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Roles and Regulation of Satellite Cells in Skeletal Muscle Regeneration

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By

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ABSTRACT

Skeletal muscle has an innate ability to self-regenerate in response to certain stimuli. In the case of trauma, muscle resident stem cells are required to meet the regenerative needs of the tissue. These resident stem cells, called satellite cells (SCs), are crucial in the regenerative process following injury; understanding the major factors which regulate satellite cell activity can provide valuable insight for regenerative medicine. The ability to implement and properly activate satellite cells has immense potential in the treatment of conditions including trauma, degenerative disorders, and age-related sarcopenia. This review will discuss the current understanding of satellite cell-mediated regeneration and the related cellular and molecular dynamics involved in regulation. Lastly, current research in this area of regenerative medicine and implications for future clinical applications will be explored.

I. Introduction

Skeletal muscle (SKM) tissue is one of the most abundant tissues in the human body, accounting for ~40% of body mass in the average adult (Zhang, Liang and Shan; Meiliana, Mustika Dewi and Wijaya). SKM is crucial for both basic motor functions and quality of life. Its most obvious role is providing movement including actions such as locomotion, posture, expression, and communication (Meliana *et al*). Even beyond this ostensible use, skeletal muscle tissue serves numerous physiological functions. A major example of this is that SKM is the predominant insulin-dependent utilizer of glucose in the human body. While not markedly high in relation to other body tissues the metabolic activity of skeletal muscle at rest is significant due to the large mass of SKM, and exercise can increase its energy (ATP) demands can increase up to nearly 50x from its resting rate (Hargreaves and Spriet). The crucial structural and physiological functions of SKM combined with its abundance in the body makes it a vital contributor to body homeostasis, including blood chemistry and thermal regulation.

The anatomy of skeletal muscle tissue is highly organized, with unique structural components that directly relate to its function. The most basic functional unit of an individual myofiber within skeletal muscle is a sarcomere which is composed of integrated actin and myosin filaments which create the characteristic observable striations characteristic of SKM fibers (*Figure 1*). Upon stimulation by motor neurons at neuromuscular junctions these filaments interact and ratchet against each other which shortens the length of the sarcomeres along the entire muscle in an ATP-dependent process. The specialized skeletal muscle cells, called myofibers or myofibers, are elongated and multinucleate due to the embryonic fusion of many precursor cells into a single myofiber. Myofibers can extend many centimeters along the length of a muscle, with a maximum length of around 12cm (Feher).

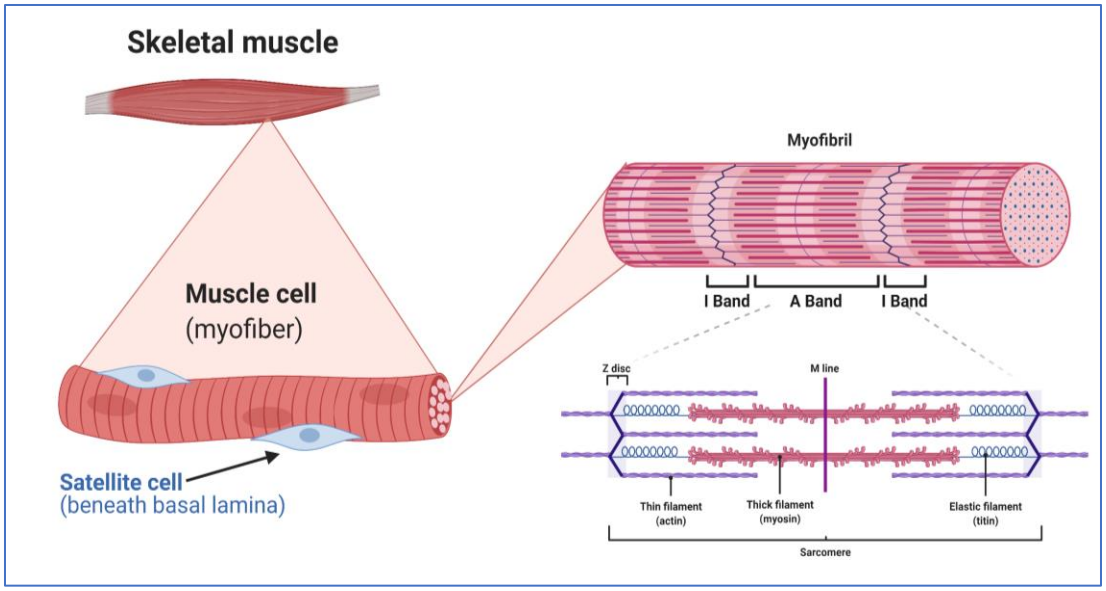

Figure 1: *Diagram of skeletal muscle organization and relative location of SCs. Created with Biorender.com.*

Myofibers themselves are embedded in an extracellular matrix, a three-dimensional mesh scaffolding that supports and organizes tissue components. In SKM, the organization provided by the ECM is critical for normal physiology. ECM components integrate skeletal muscles from their points of origin to insertion points by integrating into tendons which ultimately integrate into bone which SKM pulls upon to provide movement. The ECM is composed of collagenous components and various other macromolecules, such as elastin, glycoproteins, and proteoglycans, which provide tensile and compressive strength as well as bind and sequester a variety of molecular factors. The network of connected ECM meshes that surrounds and interweaves between the composite SKM components is generally depicted in three major layers: the endomysium, which surrounds individual myofibers, the perimysium, which surrounds bundles of myofibers, and the epimysium which envelops the entire muscle (Csapo, Gumpenberger & Wessner).

Being multinucleate, myofibers have a significant amount of plasticity and can undergo changes in fiber size and the expression of metabolic characteristics. The elevated ability to transcribe its tissue specific proteins gives SKM a great deal of regenerative ability in addition to its ability to grow, adapt and regenerate (Collins, Olsen and Zammit). The normal physiology of healthy, unstressed skeletal muscle includes constant self-regeneration in which the myofilaments and other structural proteins are regularly degraded and synthesized. The balance between degradation and muscle protein synthesis (MPS) is what determines if growth (hypertrophy), reduction (atrophy), or maintenance of muscle mass occurs. Under normal circumstances, skeletal muscle is a relatively stable tissue and regular MPS is achieved via the multiple nuclei of the fiber.

The rate of nuclear turnover is not constant and varies due to a variety of factors including muscle activity and fiber type. While there is a marked ability to self-regenerate in response to damage, its self-regenerative abilities are finite and MPS requirements can exceed the capacity of a myofiber's existing nuclei. When myofibers are damaged by acute injury or substantial overload and require protein synthesis beyond their inherent capacity, resident stem cells are activated to augment the regeneration. These cells are called satellite cells (SCs), due to their location on the periphery of muscle fibers beneath the basal lamina in the ECM, which is often described as the SC "niche." Activated satellite cells can donate their nuclei to an intact

myofiber or can provide new myogenic cells to replace tissue, including entire myofibers, lost due to injury. There is ongoing research into how much SC recruitment is involved in normal replacement outside of injury, but evidence indicates that nuclear accretion from SCs is a more extensive occurrence than previously thought (Bradley, Pulliam and Betta). The dynamics of injury-induced SC-mediated regeneration of skeletal muscle will be the primary focus of this review, as many of the key events and regulatory mechanisms following acute injury are echoed in other models of SKM regeneration and related to dysfunctions such as with age-related regenerative decline and chronic muscle diseases (Karalaki *et al*).

SCs are the major players in skeletal muscle regeneration (Charge & Rudnicki; Collins *et al*; Forcina, Miano & Pelosi; Fu, Wang & Hu; Järvinen *et al*). They are multipotent progenitor cells with other roles including the formation of skeletal muscle during embryonic development and augmentation of muscle protein synthesis in mature myofibers by nuclear accretion. In response to significant tissue damage, SCs proliferate, differentiate, and fuse to the site of injury to regenerate the affected region of muscle. There are a variety of physiological cues which modulate satellite cell activity including inflammatory responses, cell-matrix reactions, and numerous molecular signaling factors. This paper will discuss the established model of trauma-induced SC-mediated myogenesis in adult SKM using corroborated information from literature in the fields of physiology, cell biology, and molecular medicine. This review will discuss the most prominent and well understood dynamics involved in the regenerative process. Understanding the regulation and modulation of SCs in SKM regeneration not only aids the understanding of normal physiology but can provide valuable insight for the improving clinical treatments and developing future regenerative therapies. Applications of this knowledge have immense potential in treatment of acute injury, as well as clinical implications for the treatment of dystrophic disorders and age-related sarcopenia. Lastly, this work will identify unknowns and areas for future research in this realm of regenerative medicine.

II. Phases of Satellite Cell Mediated Regeneration

Recruitment of SCs in tissue regeneration is a complex process involving numerous synchronized cellular and molecular events. SCs in their peripheral niche reside in a state of quiescence but will re-enter the cell cycle upon activation; once activated, SCs follow a cell fate pathway of proliferation and differentiation to ultimately become mature myofibers, either by fusing with other nascent myofibers to replace a damaged myofiber or integrating into existing damaged myofibers to remodel a functional multinucleated fiber (Collins *et al*). Proliferation of SCs produces myogenic progeny, which may either return to the SC niche to populate the stem cell reserve or continue to differentiate into committed myoblasts. These progenitor cells terminally differentiate and fuse together and to the injury site to form a structure called a myotube which matures into a functional myofiber. Myotubes develop within and fuse to the injury site to replace the critically damaged region of a myofiber or can form an entirely new myofiber to reconstitute fully functional tissue. This integration into the myofibril repairs the damage at the injury site and contributes a new nucleus for future protein synthesis (Forcina *et al*).

Regeneration of skeletal muscle is described in three major phases: 1) destruction, 2) repair, and 3) remodeling (Karalaki *et al*; Järvinen *et al*). There is some overlap in the events of these phases, however the overall progression of regeneration is best understood with these events in sequential context. The destruction phase is initiated by the injury event, where damage to myofiber components and ECM catalyzes rapid degeneration at the site. This is propagated by various factors released upon ECM disruption and from the damaged myofiber, including various growth factors and sarcoplasmic contents. These damage-released factors

induce local responses as well as release of additional growth factors and cytokines from non-muscle tissues in a systemic response when serum levels of damaged muscle derived factors (DMDFs). In an acute injury, contraction bands form a “fire door” on the border of the injury shortly after the event, which halts the propagation of necrosis from continuing beyond the affected site. The injury induces a swift inflammatory reaction and induces the infiltration of circulating immunocytes, primarily neutrophils, monocytes, and macrophages, from local vasculature to degrade and phagocytize damaged tissue components. Immunocytes at the injury site also contribute regulatory cytokines and provoke further escalation of the pro-inflammatory milieu. These cellular debris remaining after the necrosis and degeneration are removed by phagocytic immune cells. Activated SCs migrate to the site of damage and undergo asymmetrical division, resulting in a self-renewed SC and a daughter cell which will become a committed myoblast. These nascent cells begin to differentiate and proliferate to provide more regenerative cells for repair of the damaged myofiber (Järvinen *et al*).

Following the destruction and inflammation is the repair phase which involves the activation of SCs and regulation of their myogenic progeny by secreted factors. These include various growth factors, cytokines and chemokines released upon the local damage, by infiltrating immune cells, and by SCs themselves; SC division occurs in response to shifting levels of extrinsic factors. Asymmetric division of SCs results in a self-renewal of the SC as well as the production of a myogenic daughter cell. This cell differentiates and commits to the myofiber cell fate as a myoblast, which will proliferate to expand the supply of myoblasts for regeneration of the injury (Järvinen *et al*; Kuang *et al* 2007, Chargé & Seale).

The last phase of regeneration involves terminal differentiation of myoblasts and their integration in the site to form functional myofibers. This final phase of remodeling involves the fusion of nascent myofibers to each other and to existing myofibers to reform functional tissue in the site of injury. The contribution of new nuclei as well as cytoplasmic contents completes the regenerative process and regular physiological functions of the muscle can continue (Bradley, Pulliam and Betta; Karalaki *et al*).

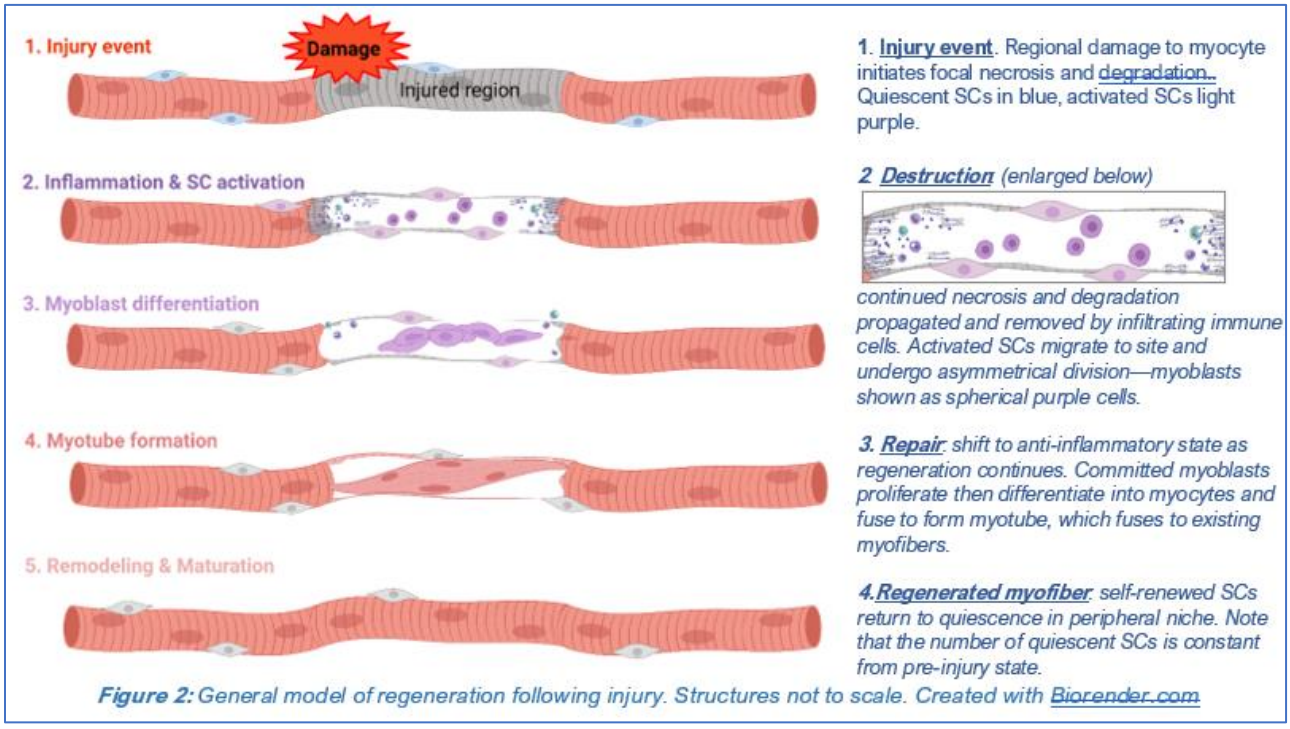

Figure 2: *General model of regeneration following injury. Structures not to scale. Created with Biorender.com*

III. Key Markers in Satellite Cell Activation and Myogenic Cell Fates

Satellite cells pass through a series of stages between their initial state of quiescence to their cell fate as a terminally differentiated and fused myofibers. As somatic cells, each contains the same full genome unique to the individual, but the characteristics of each stage of their cell cycle is determined by modulating which genes are expressed or inhibited. This progression is regulated by various transcription factors and post-transcriptional mechanisms. SCs in each stage of their life cycle are identified and characterized by the variable expression of molecular markers such as transcription factors, surface proteins, and receptors.

Proliferation and differentiation are regulated by a handful of key myogenic regulatory factors (MRFs), summarized in Table 1., and discussed in greater depth in the next section. The first MRFs expressed, myogenic factor 5 (*Myf5*) and myogenic determination factor (*MyoD*), initiate and propagate differentiation of early myoblasts by the upregulation of secondary MRFs *myogenin* and *MRF4*. *Myogenin* and *MRF4* induce terminal differentiation of myoblasts into myofibers, which express genes for myofiber proteins such as myosin heavy chain (MyHC), troponin, and muscle creatine kinase.

Table 1. Summary of Myogenic regulatory factors

Transcription Factor		Present in	Known Activities	Main References
Family				
Paired box transcription factors	*Pax7*	- Quiescent SCs - Activated SCs/Early myoblasts	Maintenance of quiescence and promotion of self-renewal.	Bentzinger *et al*; Buckingham & Relaix; Snijders *et al*; Z. Chao *et al*
	Pax3	- SCs in smooth muscle - Embryonic myoprogenitor cells	Regulates migration of muscle precursors from somite	Buckingham & Relaix; Karalaki *et al*
Myogenic Regulatory factors (MRFs)	*Myf5*	- SCs & proliferating myoblasts	Induces myoblast differentiation	Karalaki *et al*; Snijders *et al*
	MyoD	- Differentiating myoblasts	Myogenic commitment & progression towards terminal differentiation.	Karalaki *et al*; Snijders *et al*
	Myogenin	- Terminally differentiating myoblast - Early myofiber	Terminal differentiation of myoblasts	Bentzinger *et al*; Snijders *et al*
	MRF4	- Differentiating myofiber	Terminal differentiation: last MRF expressed in maturing myoblasts	(Snijders, Nederveen and McKay; Bentzinger, Wange and Dumont)

Pax7 Characterizes the Quiescent SC

The transcriptional regulation of satellite cell fates has been well characterized. The paired-box transcription factor *Pax7* is considered the master regulatory transcription factor of satellite cells (Lilija, Zhang and Magli). It is used as the canonical biomarker characterizing quiescent and early activated SCs in mature muscle (Segalés, Perdiguero and Muñoz-Cánoves; Schmidt, Schüler and Hüttner). While *Pax7* is highly expressed and is generally considered the most reliable marker, there is recent evidence indicating that the quiescent populations of SCs are heterogeneous, and *Pax7* is not present in all populations *in vivo* (Snijders, Nederveen and McKay).

The factor *Pax3* is an essential regulator of prenatal muscle development. In adult muscle *Pax3* can be found expressed in combination with *Pax7* in quiescent SCs, and is most abundantly expressed in the diaphragm and certain smooth muscles (Buckingham & Relaix; Segalés *et al*). *Pax3* is considered a second key regulator upstream of embryonic myogenesis and has been identified in rare interstitial myogenic cells in adult tissue, likely descending from embryonic cells of the mesodermal somite (Kuang *et al* 2006). While the post-natal function of *Pax3*+/*Pax7*- satellite cells in mammals remains to be definitively determined, they have been observed to proliferate upon injury and may contribute a small number of myoblasts in post-injury regeneration, potentially providing some redundancy in the essential function *Pax7* (Chargé and Rudnicki; Buckingham and Relaix).

Asymmetric Division Maintains the Reserve Satellite Cell Population

A unique ability of SCs, along with some other stem cells, is the ability to undergo asymmetrical division. This process produces heterogenous progeny, with one myogenic progenitor cell to contribute to regeneration and one "self-renewed" cell which returns to the reserve pool of quiescent stem cells for future needs (Karalaki *et al*; Kuang *et al* 2007). The myogenic daughter cell will upregulate *MyoD* and continue to commit to the myofiber cell fate, while the other will upregulate *Pax7,* downregulate *MyoD*, and return to quiescence (Bentzinger *et al*; Kuang *et al* 2007). Asymmetric division is regulated by a variety of factors, including extrinsic interactions with the ECM and PAR complex proteins (Feige, Brun and Ritso). The PAR complex has been identified through extensive research in *Drosophila melanogaster* and *Caenorhabditis elegans*, and remains an active area of study in other metazoan species in the context of asymmetric division of stem cells (Dewey, Taylor and Johnston). Ultimately, the asymmetric orientation in a dividing cell and subsequent asymmetric inheritance of PAR components contribute to differentiation of daughter cells by linking cell fate determinants with mitotic spindle orientation. The cytoplasmic polarity creates the heterogenous daughter cells by contributing different transcriptional factors, receptors and ligands to the daughter cells. Interactions with the extracellular matrix components are significantly involved in the regulation of SC polarity via PAR complex proteins. This asymmetry contrasts with pathways which lead to more equal inheritance among daughter cells, including the planar cell polarity pathway (Dewey *et al*).

Specific to the PAR-regulated asymmetrical division of activated SCs, the myogenic daughter cell has activation of mitogen-activated protein kinases (MAPKs) related to *MyoD* and *Myf5* upregulation for proliferation and differentiation (Dewey *et al*). Conversely, the cell primed for self-renewal remains $Pax7^{+}$ and $MyoD^{-}$, regulated through extracellular receptor kinase (ERK) signaling inhibitor Sprouty1 (*Spry1*) (Fu, Wang & Hu). *Spry1* has been shown to be crucial for maintenance of quiescence and for self-renewal following SC activation. It is robustly expressed in quiescent SCs and downregulated in proliferative myoblasts (Shea, Xiang and LaPorta). The $Spry1^{+}$ self-renewing daughter cell expresses higher levels of *Pax7* and other regulatory factors, and returns to the quiescent niche (Bentzinger, Wange and Dumont; Fu, Wang and Hu; Shea, Xiang and LaPorta)

Myoblast commitment and cell cycle progression occurs with upregulation of Myogenic Regulatory Factors

Along with *Pax7,* the MRFs *Myf5*, *MyoD*, *Myogenin* and *MRF4* are key biomarkers and modulators of myogenic differentiation (Relaix, Bencze and Borok; Schmidt, Schüler and Hüttner). The activation of SCs is induced at least in part downstream responses to the disruption of the SC niche. Activating stimuli include expression of activation protein-1 (AP-1)

members, MAPKs, and members of the activating transcription factor (ATF) family (Shaulian and Karin). The myogenic progeny of activated SCs show rapid upregulation of *MyoD* and/or *Myf5* following activation, which induce proliferation (Chargé and Rudnicki). At this point, the proliferative *MyoD*$^{+}$ and/or *Myf5*$^{+}$ myogenic cells are termed myoblasts. *MyoD* or *Myf5* expression is an absolute requirement for differentiation of myoblasts in regeneration, which has been demonstrated in studies with double knockout (*MyoD*$^{-}$/*Myf5*$^{-}$) cells failing to express myogenin or myosin heavy chain (MyHC) and instead adopting fibroblast-like morphology (Yamamoto, Legendre and Biswas). Myoblasts with *MyoD* and/or *Myf5* will continue to differentiate and upregulate *myogenin*, then *MRF4,* and commit to terminal differentiation to become committed myofibers. These nascent myofibers will fuse together into a myotube via expression of surface adhesion molecules then integrate into existing tissue infrastructure of adjacent myofibrils or ECM components. Ultimately these cells will express MyHC, and other characteristic proteins of mature myofibers and continue MPS as part of the functional mature muscle. (Karalaki, Fili and Philippou; Yamamoto, Legendre and Biswas).

Epigenetic considerations

There is a significant amount of recent and ongoing research into satellite cell modulation by epigenetic mechanisms beyond the normal regulatory epigenetic events that occur with transit through stages of differentiation (Massenet, Gardner & Chazaud). Causes of epigenetic changes are numerous and often difficult to predict but are known to occur by environmental factors or physiological variations which act directly or indirectly on one's genomic expressions. Genomic expression is altered by the upregulation or silencing of gene expression through interference with transcription factors, chromatin remodeling, or indirect alterations to post-translational control mechanisms. Specific to satellite cells, a large amount of literature exists discussing the effects of epigenetic influences on their differentiation and proliferation and how these would affect regeneration. While chromatin remodeling and temporal changes in transcription factor activity is a part of normal cell fate transit, such as in the modulation of regulatory proteins and heterochromatic reorganization involved with myogenic differentiation downstream of MRFs (Agarwal, Hardt and Brero; Massenet *et al*; Z. Chao, X.-L. Zheng and R.-P. Sun; Gurung & Parnaik). Epigenetic changes which may alter normal cell fates and can hinder satellite cell recruitment or reduce the capacity to self-renew (Dacwag, Bedford and Sif; Parker). These factors are important to recognize in considering individual variations in satellite cell activity, as well as age-related sarcopenia and dystrophic pathologies. Specific epigenetic mechanisms and variances in the normal regulatory pathways are an expanding field of research that may provide future insight into therapeutic interventions for a number of regenerative dysfunctions.

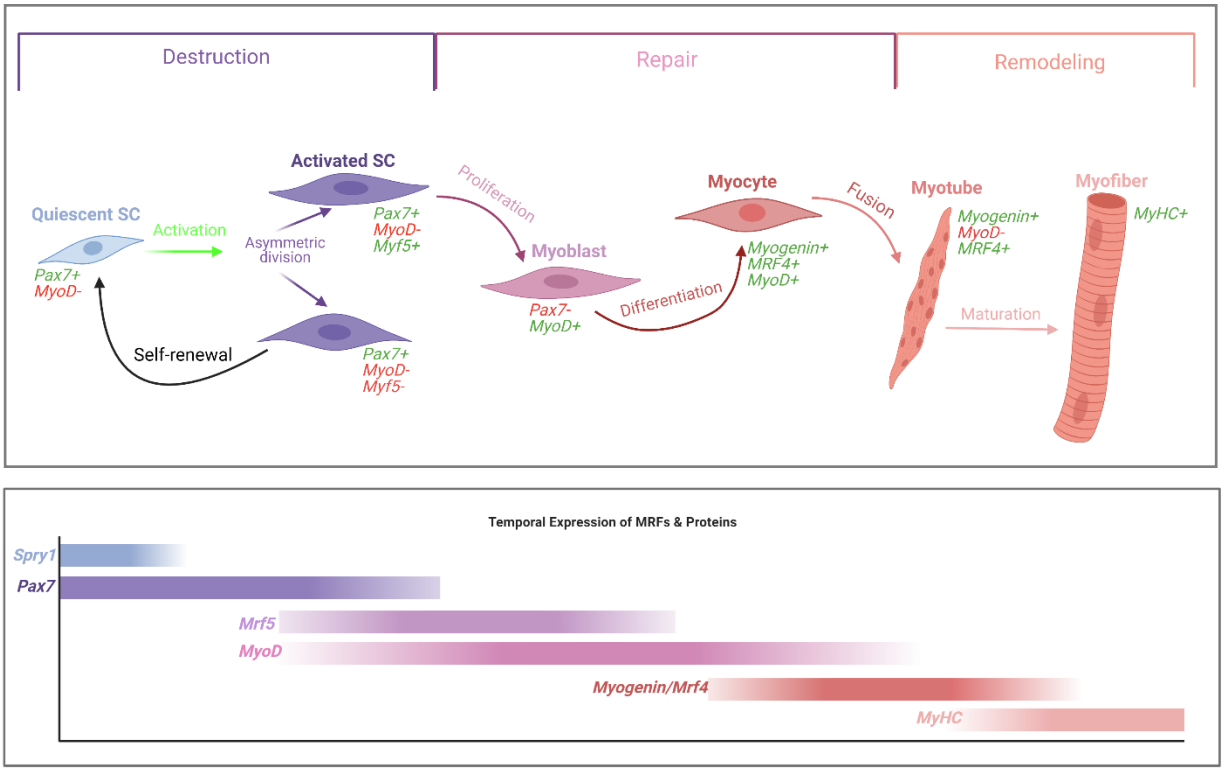

Figure 2.
Top: Phases of SC differentiation during regeneration with associated marker and protein expressions. Actively expressed factors in green, factors downregulated (absent or otherwise physiologically insignificant levels) in red.
Bottom: Temporal expression of MRFs from of SC activation to myoblast differentiation, fusion, and in resulting myofiber. Image created using Biorender.

IV. Inflammation & Immune cell dynamics

Acute perturbations of muscle tissue initiate a series of complex interactions with the immune system which contribute to the regulation of regeneration. The immune response in regeneration of mature muscle is a major difference in comparison to myogenesis during development, and the cytokines released contribute to the modulation of transcriptional activities of SCs and the subsequent myoprogenitor cells. The inflammatory phase is initially characterized by neutrophil invasion, followed by a sequential increase in other immunocytes including macrophages and monocytes (Filippin, Cuevas and Lima, The role of nitric oxide during healing of trauma to the skeletal muscle). Proinflammatory cytokines including interleukin-1 (IL-1) and tumor necrosis factor α (TNF-α) released in inflammatory responses by Th1 cells, neutrophils, and M1 macrophages propagate the tissue damage, which induce further release of NO (Filppin, Cuevas and Lima; Karalaki, Fili and Philippou; Tidball & Villalta). One way that proinflammatory cytokines contribute to tissue damage is by releasing reactive oxygen species (ROS) via activation of NADPH oxidase, which can damage lipids, proteins, and nucleic acids in macromolecular structures (Filippin, Cuevas and Lima). Major proinflammatory cytokines released by myeloid immunocytes in the early response include tumor necrosis factor (TNF-α) and interleukin-1 (IL-1). TNF-α is a known modulator of chemotaxis and has specifically been observed to inhibit terminal differentiation and muscle cell fusion while stimulating proliferation of early-stage myoblasts (Tidball).

The initial event upon injury to muscle tissue is a rapid degeneration of the damaged myofibers, which morphologically resembles necrosis (Ciciliot and Schiaffino; Chargé and Rudnicki). Serum levels of muscle proteins (e.g., creatine kinase, skeletal troponin, MHC) are subsequently elevated, inducing the complement cascade and the chemotactic recruitment of inflammatory cells to the site (Karalaki, Fili and Philippou, Muscle Regeneration: Cellular and Molecular Events; Ciciliot and Schiaffino). The role of immune cells is crucial to the first stages of regeneration, and the dynamics between different types promotes different phases of regeneration and SC cell fates. Initially following injury, resident mast cells and macrophages sense chemotactic molecules released by local damage, and secrete pro-inflammatory cytokines, such as IL-6, TNF-α, and histamines which promote activation and proliferation of SCs and recruit other immunocytes to the site (Bentzinger, Wange and Dumont).

The first inflammatory cells to infiltrate are phagocytes, predominantly neutrophils and macrophages, which enter from the blood stream and phagocytize necrotized components and debris (Tidball & Villalta). Neutrophils attracted by TNF-α and other components released by damaged tissue secrete chemokines which recruit supplemental immunocytes to the area (Bentzinger, Wange and Dumont) (Yang, Zhang and Yang). Cytokine secretions (e.g. IL-1 and IL-6) from neutrophils and resident macrophages stimulate the extravasation of monocytes to the injury site and their subsequent differentiation into additional macrophages, which are critical players in temporal and spatial events in the regenerative response (Howard, Pasiakos and Blesso; Lu, Huang and Saederup). Macrophages are polarized as M1 and M2 phenotypes, which can be considered pro- and anti- inflammatory, respectively. The M1 phenotype peaks initially within 1-2 days post-injury and contributes to the acute pro-inflammatory response with the secretion of cytokines including TNF-α, IL-1β, and IFN-γ, and ROS. These factors facilitate the removal of cellular debris and stimulate proliferation of myoblasts while suppressing differentiation in the early phases following injury.

As regenerative responses progress, M1 macrophages convert to the anti-inflammatory M2 phenotype which peaks by 3 days post injury to become the predominant phenotype at the injury site. The conversion to M2 macrophage dominance at the site shifts to an anti-inflammatory state which stimulates the differentiation of the pool of nascent myoblasts towards terminal differentiation and integration (Howard *et al*; Karalaki *et al*; Lu *et al*). M2 cells express high levels of anti-inflammatory molecules to inhibit further damage and growth factors to continue the regenerative process (Bentzinger *et al*; Howard *et al*). The transient shift in bias from a local pro-inflammatory to anti-inflammatory milieu by macrophage skewing is crucial for facilitating the regenerative response. Diminished, excessive, or chronically extended inflammatory responses attenuates proper myoblast recruitment and proliferation and is considered a major factor in age or disease-related impairment of SKM regeneration (Howard *et al*).

V. The Satellite Cell Niche and Interactions with the Extracellular Matrix

The extracellular matrix (ECM) is the acellular component present in every tissue, which provides physical infrastructure for the macro and micro constituents of the tissue as well as workspace for crucial physiological functions and signaling to occur. Without the ECM to support and connect the many cells that make up a tissue, cell-cell communication would be impossible, and processes of cell differentiation, tissue morphogenesis, and homeostasis could not occur. The histological environment that a cell resides in is commonly called its niche and includes both the structural ECM components as well as soluble materials in the surrounding interstitial space (Gillies and Lieber). In SKM, the ECM strongly contributes to muscle's normal

function and its ability to adapt and composes the biological reservoir for quiescent SCs. The importance of SC-niche interactions is indisputable, as satellite cells removed from their niche readily lose their stem cell characteristics (Bentzinger, Wang and von Maltzahn).

SCs quiescence is actively maintained and regulated through extrinsic interactions with acellular structures and soluble components. The integrity of the niche along with spatial and temporal cues based on factors such as composition, porosity and stiffness directly influence SC activity through focal adhesions. Similarly, the activation of SCs involves interactions with soluble growth factors from the niche along with circulatory supply of immunocytes and their secreted factors. After ECM disruption, the release of ECM-bound growth factors and downstream signals are detected, along with pro- and anti-inflammatory cytokines from infiltrating immunocytes. The remodeling phase of the regenerative process involves reforming the ECM elements for support of the regenerated tissue and accessory structures and return to physiological homeostasis.

As previously stated, quiescent SCs are peripherally located and surround myofibers. Specifically, they lie between the sarcolemma and the surrounding ECM, within a cleft beneath the basement membrane (BM), which forms a membrane-like sheath around myofibers. This subdivision of the BM is technically distinct from the endomysium, which generally surrounds myofibers, however for greater context it should be recognized that the two are intimately connected. The BM has two major layers: the basal lamina (BL) and the reticular lamina (RL) (Rayaggiro, Ranaldi and Raven; Thomas *et al*). Of these two layers, quiescent SCs are in the most direct contact with components of the BL. The BL is primarily composed of collagen type IV and laminin-2 (α2, β1, and γ1 chains) assembled in cross-linked networks which are connected by glycoproteins and proteoglycans. The BL integrates with the RL via proteoglycan connections between collagen in the BL and the RL. Resident fibroblasts in the BM are the major source of ECM components in skeletal muscle, contributing collagens, laminin and glycoproteins (Thomas *et al*). On the myofiber-facing side of the SC surface cadherins and glycoproteins interact with components of the sarcolemma (Nissar, Martowirogo and Gilbert). SCs in the quiescent niche directly interact with extracellular components via surface integrins, primarily isoforms α7 and β1, which form a receptor complex that binds laminin-2 in the BL (Thomas, Engler and Meyer). The physical stability of the SC in its niche is strengthened by BL binding to actin in the cytoskeleton of the muscle fiber by dystrophin complexes.

SC focal adhesions include cell adhesion molecules (CAMs) and surface attachment proteins. (e.g., M-cadherin). These surface molecules provide means of signal reception, including cell-cell interaction and cell-matrix interactions. NCAM (neural cell adhesion molecule), for example, is upregulated as myoblasts differentiate and is a likely facilitator of myoblast recognition and fusion for the formation of the myotube (Snijders *et al*). Connection of quiescent SCs and ECM-associated intracellular structures create force-sensing signals that relay the status of the tissue in regard to ECM integrity (Holle and Engler; Thomas *et al*). These external sensors are unevenly distributed on the quiescent SC surface, correlating with structural arrangement of the niche and orientation of extracellular cues.

A major role of the SC niche in regulating its physiology is the establishment of apical-basal polarity of quiescent cells (Feige *et al*). The basal and apical cell surfaces of quiescent SCs have different expressions of adhesion proteins. The basal surface has high levels of dystroglycan and integrins-α7 and -1β. On the apical surface adjacent to the sarcolemma, M-cadherin and NCAM are predominantly expressed. The polarized niche is the fundamental basis for establishing intracellular polarity, which is essential for asymmetric division involving the orientation of the mitotic spindle for the organization of PAR complex proteins and sequestration of cell fate determinants. Restriction of the PAR complex and PAR complex proteins to cell poles

is influenced by the binary localization of adhesion factors Significantly, adhesion to the dystroglycan complex which links to the BM of the immediate niche and establishes the basal pole of a dividing cell to determine the self-renewing daughter cell. Loss of contact to apical sarcolemma components or with basal dystrophin components, such as in cultured SCs *in vivo* results in loss or reversal of internal polar orientation (Feige *et al*).

The extracellular orientation provided by cell surface adhesion molecules also cues physiological changes for the stimulation of SC activation. A significant cue of structrual disruption is SC binding to the glycoprotein fibronectin, which is primarily expressed in the RL and generally absent in the BL. The binding of fibronetin to the SC co-receptor complex is a contributory factor which stimulates proliferation. Notably, SCs themselves express the fibronectin and transiently upregulate the expression upon activation in a manner of autologous feedback which further increases proliferation (Bentzinger *et al*)

In addition to providing structural integrity and spatial cues, ECM components include various soluble factors integrated within the network of macromolecules. Negatively charged proteoglycans (e.g., perlecan, decorin, small leucine rich proteoglycans (SLRPs)) distributed throughout the BM can bind and sequester a variety of growth factors and signaling molecules, which implements the role of the ECM as a source for local and systemic signal conduction (Thomas *et al*). Some of these factors are latent in the quiescent niche and are released upon perturbation to the tissue, either directly or through downstream activation.

In the context of acute injury with damage to the muscle fiber and sarcolemma, rapid dissolution of the sarcolemma occurs and further increases the permeability of the damaged myofiber. The major enzymes responsible for the catalysis of ECM degradation are matrix metalloproteinases (MMPs), which directly target the collagen IV and laminin of the BL and can indirectly activate proinflammatory cytokines and release pro-inflammatory factors bound to ECM (Quiding-Järbrink *et al*). MMPs are canonically produced by many cell types, particularly activated macrophages, lymphocytes and granulocytes. Recent *in vivo* data suggests that activated SCs express MMP isotypes which facilitate their migration from the quiescent niche to the site of injury and further increase the release of ECM bound factors (Thomas *et al*).

ECM disruption and increased permeability following MMP activity results in increased serum levels of muscle-specific components usually sequestered within the cytoplasm of a muscle fiber (Tidball & Villalta). These damaged myofiber-derived factors (DMDFs) such as muscle-specific proteins, enzymes, and mRNAs act as stimulatory cues for a variety of steps in the regenerative process. Several DMDFs have been implicated in the activation of SKM SCs, primarily metabolic enzymes and structural proteins. There is strong indication that the presence of SKM-abundant proteins and/or SKM-specific isoforms of metabolic enzymes into interstitial space acts as a physiological cue for regenerative cells, however the exact mechanisms are yet to be elucidated. Certain DMDFs, including adenylate kinase, creatine kinase, and triose phosphate isomerase, have shown significant effect on SC activation both *ex vivo* and *in vivo*. It has been proposed that these DMDFs also recruit inflammatory cells in the early phases of regeneration (Tsuchiya *et al*). While the mechanisms of activation are unclear, the data indicating direct induction by these DMDFs is statistically significant and warrants further research into their functional significance in the context of injury and degenerative disorders.

VI. Soluble Factors

Various soluble factors, including growth factors and cytokines, serve as molecular signals which provide distinct cues to their target cell to induce or inhibit various actions. The following factors discussed in detail are the most well researched and have extensive evidence supporting their roles in SKM regeneration and SC activity. The identification of other regulatory

factors in SKM regeneration and SC activity is an ongoing area of research, and includes some intriguing evidence for the influence of molecules such as bone morphogenic protein (BMF) (Stantzou, Schirwis and Swist) and hypoxia signaling factors (Xie, Yin and Nichenko), as well as factors involved in angiogenesis, neurotrophy, and neurogenesis which relate to development and regeneration of muscle tissue as a whole and its surrounding structures that may also influence myogenic activity (Ho, Chiang and Chuang) (Sugg, Korn and Sarver) (Gerli, Moyle and Benedetti) (Saini, Faroni and Reid)

Insulin-Like Growth Factor 1 (IGF-1)

The family of IGFs has a multitude of functions in mammalian cells. In skeletal muscle, IGF-1 is by far the most well-known and researched IGF, and it is clear the growth hormone-IGF axis plays a major role in muscle growth and development. IGF-1 is a circulating factor produced and released both by the liver as well as by local paracrine action of muscle cells and M2 macrophages (Lu *et al*; Mourkioti and Rosenthal). IGF-1 is known to induce muscle hypertrophy by promoting muscle protein synthesis and increasing myofiber size, as well as facilitating energy availability by increasing glucose uptake and activating enzymes for anaerobic glycolysis (Karalaki, Fili and Philippou).

In the context of muscle regeneration, IGF-1 positively stimulates most of the steps in myoblast proliferation and differentiation by upregulating the expression of intracellular mediators, such as mTORC1 and cyclin-D, and by inducing the expression of myogenin (Chargé and Rudnicki; Karalaki, Fili and Philippou; Mourkioti and Rosenthal). Likely through the combined interactions with other environmental and intracellular factors, IGF-1 induces different activity over the duration of the regenerative processes. In early stages, IGF-1 released from damaged muscle reduces myogenic factors and induces SC proliferation. Following proliferation IGF-1 expression is correlated with increased myogenin expression for differentiation of the nascent cells, and downregulation of cell cycle markers later. This shift is due to de-escalation of local inflammatory response by immunocyte release of anti-inflammatory cytokines after phagocytosis of damaged tissue components and cellular debris (Karalaki, Fili and Philippou).

The various actions of IGF-1 can be at least in part explained by the expression of its different isoforms and binding proteins (Mourkioti and Rosenthal). Multiple IGF-1 isoforms have been identified in skeletal muscle: IGF-1Ea, IGF-1Eb, and IGF-1Ec (Duan *et al*; Karalaki *et al*; Mourkioti and Rosenthal). These various isoforms are transiently related to different phases in regeneration and/or hypertrophy of SKM, and different binding proteins specific to each isotype are expressed along the process of differentiation. For example, IGF-1Ec, commonly known as mechano-growth factor, is released in response to muscle loading/training. IGF-1Ea is correlated to Mrf4 expression and increased muscle protein synthesis demands in late-phase regeneration. Ligand binding of IGF-binding proteins induces autophosphorylation and activates multiple signal transduction cascades. The notable induced pathways in skeletal muscle differentiation include P13k-Akt, Akt-mTOR, p38 MAPK, and Erk1/2 MAPK (Duan, Ren and Gaol Mourkioti and Rosenthal).

Hepatocyte Growth Factor (HGF)

HGF is a multifunctional cytokine that was first described as a mitogen in mature hepatocytes. It is produced in the spleen and liver cells and upregulated in response to muscle damage, as detected by elevated serum levels of SKM-specific components (Chazaud; Hawke and Garry; (Yamada, Tatsumi and Keitaro). HGF is one of the factors bound within the ECM of skeletal muscle, so is one of the first cues involved in SC activation and its release is proportional to the degree of muscle injury (Chazaud; Filippiin *et al*). In addition to its release from non-muscle

tissues in systemic response, HGF may additionally be brought to the site by granulocytes during the inflammatory response (Snijders, Nederveen and McKay). Further, HGF release from stretching stimulus has been found to be dependent on local NO production, either by a paired release from the ECM or by events downstream of NO-mediated ECM degradation. Both *in vitro* and *in vivo*, HGF transcription and signaling have been correlated with activation of quiescent SCs and NO synthesis, propagating the phases of regeneration (Chazaud; Filippin *et al* 2011).

HGF acts as a potent chemotactic factor involved in activating and selectively promoting satellite cell proliferation while inhibiting myoblast differentiation through the transcriptional silencing of *MyoD* and myogenin. It is believed to promote the exit from quiescence through the activation of p38 MAPK and P13K (phosphatidylinositol 3-kinase) signaling pathways, and the downregulation of caveolin-1 protein expression which has SC activating effects downstream (Karalaki, Fili and Philippou). Recent research has shown that HGF at low doses potentiates the ECM-mediated migration of myoblasts (González, de Mello and Butler-Browne). The concentration-dependent feedback of HGF correlates positively with SC activation and proliferation until a threshold is reached, then there is a negative feedback effect associated with the expression of myostatin, an inhibitor of SC activation and myoblast proliferation. This is thought to be a function for the halting of SC activation after the initial period of SC expansion to allow progression of the regenerative progress and to reestablish the quiescent pool of reserve SCs (Yamada *et al*). It has been postulated that HGF may stimulate the migration of SCs and myoblasts to the site of injury from surrounding regions, perhaps through the activation of the ERK pathway (Karalaki 2009).

Fibroblast Growth Factors (FGFs)

There are multiple different isoforms of FGFs currently identified in the body. While numerous paracrine FGFs are broadly expressed, FGF-6 is restricted to skeletal muscle (Hawke and Garry). The mRNA for four of the isoforms (FGF-1, -2, -4, and -6) are reliably detected in SCs and their expression has been shown to stimulate proliferation of cultured SCs (Hawke and Garry; Husman *et al*; Karalaki *et al*; Pawlikowski *et al*). FGFs in SKM are bound to proteohepharan sulfates in the ECM and released within the period of inflammation after tissue disruption (Husman *et al*). There is evidence that FGF the main action of FGFs is stimulation of myoblast proliferation, likely involved with regulation of *MyoD* expression. There is some data suggesting that FGF expression encourages proliferation through indirect routes involving the upregulation of certain IGF isoforms which have inhibitory effects on myoblast differentiation (Karalaki *et al*).

FGF-6 is produced by myofibers and is transiently upregulated in response to tissue disruption and inflammation (Armand, Laziz and Chanoine)Pawlikowski *et al*). FGF-6 expression is particularly elevated after SKM injury and has been observed to induce morphological changes to the ECM and alter SC adhesion. (Karalaki *et al*). Additionally, absence of FGF-6 has been shown to increase fibrosis and enable degradation of nascent myotubes after injury. Besides what is embedded in the ECM, FGF-2 is produced locally by myofibers and fibroblasts, and in an autocrine fashion by SCs themselves. FGF-2 is often reported as a SC mitogen, and its expression is correlated with the repression of myogenesis while SC proliferation occurs. Responsiveness to FGF-2 has also been correlated with SC capacity for self-renewal and cell fate progression in differentiating myoblasts (Bernet, Doles and Hall; Pawlikowski *et al*).

Outside the effects on SC activity, FGFs likely contribute to the regenerative process in tissue-specific ways involving the development of supportive components, the most significant being vascular structures and peripheral nerves. Several members of the FGF family are known agents in neuronal genesis and maintenance. Along with nerve growth factor, FGFs are more

prominently secreted by SCs and by neurons under physiological conditions; these GFs are indicated in maintaining the necessary microenvironment after trauma to facilitate axonal sprouting and elongation. FGF-2 in particular is a known neuroprotective agent closely involved with enhancement of neurite outgrowth and axonal regeneration. The neuroprotective qualities of FGFs is known to interact with various pathways including the MAPK/ERK signaling cascade (Cui). Lastly to note, regarding angiogenesis, FGF-2 and FGF-6 are soundly corroborated as potent angiogenic factors which contribute to neovascularization after injury (Murakami and Simons).

Transforming Growth Factor β (TGFβ)

TGF*β* is a multifunctional cytokine superfamily with diverse functions. In the context of SKM regeneration, subtypes of TGFβ (-1, -2, and -3) have similar and redundant activities *in vivo* in the context of SKM injury (Husmann *et al*) TGF*β* is a latent complex in a homeostatic state, stored in the quiescent ECM and not detectable in SKM until injury. It is released upon injury and degradation and is also one of the cytokines released by degranulating platelets at the injury site. In the context of injured muscle, TGF*β* generally inhibits myoblast proliferation and differentiation, but seems to not attenuate IGF-1 or FGF-mediated stimulation (Xu *et al*; Hawke and Garry). There is a positive autoregulatory effect wherein the presence of unbound TGF*β* stimulates further release from local cells; (Filippin *et al*). Outside of the effects on SCs and myoblasts, TGF functions as a chemoattractant for macrophages and other leukocytes, and induces the release of FGF, TNF, and IL-1 from monocytes, enhancing the inflammatory response. TGF*β* has also been observed to stimulate the production of platelet-derived growth factor, which would contribute to consequential stimulation of angiogenesis in the nascent tissue. TGF*β* mediates ECM remodeling by downstream inhibition of MMPs, attracting fibroblasts and stimulating the synthesis and deposition of ECM proteins. This is relevant both in a normal regenerative context and in the progression of fibrosis, a consequence of excessive ECM deposition by fibroblasts which high TGF*β* could induce. Recent evidence has also led to some postulation that TGF*β* may increase collagen deposition indirectly by inducing alternate differentiation of myogenic cells into fibroblasts (Filippin, Cuevas and Lima).

Nitrous Oxide (NO)

While many of the mechanisms are unclear, it is apparent that NO is involved in stimulating SKM regeneration and mediating fibrosis (Filippin, Cuevas and Lima, The role of nitric oxide during healing of trauma to the skeletal muscle). In muscle injury, NO release occurs as a result of acute matrix stretching stimulus or other more severe mechanical perturbations including shear stress and degradation, such as by MMPs (Filippin, Cuevas and Lima, Nitric oxide regulates the repair of injured skeletal muscle). The mechanisms of SC activation are likely related to effects on HGF release, inflammation and MMP activation. NO acts as a pro-inflammatory factor by activating cyclooxygenases and increasing prostaglandin production, which promotes inflammation and proteolysis of tissue structures.

Blocking early NO expression has been shown to increase collagen deposition and decrease SC activation and myoblast proliferation. NO inhibition also shows significant reduction in the acute inflammatory reaction following trauma, including reduced edema and immunocyte infiltration. This is likely contributed to by the vasodilatory effects of NO increasing circulation and perfusion in the injury site and allowing mobility of soluble factors and infiltration of non-resident cells. The pro-inflammatory oxidative stress induced by NO production in the early

phases of injury have been observed to skew trauma response away from production of fibrotic scar tissue and towards functional regeneration (Filippin *et al* 2011).

Interleukin 1 (IL-1)

IL-1 is a family of pro-inflammatory cytokines considered master regulators of innate immune responses including inflammation (Kaneko, Kurata and Yamamoto). There are two forms of IL-1, IL-1α and IL-1β are structurally similar, considered ultimately homologous in terms of biological functions, and expressed in a wide range of tissue and cell types. IL-1α is a characteristic danger signal that induces inflammation upon release from necrotic cells. The IL-1α precursor triggers expression of IL-1 receptors on resident macrophages, which in turn produce IL-1β along with other chemokines related to post-necrotic inflammation. IL-1β is most prominent in acute inflammation, secreted by pro-inflammatory monocytes and M1 macrophages. As with many other pro-inflammatory factors, IL-1β has an inhibitory effect on proliferation and suppresses mitogenic activity in myoblasts during the initial pro-inflammatory response. However, some data indicates that IL-1β has some inductive effect in the activation of MRFs at the commitment stage and positive proliferative effects through combined engagement with IL-6 at certain IL-6 receptors (Alvarez, DeOcesano-Pereira and Teixeira).

Interleukin 6 (IL-6)

IL-6 is another cytokine released by macrophages at muscle injury sites. It is normally a pro-inflammatory cytokine but has been shown to be anti-inflammatory in muscle tissue and have inhibitory effects on pro-inflammatory cytokines, such as TNF-α and IL-1 (Pedersen & Febbraio; Steensberg and Schjerling). IL-6 is secreted by infiltrating macrophages and neutrophiles, and myofibers themselves. It has become well-established that IL-6 is also produced by skeletal muscle, thus referred to as a “myokine” in that context (Pedersen & Febbraio; Snijders *et al*). Within target cells, including SCs, IL-6 reception induces the dimerization of tyrosine kinase receptor gp130. Downstream of this includes the activation of transcription 3 (STAT3), which activates the transcription of various genes. Additionally, gp130 dimerization activates phosphatidylinositol-3 kinase and members of the mitogen-activated protein kinase (MAPK) group. A mammalian MAPK, p38, positively regulates myoblast differentiation by upregulating *MRF4* (Raingeaud, Gupta and Rogers).

IL-6 receptor expression is upregulated in SCs following muscular damage, but seems to have a pleiotropic effect on SKM related to temporal release and local concentration. High concentrations of IL-6 may inhibit myogenic progression through disruption of intracellular GF signaling, resulting in compensatory IGF-1 production. IL-6 has paradoxically been observed in persistent inflammation and mediation of muscle wasting (Howard *et al*; Snijders *et al*). This current knowledge involving IL-6 mediation of muscle regeneration suggests a potent role in SC regulation, however there is evidence of divergent roles of IL-6 for both mediating SC activity and impairing muscle regeneration. Ongoing research into the concentration-dependent actions of IL-6 in inflammatory signaling will likely elucidate the mechanisms behind opposing roles of myogenic facilitation and persistent inflammation and muscle atrophy. While the role in persistent inflammation and promotion of muscle atrophy is not yet fully understood, the effects of IL-6 signaling in low concentrations of modulating SC proliferation and enhancing myoblast differentiation have been experimentally observed and replicated (Howard *et al*; (Serrano, Baeza-Raja and Perdoguero)Snijders *et al*) (Gao, Durstine and Koh)

VII . Current Research & Clinical Prospects

VIII. Conclusion

As the established major actors in skeletal muscle regeneration, satellite cells are an irrefutably important subject of research. The interactions satellite cells with their microenvironment, immune cells, and signaling molecules modulate their activation and the later regenerative activities of their myogenic progeny. Understanding the activation and regulation of satellite cells not only aids the understanding of normal physiology but provides valuable insight for the improvement of current clinical approaches. Ongoing research into the numerous regulatory dynamics of muscle regeneration will better the scientific understanding of regenerative dysfunctions and provide basis for the development of future treatments.

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