Notch-Mediated Restoration of Regenerative Potential to Aged Muscle

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Quiescent skeletal muscle precursor cells, or satellite cells, are positioned between the basal lamina and the plasma membrane of muscle fibers (1, 2). In response to injury, these cells undergo activation, a process defined as the break from quiescence and the initiation of cell proliferation. They then progress along a myogenic lineage pathway to generate myoblasts, which ultimately fuse to each other or to injured myofibers to promote repair and regeneration (3). Satellite cells represent the endogenous source of muscle precursor cells and account for more than 99% of the regenerative potential of adult muscle (3, 4). The efficacy of skeletal muscle regeneration is markedly impaired with age (5, 6), but the role of age-related changes in satellite cell activation in this decline is unknown. We recently showed that satellite cell activation pathway (7), a pathway well characterized for its role in myogenesis (8, 9) and in tissue formation during embryogenesis (10–12). Although alterations in Notch signaling have been associated with development abnormalities and diseases (11, 13), changes in Notch signaling have not been implicated in the aging process. These studies demonstrate that the age-related decline in muscle regenerative potential is due to a decline in Notch signaling and can be reversed by Notch activation.

The diminished regenerative potential of aged muscle was evident when injured hindlimb muscles of young (2 to 3 months), adult (5 to 7 months), and aged (23 to 24 months) mice were analyzed for activated, proliferating satellite cells associated with the myofibers (Fig. 1A; fig. S1A). Accordingly, many fewer myoblasts were generated in myofiber explant cultures (7) from...
aged mice than from young or adult mice (fig. S1B). This difference was dramatically apparent when the cells were induced to differentiate, even though there was no major defect in the propensity of aged myoblasts to fuse (Fig. 1B). This age-related decline in myoblast generation is consistent with results from single fiber preparations (14).

Previous studies, using various methodologies to study different muscles from different species, have diverged in their conclusions as to whether there is a decline, no change, or even a relative increase in satellite cell density with age (15–18). To determine whether a decrease in satellite cell number with age might account for the diminished myoblast production that we observed, we quantified purified satellite cells isolated from mouse hindlimb muscles over this age range. Fluorescence-activated cell sorting (FACS) analysis demonstrated that nearly 100% of the purified cells expressed both CD34 and M-cadherin (Fig. 2A; fig. S1C), which confirmed that these were indeed satellite cells (19). There was no significant difference in satellite cell number between young and old muscle (Fig. 2, B and C). This was confirmed by Western blot analysis of CD34 expression in myofiber explants (fig. S1D). Therefore, the dramatic age-related decline in myoblast generation in response to injury is due to an impairment of activation rather than a decline in number of satellite cells.

Because Notch signaling plays a critical role in satellite cell activation and adult muscle regeneration (7), we compared satellite cells from adult and aged muscle for the expression of Notch-1, its ligand Delta-1, and its inhibitor Numb (11, 20). In satellite cells from resting muscle of all ages, there was negligible expression of Delta-1 and high levels of Numb expression (Fig. 3A; fig. S2, B and C), consistent with previous results (7), and the levels of full-length Notch-1 were very similar (fig. S2A). In response to injury, satellite cells...
from young and adult muscle up-regulated Delta-1, whereas old satellite cells failed to do so (Fig. 3A; fig. S2C). Delta up-regulation was associated with cell proliferation as demonstrated by PCNA staining (fig. S2B). Interestingly, increased Delta expression was associated with decreased Numb expression (Fig. 3A). Analysis of multiple experiments demonstrated that there were about one-fourth as many activated satellite cells (CD34+/M-cad+/Δelta+/Numb−) in old muscle as in young or adult muscle in response to injury.

We used immunoblot analysis to test whether the reduced Delta up-regulation after injury was associated with lower levels of activated Notch in old cells. The levels of transmembrane Notch-1 were similar in cultures of all ages (fig. S2D). However, there were consistently lower levels of activated Notch in old satellite cells activated ex vivo. The biphasic pattern of Numb expression during activation of aged satellite cells was similar to that previously described in young cells (7).

We also analyzed the expression of Delta after muscle injury in vivo in young and aged mice. In young mice, Delta was induced adjacent to the injury site not only in satellite cells, but also prominently at myofiber membranes and possibly in interstitial cells in this region (Fig. 3B). This pattern was also observed at a distance from the injury site, which suggested a diffusible signal for Delta up-regulation. By contrast, there was almost no up-regulation of Delta in old muscle after injury, a finding confirmed by Western analysis, which included both satellite cells and myofibers (fig. S2C).

To further test the significance of Notch signaling for regeneration of young and aged muscle, we introduced specific inhibitors and activators of Notch at sites of injury and analyzed their effects on muscle repair. To inhibit Notch activation we used a soluble Jagged-Fc fusion protein (21), which in vitro blocked Notch activation, decreased cell proliferation, and enhanced differentiation of myogenic cells (fig. S3, A to C). When Jagged-Fc was introduced at sites of injury, there was a marked inhibition of young muscle regeneration (Fig. 4). Whereas regenerating young muscle was essentially devoid of inflammatory cells and nascent scar formation, the presence of the Notch inhibitor led to ineffective regeneration, similar to that seen in old muscle (Fig. 4). Analysis of multiple experiments showed a dramatic and consistent reduction in the number of regenerating myotubes in young muscle treated with Jagged-Fc compared with control treatment (fig. S3D).

We then tested whether forced activation of Notch could improve regeneration of aged muscle. We activated Notch directly, using an antibody to its extracellular domain (fig. S3), which in vitro blocked Notch activation, decreased cell proliferation and an inhibition of myogenic differentiation (fig. S3D). Strikingly, acute activation of Notch signaling in vivo markedly improved the regeneration of old muscle, rendering it similar to young muscle (Fig. 4, A and B). Analysis of multiple experiments showed that Notch activation at the time of injury reproducibly and significantly enhanced the formation of regenerating myotubes in aged, injured muscle (fig. S3D). Therefore, Notch activation is not only necessary for regeneration of young muscle but also sufficient to promote effective regeneration of aged muscle.

The results of this study demonstrate that inadequate activation of Notch-1 by Delta contributes to the loss of regenerative potential in old skeletal muscle. We propose that a decline in Notch signaling necessary for cell proliferation and cell fate determination may also occur in progenitor cells and stem cells in other tissues with age and may be a general mechanism underlying the diminished regenerative properties of aged tissues.

References and Notes
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Figs. S1 to S3
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