

Won't You Be My Neighbor? Muscle Stem Cells Recruit Endothelial Cells to Their Niche

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The maintenance of a pool of quiescent satellite cells (muscle stem cells) is necessary for long-term muscle health. In this issue of *Cell Stem Cell*, Verma et al. (2018) show that satellite cells recruit endothelial cells to create a vascular niche and that cross-talk between endothelial and satellite cells is vital for replenishment and maintenance of quiescent satellite cells.

Skeletal muscle has a remarkable ability to repair itself following injury due to the persistent presence of a population of dedicated muscle stem cells, the satellite cells (SCs) (reviewed by Relaix and Zammit, 2012). In response to injury, the normally quiescent SCs activate, re-enter the cell cycle, proliferate, differentiate, and fuse to form multinucleate myofibers that regenerate damaged muscle (Chargé and Rudnicki, 2004). Importantly, not all SCs are dedicated to repairing myofibers, but some SCs instead return to a niche between the myofibers and surrounding basal lamina and become quiescent (Montarras et al., 2013). Self-renewal of SCs during regeneration and maintenance of a continuous supply of quiescent SCs is essential for ensuring that future regenerative needs are met.

SCs and myofibers reside in a three-dimensionally complex environment with multiple neighboring cells that are important for normal muscle function, and recently these neighboring cells, including fibro-adipogenic progenitors (FAPs), macrophages, eosinophils, T cells, and pericytes, have been recognized to be critical regulators of SCs and regeneration (Wosczyna and Rando, 2018). Another important component of skeletal muscle is the extensive network of vasculature that enwraps the myofibers and SCs. Strikingly, endothelial cells are located in close proximity to SCs, and the number of SCs is significantly correlated with the degree of capillarization of the associated myofibers (reviewed in Latroche et al., 2015). During muscle regeneration, angiogenesis and myogenesis occur concomitantly, and there is extensive cross-talk between myogenic and endo-

thelial cells; *in vitro* transwell experiments have established that myogenic cells, through their expression of Vascular Endothelial Growth Factor (VEGF), promote angiogenesis, while endothelial cells, via their secretion of growth factors, promote SC growth (reviewed in Latroche et al., 2015). However, a role for endothelial cells in regulation of SC quiescence has not been tested. Because SC quiescence strongly depends on the three-dimensional architecture of muscle, testing the potential involvement of endothelial cells requires *in vivo* testing and analysis. Most previous studies of endothelial cell and SC interactions have used *in vitro* co-culture assays (Wosczyna and Rando, 2018), and so the innovative three-dimensional imaging as well as *in vivo* conditional mutagenesis experiments from Verma et al. (2018) (as outlined in this issue of *Cell Stem Cell*) uniquely enable their investigation of the potential role of endothelial cells in regulating SC quiescence.

The authors chose to visualize the spatial relationship between SCs and the complex vascular network by using a novel clearing technique, genetic reagents to simultaneously label endothelial and SCs in mouse muscle, and confocal microscopy. This analysis revealed that 40%–80% of SCs in uninjured, homeostatic muscle were in direct contact with capillary endothelial cells. This close proximity of endothelial cells and SCs suggested that signaling might exist between the two cell types. Consistent with previous studies (Latroche et al., 2015), transcriptome analysis identified that VEGFA is highly expressed in quiescent and activated SCs. Conditional deletion of VEGFA in SCs in homeostatic muscle

resulted in an increased distance between SCs and capillaries and thus demonstrated that SCs' secretion of VEGFA is critical for recruiting endothelial cells, which express the VEGFA receptor. Furthermore, they found that the most quiescent SCs (termed label-retaining cells because of their retention of an EdU label due to their slow proliferation rates) reside closest to the endothelial cells, and based on the work of others (Chakkalakal et al., 2012), these SCs have a high potential for self-renewal. This suggests that SC recruitment of endothelial cells may be a key step in inducing or maintaining a sub-population of more stem-like quiescent SCs during muscle homeostasis. Consistent with this notion, loss of VEGFA in SCs during muscle regeneration resulted in depletion in the number of SCs that self-renewed.

To explore how neighboring endothelial cells might regulate SC quiescence, they performed an interactome analysis between satellite and endothelial cells and identified that the Notch ligand Dll4 is strongly expressed in endothelial cells. The Notch pathway was of particular interest as previous studies had established that Notch is essential for maintaining SC quiescence (Bjornson et al., 2012; Mouriakis et al., 2012). Using experiments co-culturing SCs in direct contact with endothelial cells, the authors demonstrate that juxtacrine Notch-Dll4 signaling between satellite and endothelial cells is important for induction of SC quiescence. In summary, these data support a model whereby cross-talk between satellite and endothelial cells regulates SC quiescence; SCs secrete VEGFA and recruit endothelial cells to create a vascular



niche, and in turn, endothelial cells express Dll4, which activates juxtacrine Notch signaling in SCs to promote their quiescence (Figure 1).

Verma and colleagues thus provide evidence that SCs are actively involved in recruiting a vascular niche and SC quiescence and self-renewal are maintained by juxtacrine signals from this niche. Such recruitment by SCs of endothelial cells demonstrates that SCs are not simply passive residents of their niche, but are active participants in building the cellular milieu within which they live. A number of interesting questions are raised by this study. First, while SCs are able to locally modify the capillary network, it is unlikely that they control the overall architecture of the vasculature. The new clearing technique used in this work now allows the development and regeneration of the vascular network to be followed in three dimensions. Such an analysis would begin to elucidate the mechanisms controlling angiogenesis in muscle.

A second question raised by this study concerns the role of VEGFA in regulating SC function. The present study suggests that VEGFA secreted by the SCs mainly functions in a paracrine manner to recruit endothelial cells and indirectly to regulate SC quiescence. However, previous *in vitro* studies suggest that VEGFA can function in an autocrine manner to stimulate SC differentiation (summarized in Latroche et al., 2015). Conditional deletion of VEGF receptors in satellite and endothelial cells would allow autocrine versus paracrine functions to be ascertained. Another issue concerns the direct contact between satellite and endothelial cells necessary for juxtacrine Notch-Dll4 signaling. Quiescent SCs are well known to reside underneath the basal lamina of myofibers, and endothelial cells are surrounded by pericytes and smooth muscle cells. The basal lamina, pericytes, and smooth muscles would appear to be bar-

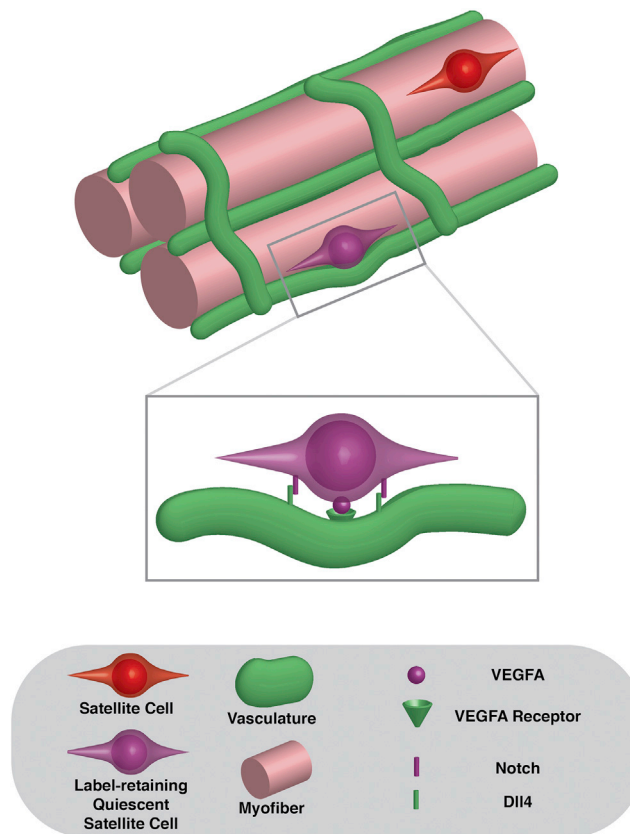


Figure 1. Cross-Talk between Endothelial Cells and SCs Regulates SC Quiescence

Expression of VEGFA by SCs recruits blood vessel endothelial cells, creating a vascular niche. Subsequently, juxtacrine Dll4-Notch signaling between adjacent endothelial cells and SCs maintains SCs in quiescence.

riers to direct contact between satellite and endothelial cells. However, as the authors reviewed, there is recent evidence that gaps in the basal lamina allow communication through this layer, and as others have noted (Latroche et al., 2015), endothelial cells are not always surrounded by these other cell types. Determination of whether satellite and endothelial cells are actually in direct contact will likely require higher-resolution imaging. Finally, the most intriguing question concerns heterogeneity in the SC population. Only a sub-population of SCs, mainly composed of label-retaining cells, reside in close proximity to endothelial cells. What determines why only some SCs recruit a vascular niche and are destined to become quiescent, label-retaining cells? Potentially only SCs destined to become label-retaining quiescent cells express high levels of VEGFA. Alternatively, all SCs may ex-

press VEGFA and are able to recruit endothelial cells, but the juxtacrine signaling necessary to establish and stabilize endothelial-SC cross-talk is only established between a subset of satellite and endothelial cells. Future *in vivo* experiments that preserve the three-dimensional architecture of muscle will be needed to resolve this issue. Increasing our understanding of the mechanisms governing SC quiescence and self-renewal will be essential for harnessing these stem cells for future therapies to rescue muscle loss during disease or aging.

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