Journal of Cell Communication and Signaling (2020) 14:131–133
https://doi.org/10.1007/s12079-019-00545-3

COMMENTARY

Hic1 deletion unleashes quiescent connective tissue stem cells and impairs skeletal muscle regeneration

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Received: 11 December 2019 / Accepted: 13 December 2019 / Published online: 21 December 2019

Abstract

Skeletal muscle fibro-adipogenic progenitors (FAPs) are tissue-resident connective tissue cells and the main cellular source of pathological fibro-fatty scar associated with muscle disorders. Although our knowledge about skeletal muscle mesenchymal progenitor cells has exploded in the past decade, we still lack information about their origin, fate, gene regulation, function, and stemness. A recent study by Underhill and colleagues, published in *Cell Stem Cell*, described the last census of *Hic1* mesenchymal progenitor/stem cells in skeletal muscle regeneration, providing valuable results and data to the ever-expanding community of scientists interested in tissue regeneration and fibrosis. This commentary contextualizes and summarizes these exciting new findings.

Keywords Fibro-adipogenic progenitors · Fibroblasts · Wound healing · Fibrosis · Mesenchymal stromal cells · Mesenchymal progenitors · Quiescence

Connective tissue (CT) is composed of heterogeneous populations of stromal fibroblasts or mesenchymal progenitors (MPs) and extracellular matrix (ECM) (Nassari et al. 2017). The skeletal muscle milieu is highly complex in structure and function and different cell populations co-exist contributing to tissue homeostasis and regeneration (Wosczyna and Rando 2018). Recent evidence established that FAPs are required for skeletal muscle maintenance and regeneration (Wosczyna et al. 2019). However, the varied behavior of FAPs in acute and chronic muscle damage suggests that these mesenchymal progenitors can be a double-edge knife depending on the type and extension of the injury (Contreras et al. 2016; Fiore et al. 2016; Gonzalez et al. 2017; Joe et al. 2010; Madaro et al. 2018; Lemos et al. 2015; Natarajan et al. 2010; Uezumi et al. 2010; Uezumi et al. 2011). Despite the important roles for stromal progenitor cells in health and disease (Lemos and Duffield 2018), our understanding of their contribution to regenerating tissues has remained elusive and has been hampered by the lack of reliable markers for these cells. Furthermore, due to the lack of in vitro characterizations and consensus in fibroblast's nomenclature, mesenchymal progenitor/stem cells remain as a heterogeneous population of stromal progenitors with enormous potential to develop novel therapeutics for the treatment of several scar-forming pathologies.

To identify mesenchymal progenitor-specific cell markers, Scott et al. (2019) performed RNA-seq analysis of mononuclear muscle fractions. They focused on a Lin^- ($CD31^-CD45^-Ter119^-$) $LY6A/Sca1^+$ population (Joe et al. 2010). This Lin^- $Sca1^+$ fraction was enriched of MP-related transcripts, such as *Pdgfra* and *Gli1*, but not of pericyte- or tenocyte-related markers (*Rgs5*, *Pdgfrb*, *Mcam*, and *Cspg4/NG2* or *Scx*, *Mkx*, and *Tnmd*, respectively) (The Tabula Muris Consortium et al. 2018). Remarkably, they observed a particular enrichment of *Hypermethylated in cancer 1* (*Hic1*) transcript in the Lin^- $Sca1^+$ MP population. Hic1 is a protein-coding gene that functions as a transcriptional repressor and regulates growth (Scott et al. 2019). Also, hypermethylation or deletion of this gene have been associated with several malignant disorders or tumors (Chen et al. 2003; Fleuriel et al. 2009). Then, they confirmed the presence of perivascular $Hic1^+$-cells in the muscle interstitial space by performing immunofluorescence analyses of Hic1 protein and using a X-gal $Hic1^{nLacZ/+}$ knockin mice. Later, they studied the participation of $Hic1^+$ progenitors in muscle regeneration by employing a

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notexin (NTX)-induced skeletal muscle damage model. Intriguingly, the authors found changes in Hic1 expression after acute damage with NTX at day 4, where the proportion of $LacZ^+$ cells (Hic1-expressing) was smaller compared to NTX at day 14 or undamaged muscle. Therefore, the expression of Hic1 was high in growth-arrested MPs (with stem-like properties) but decreased after their transient activation at day 4. The frequency of X-gal$^+$ MPs returned to basal levels at day 14 post-injury, which correlates with the establishment of regenerated myofibers. Then, the authors explored the role of *Hic1* in MP quiescence through UBC-CreERT2-mediated *Hic1* deletion. Notably, the treatment of mice with tamoxifen causes Hic1$^+$ MPs to expand by >2-fold via increased proliferation in the tibialis anterior muscles from *Hic1* cKO when compared to controls. Transcriptome analysis of *Hic1*-deleted MPs confirmed their status of activation in which molecular programs associated with cell survival, proliferation, migration, and invasion were up-regulated when compared to Hic1-expressing cells. Conditional deletion of *Hic1* not only caused the capability of homeostatic and growth-arrested Hic1$^+$ MPs to exit quiescence but also impaired normal skeletal muscle regeneration.

Next, the authors generated and used *Hic1*^{CreERT2} knockin mice -breed to a conditional tdTomato reporter line- to characterize and fate map Hic1$^+$ MPs in muscle regeneration. Scott et al. performed single-cell (sc)RNA sequencing on over 2173 Lin$^-$tdTomato$^+$ individual cells from healthy mouse muscle. Computational approaches were then used to cluster Hic1$^+$ MPs into 4 groups or clusters, fibro-adipogenic (2 sub-clusters), tenogenic, and pericytic, according to the activities of their genes or transcriptomic signatures. The authors wanted to better understand the function of Hic1$^+$ MPs during muscle regeneration and to this end, they performed a time-course RNA-seq and scRNA-seq analyses. These transcriptomic approaches reveal that Hic1$^+$ progeny secrete cytokines and enter the cell cycle, then transiently differentiate into myofibroblasts, and therefore, secrete a provisional ECM, thus, providing a favorable niche or muscle milieu to foster regeneration. These results suggest that mesenchymal stem/stromal progenitors orchestrate a complex array of cellular and molecular processes during skeletal muscle regeneration. Further experiments by Scott et al. indicated that this new Hic1$^+$ type of cells may control the timing of the different aspects of skeletal muscle regeneration after damage. Taken together, using a combination of state-of-art experimental approaches (histological methods, FACS analyses, MPs bulk RNA-seq, Single-cell RNA-seq and ATAC-seq analysis of Hic1 $^+$ MPs), the team established that mesenchymal progenitors composed multiple subpopulations of stromal cells, which they further showed to have distinct functions and lineage progressions in skeletal muscle homeostasis and regeneration. This exciting study not only provides new tools for the community interested in stromal cells but also substantial evidence on MP fate, which, remarkably, is not well understood given the importance of fibrosis in pathology, disease, and aging. Hic1$^+$ connective tissue stem/stromal cells and their derivatives could be considered not only as new therapeutic targets for preventing muscle pathologies and fibrosis but also to improve regeneration and myotendinous junction repair.

The cell-surface tyrosine kinase receptor platelet-derived growth factor receptor α (PDGFRα) or CD140a is widely used as a mesenchymal progenitor, fibro-adipogenic progenitor, and connective tissue fibroblast marker in mice and human skeletal muscle (Contreras et al. 2019a; Contreras et al. 2019b; Murphy et al. 2011; The Tabula Muris Consortium et al., 2018; Uezumi et al. 2014). Although mesenchymal stromal/progenitor cells within skeletal muscle have been also identified using several markers including LY6A (Sca-1), CD34, Osr1, Osr2, and TCF7L2/TCF4, their overlapping expression identifies discrete subpopulations of CT progenitors within embryonic and adult muscle (Contreras et al. 2019a; Mathew et al. 2011; Stricker et al. 2012; Stumm et al. 2018; Vallecillo-García et al. 2018). Altogether, these studies and the work led by Scott and colleagues suggest that stromal mesenchymal progenitors, FAPs, CT fibroblast are a complex mixture of multipotent progenitors with plasticity in their molecular programs and functions during homeostasis and regeneration. Furthermore, their results suggest that stromal Hic1$^+$ cells are heterogeneous and dynamic in injured post-natal tissues where the myofibroblast populations can be distinguished by the repression of *Hic1*. Consistent with this, we and others have identified the repression of PDGFRα expression as a hallmark of MPs differentiation into scar-forming myofibroblasts in the injured skeletal muscles and cardiac tissue (Asli et al. 2018 preprint; Contreras et al. 2019b; Farbehi et al. 2019; Fu et al. 2018; Kanisicak et al. 2016). Therefore, upcoming research should focus on identifying and understanding the stage-specific and trophic triggers of stromal mesenchymal progenitors' activation.

Acknowledgments I am grateful to my friend Camilo Riquelme-Guzmán (Center for Regenerative Therapies Dresden (CRTD) - TU Dresden) for proofreading this manuscript. Beca de Doctorado Nacional 21140378 from CONICYT to O.C. supported this work. The funding agency had no role in the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript. I apologize to my colleagues and authors whose work could not be cited or was cited indirectly, due to space restrictions.

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