoing myogenic differentiation and maintaining the muscle progenitor pool, indicating that the Dll1-expressing neural crest cells control early muscle formation.

Notch signaling in adult muscle regeneration

Notch signaling is crucial for not only muscle development, but also for the functioning of satellite cells in adult muscle. Quiescent and activated satellite cells uniformly express the paired box protein Pax7, whereas only activated cells express MyoD, a key transcription factor for the progression of the myogenic lineage and differentiation. Although the majority of the Pax7+MyoD+ activated satellite cells proliferate, then downregulate Pax7, maintain MyoD, and undergo myogenic differentiation, others maintain their Pax7 expression, downregulate MyoD, and withdraw from the cell cycle to return to a quiescent state. Accumulating evidence suggests that the Notch signaling pathway is crucial to maintain the satellite cell pool in adult muscles. Forced activation of Notch1 in cultured satellite cells has been shown to promote proliferation and inhibit myogenic differentiation. Pharmacological inhibition of Notch activation with a γ-secretase inhibitor results in an increase in the Pax7 MyoD+ cell population committed myogenic differentiation and a decrease in the Pax7’MyoD’ self-renewed population in vitro. Double-gene ablation of the Notch effector genes Hey1 and HeyL leads to a gradual decrease in the number of Pax7’MyoD’ proliferative satellite cells, but a remarkable increase in Pax7 MyoD’ differentiating cells in limb.

Fig. 1 Notch signaling cascade

Notch signaling is highly conserved and plays important roles in many biological events including survival, proliferation, and differentiation in a variety of tissues in both development and regeneration. Notch receptors are transmembrane proteins comprised of an extracellular domain (NECD), a transmembrane domain, and an intracellular domain (NICD). Notch signaling is activated when Notch receptors bind to their ligands, which facilitates the subsequent proteolytic cleavage of the Notch receptors. This proteolytic cleavage is mediated by a disintegrin and metalloprotease (ADAM) and γ-secretase, resulting in the release of NICD. Released NICD then translocates into the nucleus and interacts with the recombining binding protein-J (RBP-J), a DNA-binding protein that acts as a key mediator of Notch signaling, leading to the transcription of target genes including Hairy/ enhancer of split (Hes) and Hes-related with YRPW motif protein (Hey) family genes.
muscle in vivo\textsuperscript{39}. Furthermore, quiescent satellite cells lacking \textit{RBP-J} undergo premature differentiation without self-renewal, resulting in a subsequent depletion of the satellite cell pool\textsuperscript{40,41}. Satellite cells express three Notch receptors: Notch1, Notch2, and Notch3\textsuperscript{36,37,42,43}. Of the three receptors, Notch1 and Notch2 are the most homologous, with Notch3 having a structural difference in that it lacks the transactivation domain\textsuperscript{44}. Global disruption of either the \textit{Notch1} or \textit{Notch2} gene in mice resulted in early embryonic lethality\textsuperscript{24,45-47}, while mice lacking \textit{Notch3} are viable and fertile, but exhibit a reduction in arteriogenesis of vascular smooth muscle cells in distal arteries\textsuperscript{48}. In adult skeletal muscle, \textit{Notch1} and \textit{Notch2} genes were expressed in all satellite cells, while the \textit{Notch3} gene was only expressed in the Pax7\textsuperscript{+}Myf5\textsuperscript{−} subpopulation (named “satellite stem cell”)\textsuperscript{37}. Thus, Notch3 was considered to play an important role in the self-renewal of the Pax7\textsuperscript{+}Myf5 satellite cell population\textsuperscript{37}. Interestingly, however, genetic disruption of \textit{Notch3} in mice enhanced satellite cell proliferative ability and increased muscle growth following repetitive muscle injuries\textsuperscript{16}. This may be due to an increased number of satellite cells after repeated muscle injury in \textit{Notch3} null mice\textsuperscript{40}, suggesting that Notch3 might negatively regulate satellite cell self-renewal. Conversely, a recent study has demonstrated that Notch3 knockdown by shRNA increased the Pax7 MyoD\textsuperscript{+} differentiating cell population, but decreased both Pax7 MyoD\textsuperscript{−} proliferative and Pax7 MyoD self-renewed cell populations\textsuperscript{49}. This discrepancy may be explained by the difference in experimental models. Further studies will be necessary to better understand the precise function of Notch3 in satellite cells in adult muscle. Indeed, additional experiments using conditional knockout mouse models for Notch3 may be particularly informative.

We have recently characterized the role of Notch1 and Notch2 in satellite cells in adult muscle\textsuperscript{50} by generating tamoxifen-inducible satellite cell-specific \textit{Notch1} and/or \textit{Notch2} knockout mice by crossing \textit{Notch1}-floxed\textsuperscript{51} or \textit{Notch2}-floxed\textsuperscript{47} mice with Pax7\textsuperscript{CreERT2} mice\textsuperscript{52}. We showed that the number of satellite cells per myofiber was reduced in \textit{Notch2}-(N2-KO) but not \textit{Notch1}-inactivated (N1-KO) mice, while satellite cells with both \textit{Notch1} and \textit{Notch2} knocked out (DKO) led to an almost complete depletion of the quiescent satellite cell population\textsuperscript{50}. This may be due to the premature activation of quiescent satellite cells as an abnormal upregulation of MyoD was detected in DKO satellite cells. Thus, our results revealed that Notch1 and Notch2 coordinately maintain the quiescent state of satellite cells by preventing their activation. We next examined the effect of Notch1 and/or Notch2 deletion in activated satellite cells. The proportion of cells positive for Ki67, a proliferation marker, decreased significantly following the inactivation of either \textit{Notch1} or \textit{Notch2}, with this number being the lowest in DKO satellite cells. Correspondingly, N1-KO, N2-KO, and DKO satellite cells all displayed an increase in the differentiating cell population and a reduction in the self-renewed cell population\textsuperscript{50}. Our findings indicate that Notch1 and Notch2 prevent myogenic differentiation and promote self-renewal when satellite cells are activated in adult muscle (Fig. 2). These results are in line with the recent studies describing the loss of self-renewal ability in Hey1/HeyL double-inactivated satellite cells\textsuperscript{39} and the acceleration of satellite cell self-renewal by constitutive Notch1 activation\textsuperscript{53}. Altogether, a “Notch1/Notch2-RBP-J-Hey1/HeyL” axis is likely to be a predominant Notch signaling pathway that maintains the stem cell pool in adult skeletal muscle.

Fig. 2 Roles of Notch1 and Notch2 in satellite cells during myogenic progression

In healthy adult muscle, satellite cells are mitotically quiescent, but are activated in response to stimulation, including muscle injury. Activated cells become myoblasts and then proliferate extensively, with the majority of the satellite cell progeny undergoing myogenic differentiation to provide myonuclei for newly formed myofibers. Satellite cells are able to return to a quiescent state to self-renew and maintain the stem-cell pool. Notch1 and Notch2 coordinately keep satellite cells quiescent in adult muscle. Once activated, Notch1 and Notch2 prevent premature myogenic differentiation and promote self-renewal to replenish the stem-cell pool.
Notch signaling in skeletal muscle disorders

Satellite cell dysfunction has been shown in muscle diseases such as age-related sarcopenia. The number of satellite cells and their function decline with age, which may be caused by decreased Notch activity with aging. For example, aged muscle loses the ability to regenerate following muscle injury due to an insufficient upregulation of Delta ligand in regenerating fibers and a subsequent reduction in Notch signaling in satellite cells. Importantly, Conboy et al. demonstrated that satellite cells in aged mice could be rejuvenated when exposed to serum from young mice by heterochronic parabiosis, which leads to an upregulation of Delta expression in the regenerative niche and restored Notch signaling. Aged muscle produces excessive amounts of transforming growth factor (TGF)-β, which activates Smad3 in satellite cells and results in failed population expansion and impaired muscle regeneration. Forced Notch activation restores muscle regeneration by inhibiting the TGF-β-Smad3-dependent up-regulation of the cyclin-dependent kinase inhibitors, p15, p16, p21, and p27 in aged satellite cells. This rejuvenation effect could be observed in human muscle stem cells, where NOTCH expression is downregulated. Indeed, the dysfunction of satellite cells with aging may be caused by an insufficiency in Notch signaling.

Recent studies have also shed light on the relationship between aberrant Notch signaling and the development of muscle diseases including muscular dystrophy, which is characterized by progressive muscle weakness and degeneration. A missense mutation in POGLUT1 (protein O-glucosyltransferase 1), which encodes an enzyme that posttranslationally modifies Notch, is responsible for muscular dystrophy with reduced Notch signaling and the subsequent loss of satellite cells. This mutation attenuates Notch signaling in satellite cells by reducing the activity of O-glucosyltransferase, and consequently, myoblasts isolated from patients exhibit a proliferation defect and precocious differentiation; these patients also have a decreased pool of reserve cells. Early-onset myopathy, areflexia, respiratory distress, and dysphagia (EMARDD) is a rare congenital muscle disease, caused by mutations in the multiple epidermal growth factor-like protein 10 (MEGF10), which is involved in satellite cell function. Remarkably, Notch1 interacts with Mefg10, but this interaction is impaired by the pathogenic mutation of MEGF10, indicating that Mefg10 regulates satellite cell function by mediating, at least in part, the Notch signaling pathway, thereby contributing to the pathogenesis of EMARDD. In mdx mice, a well-established model for Duchenne muscular dystrophy (DMD), the self-renewal ability of satellite cells seems to decline as the disease progresses. Notch signaling is also insufficient in mdx satellite cells, concomitant with the reduced expression of Notch1, Notch3, Jag1, Hey1, and HeyL, which may contribute to the depletion of satellite cells in DMD. Furthermore, whole-genome sequencing and transcriptome analyses identified that the Jagged1 ligand is expressed at relatively high levels in muscles of dogs with the mild DMD phenotype, as compared with severely affected DMD dogs. Moreover, the pathological phenotype of DMD is ameliorated by the overexpression of Jagged1 in a zebrafish model. Collectively, these findings suggest that dysfunction of Notch signaling in satellite cells is associated with the pathogenesis of muscle diseases, and therefore selective targeting for Notch signaling may be a therapeutic option to treat muscular diseases.

Concluding remarks

The present review described the roles of Notch signaling in the muscle stem cells in postnatal muscle growth and regeneration as well as in embryonic myogenesis. Notch signaling is crucial for maintaining the quiescent state of satellite cells in adult muscle, while it promotes self-renewal and inhibits myogenic differentiation when satellite cells are activated. Notch receptors may vary in their expression and their regulation of satellite cell function. The transcription factor forkhead box O-3 (FOXO3) promotes satellite cell self-renewal by directly regulating the expression of Notch1 and Notch3, but not Notch2. A transcription factor CCAAT/enhancer binding protein β (C/EBPβ) acts as a direct positive regulator for Notch2 expression, but not Notch1 and Notch3, and this controls satellite cell self-renewal during muscle regeneration. A recent report from Bi et al. described the stage-specific roles of Notch1 during myogenesis. Further studies are required for a better understanding of the molecular relationship and regulation between Notch1, Notch2, and Notch3 in satellite cells. More recently, Nandagopal et al. discriminated the dynamics of Notch signaling between Dll1 and Dll4 during embryonic myogenesis. Dll1 induces pulsatile activation of Notch1 that specifically upregulates Hes1 and promotes myogenesis, whereas, Dll4 induces sustained activation of Notch1 that predominantly upregulates Hey1 and HeyL, resulting in the inhibition of myogenesis. This mechanism might be involved in regulating satellite cell function during muscle regeneration in adults. Although recent conclusive works using genetically modified mouse lines have shown the importance of Notch signaling in satellite cell function, it will be important to more deeply elucidate how Notch signaling governs the stem cell-fate decision by striking a balance between quiescence, proliferation, differentiation, and self-renewal during muscle development, growth, and regeneration.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.
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