Do fast and slow muscle fibers have dedicated stem cell populations?

Veterinary Research Scholars Program
University of Missouri

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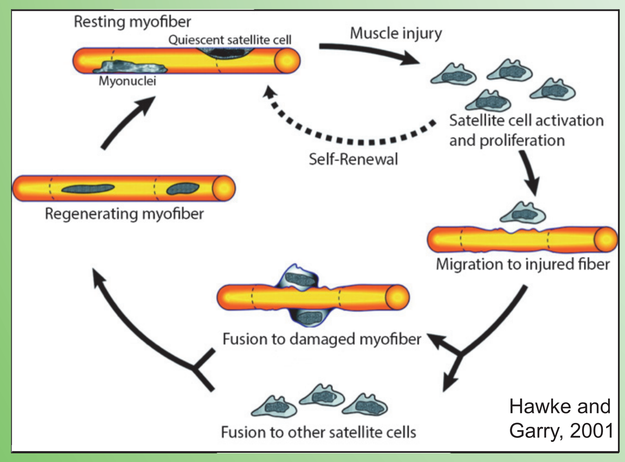
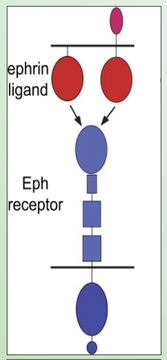
IDEXX BioResearch

Abstract

Three types of muscle are present in the body: smooth, cardiac, and skeletal. This lab focuses on skeletal muscle. It is attached to the skeleton with functions including voluntary limb movement, posture retention, and respiration. Skeletal muscle is composed of two types of myofibers, fast and slow. Fast fibers are more common overall, functioning in rapid strong contracting muscles. Slow fibers are in muscle sustaining less forceful contractions such as maintaining stability. Each skeletal muscle has a unique composition of fast and slow fibers. The lab studies adult stem cells of skeletal muscle, termed satellite cells, responsible for regeneration of injured muscle. Satellite cells are found on both fast and slow fibers and are quiescent until activated by injury. The pattern of slow and fast fibers after satellite cells regenerate injured muscle is maintained in the same composition present prior to injury- how? Recently this lab showed that the cell surface repulsive ligand ephrin-A3 is specific to slow myofibers. This suggested the possibility that a receptor for ephrin-A3 may be expressed by fast satellite cells, which could let them differentiate between the two populations of muscle fibers and thus preserve the fiber type patterning after regeneration. I am learning muscle and satellite cell biology as well as technical skills including fiber isolation, immunostaining, and culture. I am collaborating with lab member Jacqueline Ihnat to test this hypothesis: we are designing experiments to ask a series of questions, give possible answers, and create a stepwise decision tree to come to experimental conclusions based on data as it is collected.

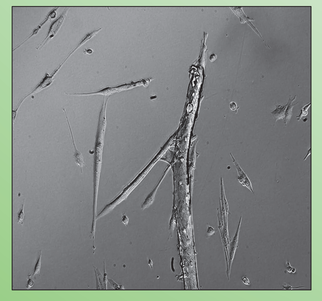
Introduction





Nikolov, 2013

Upon injury, satellite cells activate and migrate along or away from their fiber to the point of injury. They proliferate, differentiate, and fuse to create new fibers or repair existing ones. Our lab focuses on the interaction between Ephs and ephrins as a possible influence on satellite cell activity. Eph/ephrins are membrane bound and signal through cell-to-cell contact.



Slow versus fast fibers

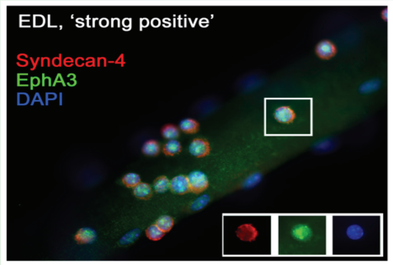
	Slow oxidative	Fast oxidative	Fast glycolytic
Myosin heavy chain	MyHC-I	MyHC-IIa	MyHC-IIx, -IIb
Contraction velocity	slow	fast	fast
Rate of fatigue	slow	intermediate	fast

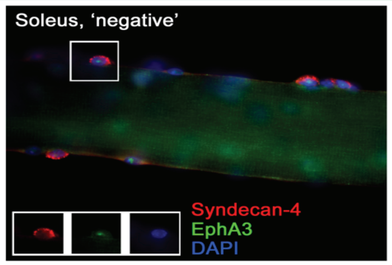
Methods

Single living intact myofibers and their associated satellite cells are isolated from the extensor digitorum longus, a primarily fast muscle, and the soleus, which is 42% slow. They are digested in an enzyme, collagenase, to break down connective tissue. Fibers are cultured to allow satellite cells to activate, proliferate, and upregulate myogenesis-associated genes. Fibers are fixed, stained, and imaged to evaluate for the presence of EphA3.

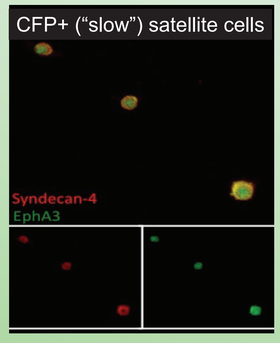
Fibers were isolated for wildtype mice and Myh7-CFP mice. Myh7 encodes for MyHC-I (slow fibers). In these mice, all slow twitch fibers are cyan fluorescence protein (CFP) positive.

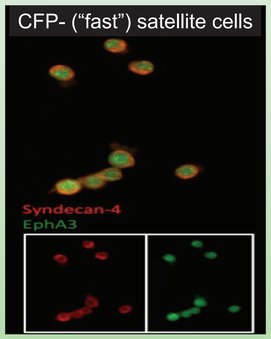
Results





We isolated fibers from the EDL and the soleus and cultured them for 96hrs. We used syndecan-4 as a marker satellite cells. Satellite cells from the EDL are more likely to be EphA3 positive whereas satellite cells from the soleus are more likely to be EphA3 negative.





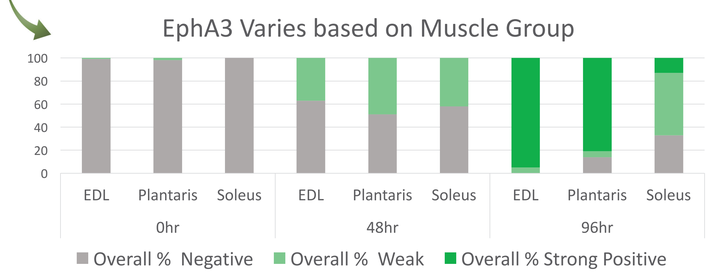
We isolated fast and slow fibers from Myh7-CFP mice. We can sort fibers based off the presence or lack of CFP. Both "fast" and "slow" satellite cells are off the fiber, they are positive for EphA3.

Decision tree

Is EphA3 expression on satellite cells consistent within fiber type?

Yes → Fiber type and EphA3 expression are related

No → Variable EphA3 expression of satellite cells suggests other influence



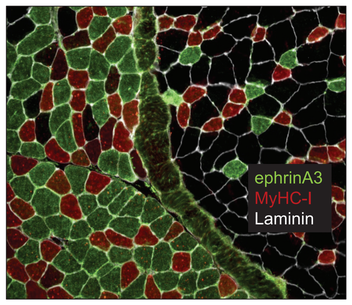
Per fiber, when satellite cells leave their fiber do they still maintain the same EphA3 expression?

Yes → EphA3 expression may be predetermined.

- Can a satellite cell be influenced to differentiate into any type of fiber?
- What factors are present or interacting to influence differentiation and fiber type specification?

No → EphA3 expression may be dependent on contact with the fiber or other signals may be present.

Is ephrinA3 on slow fibers preventing the expression of EphA3 on satellite cells associated with slow fibers?



Our lab has demonstrated the presence of the membrane-bound ligand ephrinA3 on slow fibers and its nonexpression on fast fibers. ephrinA3 and EphA3 have been shown to possess a repulsive relationship. The high ephrinA3 expression on slow fibers could be responsible for the lack of EphA3 expression on associated satellite cells.

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