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Perivascular cell α_v integrins as a target to treat skeletal muscle fibrosis

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Abstract

Fibrosis following injury leads to aberrant regeneration and incomplete functional recovery of skeletal muscle, but the lack of detailed knowledge about the cellular and molecular mechanisms involved hampers the design of effective treatments. Using state-of-the-art technologies, Murray et al. (2017) found that perivascular PDGFR β -expressing cells generate fibrotic cells in the skeletal muscle. Strikingly, genetic deletion of α_v integrins from perivascular PDGFR β -expressing cells significantly inhibited skeletal muscle fibrosis without affecting muscle vascularization or regeneration. In addition, the authors showed that a small molecule inhibitor of α_v integrins, CWHM 12, attenuates skeletal muscle fibrosis. From a drug-development perspective, this study identifies a new cellular and molecular target to treat skeletal muscle fibrosis.

Keywords

perivascular cells; skeletal muscle; fibrosis; integrins; PDGFR β

Deposition of connective tissue is beneficial for repair in the short-term (Eming et al., 2014). However, over a prolonged period, fibrosis, characterized by excessive deposition of extracellular matrix constituents, becomes detrimental (O'Reilly, 2017). Associated with most chronic diseases and postnatal healing processes, it is an increasing cause of morbidity and mortality worldwide, responsible for approximately 45 percent of mortality in the

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DISCLOSURES

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United States (Thannickal et al., 2014), (Gurtner et al., 2008). It may be triggered in response to various insults, including chronic inflammation, tissue injury, and autoimmune reactions. It occurs in a wide range of organs and may affect their architecture, becoming irreversible over time, and leading to organ failure (Affo et al., 2017; Birbrair et al., 2014a; Birbrair et al., 2013d; Martinez et al., 2017; Munoz-Felix et al., 2015; Nishiga et al., 2017; Rockey et al., 2015). Although reducing fibrosis would probably protect organs, treatment options are very limited.

The pathophysiological etiology of fibrosis in skeletal muscle is less understood than in other organs (Birbrair et al., 2014b). Fibrosis interferes with skeletal muscle regeneration (Huard et al., 2002); alters the skeletal muscle microenvironment to increase susceptibility to re-injury (Birbrair, 2017; Carlson, 1986; Huard et al., 2002; Mu et al., 2010); and impairs function (Lieber and Ward, 2013). It causes devastating clinical problems; for example, when retracted rotator cuff skeletal muscles separate from the bone (Zumstein et al., 2008) or contractures permanently fix joints in a position that requires surgical relief (Lieber and Friden, 2002). Fibrosis is characterized by mechanical stiffness of muscle fiber bundles and increased collagen accumulation. Understanding the origin and processes that drive fibrous tissue formation is a central question in skeletal muscle biology.

Myofibroblasts regulate tissue fibrosis by producing several extracellular matrix proteins (Wynn, 2008). Understanding which cells generate them may allow us to gain control of or even reverse fibrosis in pathologic conditions (Friedman et al., 2013), and recent studies in various organs have focused on them to accelerate the design of targeted anti-fibrotic treatments. So far, many cell populations have been implicated, including circulating progenitor cells (Scholten et al., 2011), endothelial cells (Zeisberg et al., 2007), resident fibroblasts (Barnes and Glass, 2011), epithelial cells (Kim, K.K. et al., 2006), and pericytes (Birbrair et al., 2015). The contribution of each cell type varies among organs (LeBleu et al., 2013). Other studies have elucidated the cellular complexity of the skeletal muscle microenvironment (Birbrair et al., 2014b). Nonetheless, the particular cells and underlying cellular and molecular processes directly responsible for skeletal muscle fibrosis remain unknown.

Studies in several organs have pointed to the plasticity of perivascular cells, which allows them to differentiate into other cell types (Birbrair et al., 2017b; Birbrair et al., 2017c), including extracellular matrix-forming cells (Birbrair et al., 2014a; Birbrair et al., 2013d, 2014b, 2015). Murray and colleagues' recent article in *Nature Communications* identifies α_v integrins on perivascular PDGFR β -expressing cells as a therapeutic target for skeletal muscle fibrosis (Murray et al., 2017). They used state-of-the-art techniques, including *in vivo* lineage-tracing, confocal microscopy, sophisticated Cre/loxP technologies, and *in vivo* pharmacological blockade, to determine the role of these cells in skeletal muscle fibrosis formation. PDGFR β -expressing cells are perivascular and located in close proximity to CD31+ muscular endothelial cells. Using PDGFR β -Cre/mTmG mice, the authors labeled both quiescent PDGFR β + cells and activated myofibroblasts and found that the perivascular PDGFR β -expressing cells originated fibrotic cells in the skeletal muscle (Murray et al., 2017). Strikingly, genetic deletion of α_v integrins specifically from the perivascular PDGFR β -expressing cells significantly inhibited skeletal muscle fibrosis without affecting

muscle vascularization or regeneration. The authors also showed that a small molecule inhibitor of α_v integrins, CWHM 12, attenuates skeletal muscle fibrosis even when administered after fibrosis is established. Moreover, α_v integrins are expressed and targetable in PDGFR β -expressing cells from human skeletal muscle (Murray et al., 2017). This study will inform direly needed new clinical therapies.

Below, we discuss this work's findings in the context of recent advances in our understanding of the role of perivascular cells in the skeletal muscle microenvironment and fibrosis generation.

PERSPECTIVES / FUTURE DIRECTIONS

The molecular functions of specific genes seem to depend on the cellular population that expresses them. Restricting gene manipulation to specific cells in the skeletal muscle has clarified the role of key proteins in physiological and pathological states and offers a very powerful tool. The main findings from the Murray study are based on data obtained using PDGFR β -Cre/ α_v integrin-floxed mice (Murray et al., 2017). Although the authors show that these cells are perivascular in the skeletal muscle, their identity remains unknown. PDGFR β is expressed in many cellular lineages throughout the embryo during development, so PDGFR β -Cre transgenic mice are not the best model for lineage tracing; many cell populations are probably being labeled at the same time (Birbrair et al., 2017a; Guimaraes-Camboa et al., 2017). Since PDGFR β expression is more restricted in adult animals, using PDGFR β -CreERT2 mice would be more suitable (Gerl et al., 2015). This model would allow genetic elimination of the α_v integrins even after the fibrotic disease is established and define the role of α_v integrins on perivascular PDGFR β -expressing cells during muscular fibrosis.

In addition, PDGFR β expression, even in adult animals, is not restricted to a single perivascular cell type. Several stromal cells, such as pericytes (Birbrair et al., 2013c; Costa et al., 2018), vascular smooth muscle cells (Lindhahl et al., 1997; Winkler et al., 2010), and fibroblasts (Ohlund et al., 2017) express this cell-surface tyrosine kinase receptor (Armulik et al., 2011). The contribution of these distinct cell populations to fibrous tissue deposition in the skeletal muscle remains unknown. The problem's complexity is increased by the fact that subpopulations of these cell subsets with distinct functions are found in the skeletal muscle. We have identified two pericyte subpopulations based on nestin-GFP expression in muscle blood vessels: type 1 (nestin-GFP-/NG2-DsRed+) and type 2 (nestin-GFP+/NG2-DsRed+) (Birbrair et al., 2011; Birbrair et al., 2014c). Although both express PDGFR β , only type-1 pericytes have the fibrogenic capacity (Birbrair et al., 2013d). Future studies should specifically block α_v integrins in type-1 pericytes.

Besides perivascular PDGFR β -expressing cells, several other skeletal muscle cells have been proposed as the source of collagen production, including fibrocytes (Herzog and Bucala, 2010), resident fibroblasts (Lieber and Ward, 2013), satellite cells (Alexakis et al., 2007), endothelial cells (Zeisberg et al., 2007), nerve-associated cells (Hinz et al., 2012), muscle-derived stem cells (Li and Huard, 2002; Silva et al., 2018), and fibroadipogenic progenitors (Uezumi et al., 2011). Their exact physiological roles in skeletal muscle fibrosis

remain unclear. Several of these studies were at least partially *in vitro*, and cell preparation and grafting may have modified properties that influence their fate *in vivo*.

An elegant fate-mapping study revealed that during development, perivascular ADAM12-expressing cells give rise to most of the cells that produce collagen in response to skeletal muscle injury (Dulauroy et al., 2012). Another recent study used genetic lineage tracing analysis to show that perivascular tissue-resident Gli1+ cells are key contributors to injury-induced organ fibrosis (Kramann et al., 2015; Sena et al., 2017a; Sena et al., 2017b). Future studies are needed to determine the overlap between PDGFR β + and PDGFR α -, ADAM12-, and Gli1-expressing cells and their relative contributions to fibrous tissue deposition in skeletal muscle.

Although collagen I is often cited as the primary extracellular matrix protein expressed by fibroblastic cells in skeletal muscle fibrosis (Gillies and Lieber, 2011), fibrotic cells produce myriad other extracellular matrix proteins, such as collagen types III, IV, V, and VI (Zhang et al., 1994), as well as glycoproteins and proteoglycans, such as fibronectin, laminin, and tenascin (Berndt et al., 1994; Hinz, 2007; Magro et al., 1997; Mahida et al., 1997). Perivascular cells are not the only source of all these proteins since resident fibroblasts, inflammatory, and endothelial cells may produce these proteins as well (Azevedo et al., 2017a; Paiva et al., 2017). Production of these various proteins in the skeletal muscle may increase with the specific disease state. Future studies should determine the specific components of the extracellular matrix and their cellular source and clarify the relative contributions of PDGFR β -, ADAM 12-, and Gli1-expressing cells to fibrotic, type I collagen, and any other skeletal muscle cells that produce extracellular matrix components.

Up to now, all studies tracking fibrotic cell formation have analyzed it after skeletal muscle injury. The cellular source of fibrosis during aging and in chronic diseases, such as Duchenne muscular dystrophy, remains unknown, impeding the development of effective therapies to repair skeletal muscle. Detailed fate-mapping and lineage-tracing experiments in dystrophic and aged mouse models will advance the field significantly.

Murray and colleagues analyzed the role of α_v integrins in PDGFR β -expressing skeletal muscle cells (Murray et al., 2017). However, α_v integrins are widely expressed in a variety of cell types in various tissues and are essential to blood vessel formation (Bader et al., 1998). As PDGFR β -expressing cells are also widely distributed, in PDGFR β -Cre mice, recombinase expression is not limited to skeletal muscle perivascular cells; PDGFR β + cells in other organs are marked as well. Thus, in PDGFR β -Cre/ α_v integrin-floxed mice, α_v integrin deletion is not restricted to skeletal muscle perivascular PDGFR β -expressing cells. Future studies should evaluate the contribution of cells expressing PDGFR β outside the skeletal muscle to fibrous tissue accumulation.

The focus on perivascular PDGFR β -expressing cells has increased with the advent of modern technologies, such as transgenic mouse models and confocal microscopy, and we have greater insight into their varying, sometimes unexpected, physiological and pathological functions. In addition to physical stabilization of the vasculature, these cells participate in the vascular development, maturation, and remodeling. They also regulate

vascular permeability and blood flow (Almeida et al., 2017; Enge et al., 2002; Hellstrom et al., 2001; Leveen et al., 1994; Lindahl et al., 1997; Pallone and Silldorff, 2001; Pallone et al., 1998; Pallone et al., 2003; Soriano, 1994) and may affect blood coagulation (Bouchard et al., 1997; Fisher, 2009; Kim, J.A. et al., 2006). In the central nervous system, they collaborate with astrocytes to regulate the functional integrity of the blood–brain barrier (Al Ahmad et al., 2011; Armulik et al., 2010; Bell et al., 2010; Cuevas et al., 1984; Daneman et al., 2010; Dohgu et al., 2005; Kamouchi et al., 2011; Krueger and Bechmann, 2010; Nakagawa et al., 2007; Nakamura et al., 2008; Santos et al., 2017; Shimizu et al., 2008; Thanabalasundaram et al., 2011). Recent studies showed they can function as stem cells (Almeida et al., 2017; Andreotti et al., 2018b; Birbrair and Delbono, 2015; Birbrair et al., 2013a, b; Dias Moura Prazeres et al., 2017; Prazeres et al., 2017), generating other cell types (Coatti et al., 2017), and regulate the function of other stem cells (Andreotti et al., 2017; Asada et al., 2017; Azevedo et al., 2017b; Birbrair and Frenette, 2016; Borges et al., 2017; Guerra et al., 2017; Khan et al., 2016; Lousado et al., 2017). Note that they have some immune functions, regulating lymphocyte activation (Andreotti et al., 2018a; Balabanov et al., 1999; Fabry et al., 1993; Tu et al., 2011; Verbeek et al., 1995), contributing to the clearance of toxic cellular byproducts, attracting innate leukocytes that exit through the sprouting vessels (Stark et al., 2013), and immunosuppressive mechanisms (Sena et al., 2018). What role α_v integrins play in the many functions of perivascular PDGFR β -expressing cells is unknown. Future studies should explore whether blocking them in these cells affects functions other than tissue fibrosis.

Integrins are transmembrane surface proteins that link the extracellular matrix with the cytoskeleton (Hynes, 2004). Considerable research indicates their pivotal roles and wide involvement in physiological and pathological processes (Ley et al., 2016). Integrins function as heterodimers, with α and β subunits (Lowell and Mayadas, 2012). Future studies should investigate which β integrins known to be associated with α_v integrins are essential for skeletal muscle fibrosis. Although fibrous tissue formation and deposition are distinct for each organ physiopathogenesis seems to be similar. In 2013, using the same mouse model, Henderson and colleagues showed that depleting α_v integrins inhibits fibrosis in the liver, lung, and kidney (Henderson et al., 2013). Future studies should distinguish α_v integrins' role in skeletal muscle fibrosis.

CWHM 12, a small molecule inhibitor of α_v integrins, decreases fibrosis development in the liver and lung (Henderson et al., 2013). Murray and colleagues propose its potential use as a therapy for skeletal muscle fibrosis. Before pursuing this avenue, possible side effects should be considered, as mice deficient in α_v integrins evidence dramatic vascular disarray (Bader et al., 1998). For instance, several cell types that express α_v integrins have been targeted for tumor growth prevention strategies using peptide inhibitors to block neovascularization (Desgrosellier and Cheresh, 2010; Paiva et al., 2018). Integrins also play important roles in normal wound healing and scarring processes (Koivisto et al., 2014; Silva et al., 2018). Unsuccessful attempts to use integrin blockers include the use of the humanized, α_4 integrin, monoclonal blocking antibody, natalizumab, for Crohn's disease treatment, which increased risk of progressive multifocal leukoencephalopathy (Kleinschmidt-DeMasters and Tyler, 2005; Langer-Gould et al., 2005; Van Assche et al., 2005). Long-term toxicity studies will be needed before CWHM 12 can be used in humans, and its efficacy in models other than

mice should be determined. A phase I study designed to examine the safety of a single dose of the integrin α_v antagonist GSK3008348 in healthy volunteers began recently and, if successful, will be tested in patients with pulmonary fibrosis (NCT02612051). CWHM 12 was tested only in chemical- or injury-induced fibrosis models; will it be effective in treating the fibrosis associated with aging and chronic diseases?

In conclusion, the study by Murray and colleagues reveals a novel and important role for perivascular cell α_v integrins in skeletal muscle fibrosis. However, our understanding of perivascular cell biology in fibrosis is still limited, and future work must elucidate the complexity and interactions of various cellular components and molecules in the skeletal muscle microenvironment during disease progression.

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Figure 1. α_v integrins on PDGFR β ⁺ cells in the skeletal muscle support injury-induced fibrosis. Perivascular PDGFR β -expressing cells are associated with skeletal muscle blood vessels. Murray and colleagues discovered that they produce fibrous tissue after skeletal muscle injury (Murray et al., 2017). Further, specific genetic ablation of α_v integrins from these cells inhibits skeletal muscle fibrosis. With state-of-the-art technologies, future studies will reveal in detail the cellular and molecular components of the skeletal muscle fibrotic microenvironment.