Review

Biomechanical Origins of Muscle Stem Cell Signal Transduction

James B. Morrissey[1,2], **Richard Y. Cheng**[1,2], **Sadegh Davoudi**[1,2] and **Penney M. Gilbert**[1,2]

*1 - **Institute of Biomaterials and Biomedical Engineering,** Toronto, ON, Canada M5S3G9*
*2 - **Donnelly Centre for Cellular and Biomolecular Research,** Toronto, ON, Canada M5S3E1*

Correspondence to Penney M. Gilbert: *penney.gilbert@utoronto.ca*
http://dx.doi.org/10.1016/j.jmb.2015.05.004
Edited by Y.-G. Chen

Abstract

Skeletal muscle, the most abundant and widespread tissue in the human body, contracts upon receiving electrochemical signals from the nervous system to support essential functions such as thermoregulation, limb movement, blinking, swallowing and breathing. Reconstruction of adult muscle tissue relies on a pool of mononucleate, resident muscle stem cells, known as "satellite cells", expressing the paired-box transcription factor Pax7 necessary for their specification during embryonic development and long-term maintenance during adult life. Satellite cells are located around the myofibres in a niche at the interface of the basal lamina and the host fibre plasma membrane (i.e., sarcolemma), at a very low frequency. Upon damage to the myofibres, quiescent satellite cells are activated and give rise to a population of transient amplifying myogenic progenitor cells, which eventually exit the cell cycle permanently and fuse to form new myofibres and regenerate the tissue. A subpopulation of satellite cells self-renew and repopulate the niche, poised to respond to future demands. Harnessing the potential of satellite cells relies on a complete understanding of the molecular mechanisms guiding their regulation *in vivo*. Over the past several decades, studies revealed many signal transduction pathways responsible for satellite cell fate decisions, but the niche cues driving the activation and silencing of these pathways are less clear. Here we explore the scintillating possibility that considering the dynamic changes in the biophysical properties of the skeletal muscle, namely stiffness, and the stretch and shear forces to which a myofibre can be subjected to may provide missing information necessary to gain a full understanding of satellite cell niche regulation.

Introduction

The remarkable regenerative ability of skeletal muscle is underpinned by satellite cells, recently recognised to contain the principal adult muscle stem cell population [1–6]. Satellite cells reside beneath the basal lamina that surrounds skeletal muscle fibres, integrated with an extracellular network of laminins, collagens and assorted proteoglycans and glycoproteins (Fig. 1; reviewed in Ref. [7]). This stem cell niche is a dynamic environment that remodels in response to various cues presented during injury. In healthy uninjured muscle satellite cells remain mitotically quiescent under the basal lamina. Following mechanical or chemical injury of myofibres, satellite cells become activated and rapidly proliferate, simultaneously migrating out of the basal lamina to necrotic myofibres in the form of myoblasts. These myoblasts eventually leave the cell cycle and align together, fusing and differentiating into myofibres to repair the damaged muscle.

Satellite cell fate decisions in mature muscle are governed by the expression of transcription factors Pax7 (expressed in quiescent and activated satellite cells, reviewed in Ref. [8]), MyoD (in activated satellite cells and during differentiation, reviewed in Ref. [9]) and Myf5 [10,11]. Lineage tracing using these transcription factors as markers has indicated that muscle stem cells may adopt asymmetric division and diverge in their fate decisions (reviewed in Ref. [12]) to produce both self-renewing and committed differentiating daughter cells. It has even

been suggested that some may reenter a form of quiescence [13], sustaining the stem cell population for future regeneration.

The basal lamina forms a niche where quiescent satellite cells are segregated from other cell types but is permissible to signalling cues, and it allows the maintenance of a slowly dividing stem cell pool in healthy muscle [14]. The regenerative capacity of satellite cells is understood to rely on preservation of key niche factors [1], and it conversely appears to diminish with time cultured *in vitro* [15,16]. Growth factor, chemical, juxtacrine contact and mechanical cues within and outside of the basal lamina have all been speculated to regulate satellite cell fate by containing them in quiescence, activating proliferation following myofibre injury or promoting differentiation into myofibres following necrosis. During regeneration of myofibres, the niche itself becomes extensively remodelled as satellite cells emerge into the site of injury. Numerous investigations have uncovered the effect of growth factors, cytokines and other chemical signals on the behaviour of satellite cells (reviewed in Refs. [17] and [18]). However, only recently have the significance of biomechanical stimuli and extracellular matrix (ECM) components also been highlighted, as we will discuss in upcoming sections of this review.

As this review will speculate, mechanical forces likely have a notable impact on the behaviour of muscle stem cells, similar to what has been found in other tissues facing continual physical demands [19–21]. Indeed, the striking influence that tuning biophysical properties can have on the fate of known multipotent stem cells in culture [22–24] is paralleled in muscle stem cells. Skeletal muscle is one of the most energetic organs of the body, and the constant mechanical strains placed on myofibres subject satellite cells to a range of stiffness, stretch and shear forces. Migration from the basal lamina exposes satellite cells to a new ECM environment and contact with other cell types, both of which may be disrupted by age and disease to the detriment of regeneration. In order to understand the coordination of the healing process in muscle and to treat its dysfunction, we envision that a further understanding of how satellite cells behave during the upheaval of their microenvironment's biophysical characteristics is required.

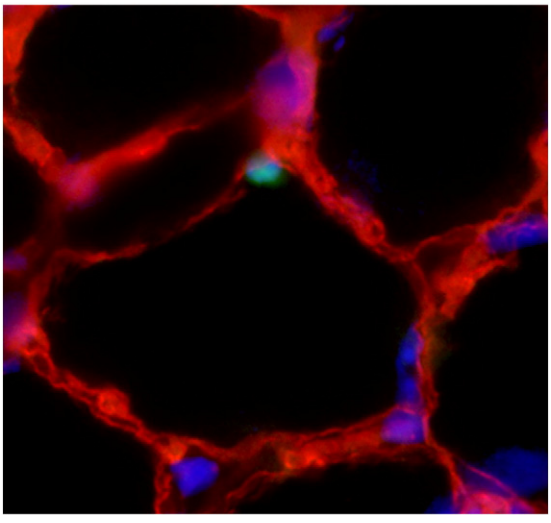

Fig. 1. The satellite cell niche. Mononucleate (blue) satellite cells (green) reside on top of muscle fibres and beneath a layer of basement membrane as indicated in this transverse histological section of a murine *tibialis anterior* muscle. Individual myofibres are ensheathed within and separated from one another by the endomysium, an ECM containing numerous proteins including collagen (red).

Substrate Stiffness

The stiffness of a surrounding substrate exerts direct influence on a cell when ECM components form complexes with integrins, which are linked to the cytoskeleton, creating focal adhesions that influence cell shape and motility (recently reviewed in Refs. [25] and [26])—broadly speaking, many cells respond to more rigid substrates by spreading their plasma membrane to cover a greater surface area. Furthermore, integrins have been implicated in the mechanotransduction of stiffness in various somatic and tumour cell lines by impacting on the phosphorylation of focal adhesion kinases (FAK), protein kinase B (also known as Akt) and extracellular signal-regulated kinases (ERK; Fig. 2) [27–29]. Other signalling cascades known to be sensitive to substrate stiffness, such as Yes-associated protein (YAP)/transcriptional coactivator with PDZ domain (TAZ) nuclear effectors in mesenchymal stromal cells (MSCs) [30,31] and myocardin-related transcription factor (MRTF) signalling in 3T3 fibroblasts [32], may be influenced by cytoskeletal polymerisation. Beyond defining cytoskeletal assembly, the stiffness of a growth substrate has been shown to direct breast tumour cell malignancy in culture and *in vivo* [29,33–35], MSC [22,23,31] and neural stem cell [24,36] differentiation, and it can be manipulated for embryonic stem cell culture maintenance *in vitro* [37,38]. Given the wide variation in stiffness between mammalian tissues [39], it is unsurprising that the stiffness of a cell's microenvironment is relevant to its functional behaviour—for example, stiffer substrates promote osteogenic differentiation in MSCs whilst a softer substrate promotes neuronal differentiation [23].

It is worth considering the biomechanical impact that the surrounding material may have on tissue function and regeneration and, in particular, the effect on the stem cell niche. With regard to skeletal muscle, waves of regeneration and degeneration and the striking changes that can occur during ageing and disease mean that the local environment is in flux and that cells may have to respond