A new role for satellite cells: control of reinnervation after muscle injury by semaphorin 3A. Focus on “Possible implication of satellite cells in regenerative motoneuritogenesis: HGF upregulates neural chemorepellent Sema3A during myogenic differentiation”

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IN THE ALMOST 50 YEARS SINCE Mauro (21) first identified satellite cells in mature skeletal muscle, a great deal has been discovered about the role these myogenic precursor cells play in muscle repair and regeneration. Skeletal muscle regeneration involves coordination of a number of processes in the injured muscles. Not only do injured and/or dying myofibers need to be repaired and/or replaced, but these regenerating myofibers need to be appropriately innervated and vascularized. In muscle disease and after certain forms of denervation injury, muscle regenerative capacity is impaired. Understanding the control of the regenerative population in adult skeletal muscle, the satellite cells, will allow the development of successful strategies to improve return of function after injury or in disease.

A great many experiments have focused on understanding what controls satellite cell function in adult muscle during regeneration after injury. The first step is the activation of these normally quiescent satellite cells so that they enter the cell cycle. In an extremely novel series of studies, Anderson, Tatsumi, and Allen identified two of the earliest signaling molecules involved in activating these cells to divide. First, nitric oxide (NO) is released locally at the point of injury, serving as the initial activator of satellite cells to enter the cell cycle (5). Following NO activation of the satellite cells, hepatocyte growth factor (HGF) is released from damaged myofibers, binds to c-met receptors on the satellite cells, and stimulates those that are distant from the initial site of injury to proliferate (4). The satellite cells themselves begin to secrete HGF, thus maintaining their activated state through an autocrine feedback loop. HGF also stimulates satellite cell migration toward the site of the injured muscle, another critical factor in enhancing muscle regeneration after injury (7). Ultimately, new myofibers are formed by fusion of satellite cells to form myotubes; additionally, the activated satellite cells can repair damaged myofibers by fusing directly with them. These events were confirmed using irradiation, which abrogates the regenerative response (16), and titrated thymidine labeling, demonstrating fusion of muscle precursors to form new myofibers (22).

Control of satellite cell proliferation and differentiation is quite complex, with some myogenic growth factors such as HGF, fibroblast growth factor (FGF), and cardiotoxin-1 promoting proliferation (3, 25, 35), while others, such as insulin growth factor (IGF)-I and -II, promote differentiation (2). Often the same factors, such as HGF, FGF, and IGF, control opposing functions depending on timing of release and the local concentration within the tissue (12). Satellite cell regenerative activity is altered by sequential synthesis and secretion of these factors; for example, HGF treatment accelerates the production of FGF in these cells (34). A great many factors have been added to the list of secreted molecules that alter the rates of proliferation and differentiation of satellite cells, including such diverse molecules as cardiotrophin-1 (25), transforming growth factor-β1 (23), and myostatin (24).

Muscle injury affects more than the muscle tissue itself. Complex control mechanisms also exist for successful regeneration of the motor nerve to the injured muscle to reestablish functional reinnervation. After the initial injury, the motor axons retract. As new myofibers begin to form, nerves need to be attracted and guided to form neuromuscular junctions with the new myofibers. Many of the same factors that promote myofiber regeneration and repair, including HGF and IGF-I, also function as survival molecules for the motor neurons and serve as promoters of axonal sprouting and guidance as the nerves grow toward the newly regenerated myofibers (8, 30). One large family of well-studied molecules best known for their axon guidance abilities are the semaphorins, first identified by their ability to cause neurite growth cone collapse (19). Semaphorin proteins are expressed by brain tissue, but also by a wide range of nonneuronal tissues, where they play a role in such diverse processes as angiogenesis and regulation of inflammation (32). Semaphorin 3A belongs to the group of secreted semaphorins that controls axon guidance and growth (29) as well as cell migration (11). In denervated muscle, terminal Schwann cells have been postulated to control guidance of regenerating axons to their original neuromuscular junctions (17). Interestingly, a subset of terminal Schwann cells upregulate semaphorin 3A after muscle denervation, but only on type IIb myofibers (10). First, this tells us that the microenvironment that controls these processes appears to differ at the single myofiber level. Second, this means another cell type would need to control the reinnervation process. Most of the above regenerative processes have been studied on a “whole muscle” scale, but to understand these processes, it is critical to know the details of the complex control and timing of these events and the microenvironment at the single cell level.

In the American Journal of Physiology-Cell Physiology, Tatsumi and colleagues (36) demonstrate for the first time that semaphorin 3A is expressed by satellite cells in injured muscle. Thus, in addition to the role of satellite cells in the formation and/or repair of individual myofibers, they also have the...
potential to play a role in controlling myofiber innervation. This is a very exciting observation from a number of viewpoints, and it opens up a new area of investigation that will hopefully answer many important questions about control of muscle repair after injury at the single cell level. Specifically, Tatsumi et al. (36) make the novel observation that in injured skeletal muscle, satellite cells upregulate their expression of semaphorin 3A in response to secretion of HGF, itself an important paracrine and autocrine factor whose increase very early after muscle injury controls satellite cell activation for their entry into the cell cycle (4). Other myogenic factors, such as IGF and platelet-derived growth factor, known to be secreted later in the process of muscle regeneration, have no effect on this semaphorin 3A upregulation in satellite cells. Only epidermal growth factor produces a small, but significant, elevation in semaphorin 3A by the satellite cells. Even more intriguing is the demonstration that the HGF does not facilitate semaphorin 3A expression by the c-met receptor, suggesting that a second receptor is involved in control of semaphorin 3A expression in satellite cells.

The timing of semaphorin 3A expression correlates well with the timing of specific processes occurring within the damaged muscle. Generally, it takes 24–48 h for satellite cells to activate, divide, and synthesize and secrete HGF after muscle injury. At 48 h after injury, satellite cells are proliferating, and the chemorepulsive function of semaphorin 3A secreted at this time would prevent neuritogenesis from occurring prematurely, when the regenerating myofibers have not yet formed. It is known that initial acetylcholine receptor patterning in myofibers occurs in the absence of motor innervation (38), so the nerves are not needed at this point in the regenerative process. In general, it takes several additional weeks to complete regeneration of the muscle fibers, and the sustained expression of semaphorin 3A during this period, as shown by Tatsumi et al. (36), means that there is ongoing repulsion of growth cones during this period of muscle formation and remodeling. This correlation does not prove that the secreted semaphorin 3A is responsible for the delay of neurite in-growth; further studies that specifically examine timing of in-growth of axons are needed. However, the levels of semaphorin 3A secreted by the satellite cells are sufficiently high to make their potential control of this process quite compelling.

Control of axon growth and guidance is extremely complex and multifactorial. However, gradients of secreted semaphorin 3A can produce seemingly opposite effects: neurite collapse (19), neurite growth (6), and guidance toward or away from a location (20). Thus, it would be interesting to know whether there is differential expression of semaphorin 3A at differing distances from the site of the original muscle injury and how this is reflected in the timing of neuritogenesis in each area. Semaphorin 3A is also known to enhance the rate of fast anterograde and retrograde axoplasmic transport in axons (14); this long-range signaling from the periphery to the neuronal cell body would allow neurons to adjust their growth rate in response to what is ongoing in the periphery. Following muscle injury, there is a decline in HGF expression to basal levels, and as one would predict, this results in a concomitant decline in semaphorin 3A expression by the satellite cells. This decreased semaphorin 3A would increase the environmental receptivity for motor nerves to grow toward the newly formed muscle fibers and form neuromuscular junctions. The patterns and timing of nerve in-growth will confirm whether these pathways are indeed controlled in this way.

Interestingly, Tatsumi and colleagues (36) showed that a basal level of semaphorin 3A expression is maintained in quiescent satellite cells. This low level of expression may be involved, in concert with terminal Schwann cells, in limiting the amount of neuromuscular junction remodeling that occurs in normal, uninjured muscle (37). Future studies at the site of newly forming neuromuscular junctions are needed to answer this question. Certainly, the presence of semaphorin 3A and the position of the satellite cells relative to the neuromuscular junction support their potential role in this process, as well as their potential role in refining the final size of the neuromuscular junctions by preventing terminal sprouting (38).

Tatsumi et al. (36) show that, even after antibody neutralization of the HGF-specific receptor c-met, there was no change in the upregulation of semaphorin 3A secretion after HGF stimulation. Despite the apparent lack of control by the c-met receptor of semaphorin 3A secretion demonstrated in this study, other potential binding partners exist (27). Competition between semaphorin activation and vascular endothelial growth factor (VEGF) has been described, and a similar scenario may be occurring here with HGF and semaphorin 3A (1). Hopefully, future studies will determine the specific HGF receptor that controls semaphorin 3A secretion from satellite cells in injured muscle. It is particularly interesting that HGF did not increase semaphorin 3A secretion in the satellite cells through the c-met receptor, because it is expressed in abundance on the satellite cells themselves (9). This suggests that the semaphorin 3A will not act in an autocrine fashion on the satellite cells, as is the case with HGF.

Semaphorins mediate changes in axon growth by binding to their receptor and modifying endocytosis at the growth cone plasma membrane (20). Classically, semaphorins, and semaphorin 3A specifically, activate downstream cell signaling pathways by binding to a neuropilin receptor and forming a neuropilin-plexin complex, which then results in axon repulsion (28). Interestingly, the hepatocyte growth factor receptor shares a structural homology to the plexins, a transmembrane semaphorin receptor, and these two receptors can interact. When direct association occurs between plexin and the HGF receptor met, binding of semaphorin to the plexin causes tyrosine phosphorylation of both receptors (13). This signaling can occur in the absence of neuropilin binding. Another example of direct binding independent of neuropilin was the interaction between semaphorin 3E and plexin-D1, which was shown to control vascular patterning in developing somitic muscle (15). Thus, semaphorins can signal using a diversity of pathways, either through a neuropilin-plexin complex, directly with plexin itself, or through a met-plexin complex. In addition to the c-met receptor complex, four additional semaphorin-binding receptor complexes have been described, including one with L1, a molecule associated with cell adhesion (26). Future studies are needed to determine which cells are binding the semaphorin 3A secreted by the satellite cells and as a result, which pathways are activated. Because of the number of cell...
types that bind semaphorins, these actions will help determine which cells respond in the regenerating muscle, and at what time during the various processes they are functioning. This is very important, because reinnervation is only one of the processes occurring after muscle injury.

What else might the semaphorin 3A be doing in the regenerating muscle? While semaphorins have a very important role in axon guidance and growth, they play a role in a myriad of other processes (32). Many of these are critical to successful muscle regeneration, including inflammation, angiogenesis, and cell migration. After muscle injury, necrotic muscle tissue needs to be removed. Semaphorin 3A can alter vascular permeability (1) as well as T-cell migration (18), both of which would result in infiltration of inflammatory cells at the site of injury. This is important for removal of the dying myofibers. Semaphorin 3A also suppresses activation of T cells; thus, in the context of muscle injury it could prevent development of an immune response at the injury site.

Additionally, semaphorin 3A can modulate vasculogenesis and competes with VEGF in this role (1). Semaphorin 3A affects vessel remodeling by inhibiting extracellular matrix ligand recognition of integrins, freeing cells from adhesion to the extracellular matrix (33). Interestingly, in a recently published study, satellite cells were shown to produce a secreted factor that significantly increases angiogenesis when cocultured with microvascular segments from the epididymus (31). While this was attributed to hypoxia-inducible factor-1α (HIF-1α), a known transcriptional activator of VEGF expression, the timing of this process also correlates with that of increased semaphorin 3A secretion by satellite cells after injury (36). Because both HIF-1α and semaphorin 3A affect the function of VEGF, it will be interesting to examine the timetable of each of these molecules and compare their behavior in a combinatorial assay.

It would appear that semaphorin 3A can mediate multiple, and often opposing, functions within a wide array of tissues. After muscle injury, control of myofiber regeneration and reinnervation, angiogenesis, and inflammatory cell infiltration all must be carefully controlled. Tatsumi et al. (36) have now added semaphorins to the mix of factors that control these processes, all of which occur simultaneously, with HGF activating the satellite cells and upregulating their expression and secretion of semaphorin 3A, which in turn repels neurites and promotes vasculogenesis (Fig. 1). The complex role of semaphorin 3A in these diverse processes is presumably mediated by 1) different semaphorin 3A receptor complexes, 2) concentration gradients around individual cellular elements, 3) the use of different intracellular signaling pathways within a given cell type, and 4) different molecules controlling semaphorin 3A upregulation and secretion. To differentiate between these possibilities, the timing of expression of semaphorin 3A relative to its specific cellular targets will need to be examined in combinatorial assays, because molecules such as HGF, semaphorin, plexin, and the c-met receptor interact in ways that are just beginning to be understood. The Tatsumi, Anderson, and Allen groups have been instrumental in defining a number of the important players that control satellite cells and muscle regeneration, and the addition of semaphorins to this already long list brings us closer to understanding these complex processes. This study by Tatsumi and colleagues (36) reminds investigators that the “push and pull” of the various molecules controlling muscle regeneration is exceedingly complex, and identifying all the players involved is extremely important to unraveling how muscle regeneration really works.

Fig. 1. Model of a single myofiber before and after injury. A: a single myofiber (orange) is surrounded by a basal lamina (black). Quiescent satellite cells (blue) are located in the space (cream) between the basal lamina (black) and the sarcolemma of the myofiber. A single axon forms a neuromuscular junction in the central region of this fiber (yellow). The myofibers are surrounded by intact blood vessels, depicted here as strands (red). B: after injury, the myofiber begins to necrose and die (brick red). Initially, hepatocyte growth factor (HGF, pink) is released into the space between the basal lamina (black) and the sarcolemma, activating the satellite cells (green). Within 48 h, the activated satellite cells secrete semaphorin 3A (purple), which in postulated to repel in-growing neurites (yellow) and cause blood vessel remodeling (red).
REFERENCES


