

The Role of Fibro-Adipogenic Progenitors in Musculoskeletal Disease

G. Kaur^{1,2,3}, M. R. Davies^{1,2}, X. Liu^{1,2}, B. T. Feeley^{1,2}

¹ Department of Veterans Affairs, San Francisco Veterans Affairs Health Care System, San Francisco, California, U.S.A.

² Department of Orthopedic Surgery, University of California, San Francisco, California, U.S.A.

³ School of Medicine, University of California, San Francisco, California, U.S.A.

CORRESPONDING AUTHOR:

Department of Veterans Affairs
San Francisco Veterans Affairs Health Care
System
1700 Owens Street
San Francisco, (CA), U.S.A.
E-mail: gurbani.kaur@ucsf.edu

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SUMMARY

Skeletal muscle consists of heterogeneous cell populations that work in concert to support musculoskeletal homeostasis and regeneration in the context of acute injury and chronic disease. In this past decade, the recently discovered sub-population of muscle resident stem cells known as fibro-adipogenic progenitors (FAPs) have been implicated in the pathogenesis and regenerative response to a number of musculoskeletal conditions ranging from acute muscle injury to neurodegenerative disease, muscular dystrophy, rotator cuff tears, sarcopenia and heterotopic ossification. The purpose of this literature review is to synthesize a complete collection of basic, translational, and clinical science evidence implicating FAPs in the pathogenesis and regenerative response to acute and chronic musculoskeletal diseases. This study is an extensive literature review of papers recently published on FAPs in the development of acute and chronic musculoskeletal degeneration, and their potential role in therapeutic regeneration of these injuries. In this review, we highlight the multipotency of FAPs on a molecular level and review their role in the pathogenesis of acute, chronic, and degenerative musculoskeletal disorders with implications for the development of future novel therapeutic interventions.

KEY WORDS

Acute muscle injury; amyotrophic lateral sclerosis; cardiac fibrosis; chronic kidney disease; chronic obstructive pulmonary disease; fibro-adipogenic progenitors; heterotopic ossification; muscular dystrophy; neurodegenerative disease; obesity; rotator cuff tear; sarcopenia; spinal muscle atrophy.

INTRODUCTION

Fibrofatty degeneration of skeletal muscle is common to many disease and injury processes, but as yet lacks an effective treatment. Over the past decade, advances have been made in uncovering cellular and molecular processes that play a role in the degeneration of muscle. Fibro-adipogenic progenitors (FAPs) are a subpopulation of resident skeletal muscle stem cells arising from a distinct developmental lineage which have since been shown to play an important role in response to both acute injury and chronic musculoskeletal disease. Joe *et al.* (2010) defined this population as $\text{lin}^{-}/\text{a7}/\text{Sca1}^{+}$ cells which were also uniformly positive for

the surface marker PDGFR α (1). Uezumi *et al.* (2010) likewise noted ubiquitous PDGFR α expression of this population of mesenchymal progenitors (2). In healthy conditions in adult skeletal muscle *in vivo*, FAPs primarily localize to the interstitial space of skeletal muscle and are quiescent (1). Natarajan *et al.* (2010) summarized early findings that in the setting of an acute skeletal muscle injury, FAPs proliferate in the interstitial space between myofibers and produce trophic factors aiding in myofiber regeneration (3). However, when muscle fiber regeneration is impaired, FAPs continue to proliferate within injured muscle tissue and differentiate into adipocytes and myofibroblasts, leading to worsening muscle fatty degeneration and fibrosis. It

has therefore been recognized from early on that the skeletal muscle 'niche' in which FAPs are residing and proliferating is critical in determining their role in muscle degeneration versus regeneration.

In this review, we present a summary of the role of FAPs in a wide range of musculoskeletal disease processes in an effort to better understand the complex, clinically relevant role of these cells in muscle degeneration and regeneration.

We affirm that this literature review was synthesized in ethically in compliance with the international standards and required by Muscle, Ligaments, and Tendons Journal (4).

Acute Muscle Injury

FAPs were first recognized for the role that they play in response to a direct injury to muscle fibers. It was shown that a notexin (myotoxic venom) acute muscle injury in healthy mice results in rapid proliferation of FAPs in the injured muscle before a return to pre-damage levels between 4-5 days after injury (1). Similarly, in an acute injury setting created with either cardiotoxin (CTX) or glycerol, it was demonstrated that glycerol but not CTX led to adipogenic differentiation of FAPs (2). FAPs transplanted from the glycerol-injured muscle did not differentiate into adipocytes when transplanted into the CTX-injured muscle, while FAPs from the CTX-injured muscle underwent adipogenic differentiation when transplanted into glycerol-injured muscle (2). This highlights the importance of the local injury environment on FAP differentiation and suggests that adipogenic differentiation of FAPs primarily occurs when myogenic progenitor (MP) myofiber regeneration is impaired, as was seen with glycerol-mediated injury (2). It was further shown that hematopoietic cells partially mediate clearance of FAPs from muscle following a direct injury with NTX, and that this response is impaired in CCR2 knockout mice, in which bloodborne monocytes are unable to invade the parenchyma of the damaged muscle tissue following direct injury (5). This contraction of the FAP population that occurs several days after an acute muscle injury is thought to be largely mediated by TNF- α secreted by CD68⁺ macrophages, which leads to rapid apoptosis of FAPs following acute injury (5).

Neurodegenerative diseases: ALS and SMA

While FAPs may participate in a regenerative role in acute muscle injuries, their role in chronic, degenerative skeletal muscle diseases appears to be less beneficial. Here we will review the role of FAPs in the pathogenesis of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), specifically in the disease characteristic wasting and scarring of muscle tissues (6-8).

In ALS and spinal muscular atrophy (SMA), loss of the neuromuscular junction (NMJ) precedes progressive myofiber atrophy. FAPs play a key role in this progression from denervation to progressive myofiber atrophy (6-8).

In mouse models of mild acute muscle injury induced by cardiotoxin injection, FAPs were recruited to damaged muscle tissues and cleared between five to seven days, correlating with complete regeneration (6, 7, 9). In contrast, denervation by sciatic nerve transection, resulted in FAPs recruitment and retention at the injury site without concomitant macrophage infiltration or satellite cell-mediated regeneration (6, 7, 9). Denervation persistently activates STAT3 signaling in FAPs and simultaneously promotes their secretion of IL-6 through reciprocal positive feedback, promoting muscle atrophy and fibrosis (7). This muscle atrophy and fibrosis mediated by aberrant STAT3-IL-6 signaling in FAPs was also observed in murine models of spinal cord injury, SMA, and ALS and as well as in muscle samples from ALS patients (7). Thus, FAPs may represent a target for treatment of neuromuscular diseases. For example, antibody-mediated inhibition of FAP-specific STAT3-IL6 signaling prevents muscle atrophy and fibrosis in murine models of acute denervation and ALS.

FAPs also generate muscle fibrosis in these diseases by increasing extracellular matrix (ECM) deposition in the interstitial space between myofibers (6, 7). Induction of the TGF- β signaling pathway in skeletal muscle of murine models of ALS as well as in symptomatic ALS patient muscle is correlated with increased ECM deposition resulting in fibrosis (6, 10). FAPs and other mesenchymal progenitors residing in connective tissue express fibrotic genes: collagens, CTGF, and α -SMA, and deposit fibrotic ECM proteins in the interstitial space between muscle fibers resulting in muscle fibrosis (3, 6, 9). Because FAPs are sensitive to TGF- β signaling, one proposed model for the pathogenesis of fibrosis in ALS is that of a TGF- β -mediated increase in FAP cell number and FAP-mediated deposition of ECM in the muscle interstitium (6). Thus, inhibition of TGF- β may help prevent FAP-mediated skeletal muscle fibrosis in the setting of neurodegenerative diseases such as ALS. **Figure 1**, below, illustrates these molecular mechanisms promoting FAP mediated skeletal muscle atrophy and fibrosis in ALS.

Muscular dystrophy

Patients with muscular dystrophies suffer from characteristic progressive weakness and loss of muscle mass due to genetic perturbations of protein production requisite for healthy muscle development. Onset, signs, and symptoms of muscular dystrophies are dependent upon specific genetic

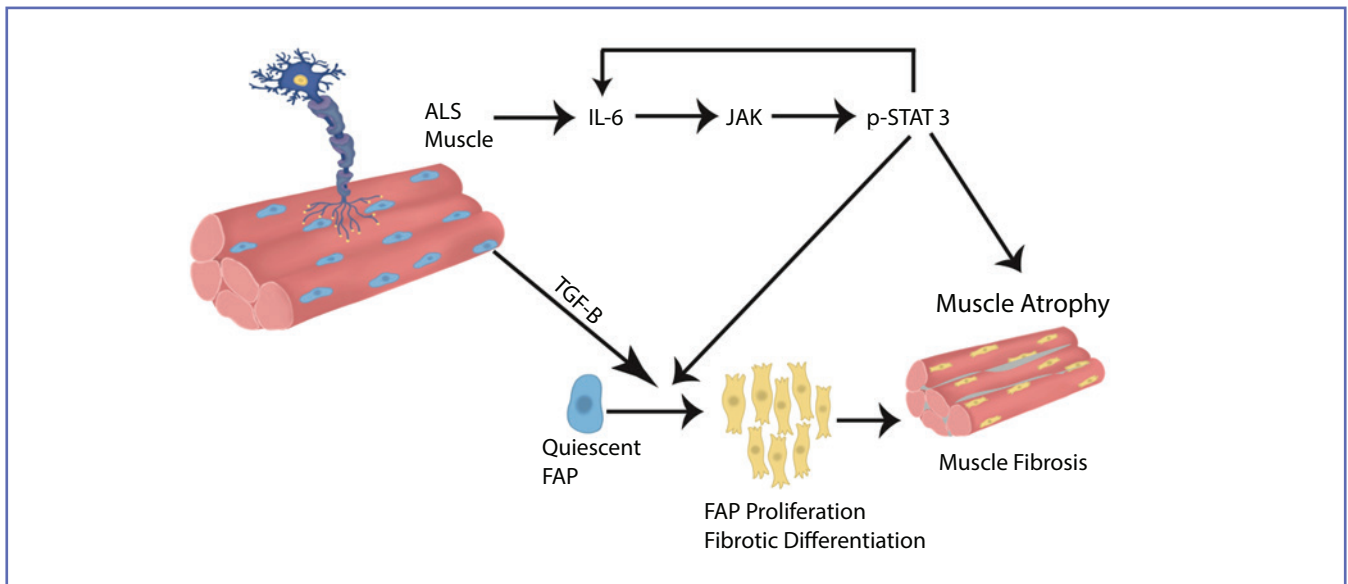


Figure 1. IL-6-Stat3 and TGF β signaling stimulates Fibro-Adipogenic skeletal muscle degenerative remodeling in neurodegenerative diseases like ALS. Increased TGF β signaling activates FAPs to proliferate and differentiate into fibroblasts. These fibroblasts deposit ECM proteins in the interstitial space causing muscle fibrosis. Simultaneously, increased IL-6-Stat3 signaling in neurodegenerative diseases such as ALS leads to reciprocal positive feedback and sustained signaling as well as muscle atrophy.

mutation-defined sub-classifications of these chronic myopathies. FAPs have been implicated in both the fibro-adipogenic remodeling of muscle and in some cases putative therapeutic intervention in Duchenne's Muscular Dystrophy (DMD), Facioscapulohumeral Dystrophy, and Limb-girdle dystrophy.

Duchenne's Muscular Dystrophy

Duchenne's Muscular Dystrophy (DMD) is caused by an X-linked recessive mutation in the dystrophin gene which results in a complete loss of dystrophin in the sarcolemma. In healthy skeletal muscle tissue, dystrophin anchors the cytoskeletons of myofibers to the extracellular matrix by complexing with glycoproteins in the sarcolemma. In DMD, the unanchored sarcolemma is rendered vulnerable to mechanical stress. Therefore, DMD myofibers undergo permanent and asynchronous cycles of degeneration and regeneration which leads to chronic inflammation, fibro-adipogenic remodeling and atrophy of skeletal muscle tissues (11).

Myogenic progenitors (MP) and myotubes regulate the proliferation and fibro-adipogenic differentiation of FAPs, but this regulation is altered in DMD. Analysis of FAPs cultured in MP conditioned media revealed that MPs stimulate FAP proliferation by activation of the PI3K/Akt pathway, as further evidenced by reduction of Akt phosphorylation in FAPs and their proliferation rates in conditioned MP

media with addition of an PI3Kinase inhibitor (12). However, the secretome of MPs is altered in a dystrophic environment. DMD MPs do not stimulate proliferation of DMD FAPs despite elevated Akt phosphorylation being observed in these FAPs, which was rescuable by healthy MP conditioned media (12). Further analysis of the MP secretome in healthy *versus* DMD muscle is necessary to elucidate specific factors responsible for FAP proliferation whose expression may be altered in the context of DMD, such as putatively platelet-derived growth factor-AA (3, 13). Whereas the MP secretome regulates FAP proliferation, the myotube secretome regulates FAP differentiation, inhibiting adipogenesis but promoting fibrogenesis. This is evidenced by mRNA expression of fibrotic and adipogenic markers in human FAPs treated with myotube-conditioned media compared to those treated with MP-conditioned media (12). Further molecular analysis of these cultures revealed that this anti-adipogenic and pro-fibrotic differentiation by myotube cytokines is mediated through activation of the ALK4/5/7 receptors, resulting in Smad2 phosphorylation and in parallel increasing GLI1 to activate Hedgehog signaling in FAPs (12, 14-16). Finally, these experiments revealed that MPs, rather than myotubes, secreted factors to inhibit FAP adipogenesis, and that DMD myotubes did not promote FAP-mediated fibrogenesis, suggesting that DMD alters the secretome of both MPs and myotubes (12). However, these findings

suggest that non-myocyte cells are responsible for promoting FAP-mediated muscle degeneration in DMD.

Chronic inflammation in DMD muscle involves the infiltration of pro-inflammatory macrophages, one subset of which is defined by Ly6C expression, found to be elevated in skeletal muscle of DMD patients and in Mdx mice (17). Elevated LTBP4 expression in DMD muscle stimulates Ly6C positive macrophages to produce latent-TGF- β 1, which is activated by enzymes secreted by FAPs (17). Activated TGF- β 1 then promotes FAP survival as well as myofibroblast differentiation and subsequent collagen deposition, thereby promoting pathological fibrosis of skeletal muscle (9, 17-19). Emerging evidence suggests that sub-populations of Vcam-1 expressing pro-fibrotic FAPs are persistent as DMD progresses (20). Furthermore, in early stages of DMD, FAPs can be reprogrammed to adopt pro-myogenic fates rather than fibro-adipogenic fates with histone deacetylase (HDAC) inhibitors, but lose this potency in late stages of the disease (21). HDAC inhibitors increase microRNA 206 in extracellular vesicles secreted by FAPs, which enhances satellite cell proliferation and differentiation and subsequent muscle regeneration in DMD human cells and Mdx mice (22). Similarly, TGF- β treatment of murine muscle progenitors and primary human cell cultures demonstrated increased expression of HDAC4, a key inhibitor of myogenic differentiation (23). However, HDAC4 expression can be directly attenuated by microRNA 206 and microRNA 29 through Smad3 inhibition, thereby supporting skeletal muscle regeneration in the context of aberrant TGF- β signaling (23). Since Metformin, an AMPK activator, reduces fibrosis and increases muscle regeneration and strength in murine models of DMD, it has been suggested that decreased AMPK expression in the DMD damaged myofiber niche initiates the macrophage and FAP mediated fibrosis of DMD skeletal muscle (17). Additionally, dysbiosis in macrophage subpopulation representation among the inflammatory infiltrate may support FAP adipogenesis. Whereas IL-4 polarized macrophages (M2) secrete cytokines to promote white adipocyte differentiation of FAPs, IL-1 β macrophages (M1) secrete cytokines that promote phosphorylation of Smad2 in FAPs thereby inhibiting their adipogenic differentiation (18, 24). Thus, overrepresentation of IL-4 polarized macrophages within the inflammatory infiltrate in DMD tissues could support FAP-mediated white adipogenesis in skeletal muscle. In summary, the abnormal environment resulting from a lack of dystrophin creates an imbalance in IL-4 polarized macrophage-mediated signaling which ultimately results in increased white fat differentiation of FAPs, as illustrated in **figure 2**.

Facioscapulohumeral Dystrophy

Facioscapulohumeral muscular dystrophy (FSHD) is caused by an autosomal dominant mutation resulting in ectopic expression of the double homeobox 4 retrogene in skeletal muscle. This results in progressive weakness and wasting of muscles of the face, scapula, upper arms, trunk, and legs due to fibro-adipogenic remodeling of muscle. In a murine model of FSHD that closely recapitulates the pathophysiology of the disorder in humans, mice were observed to have a dose-dependent accumulation of intramuscular FAPs that positively correlated with inflammatory infiltrate, muscle fibrosis, and reduction in satellite cell numbers in diseased muscle (25).

FAPs that aberrantly accumulate in DUX4-expressing dystrophic muscle seem to be more sensitive to inflammatory stimuli during the progression of the disease as noted by differential gene expression of FAPs in acute vs. chronic doxycycline treated FSHD mice (25). The transcriptional profiles of the FAPs in FSHD patient muscles and from the hindlimbs of FSHD mice were strikingly similar (25). The transcriptional profile of these FAPs diverged from pro-myogenic FAPs seen in acute injury settings, and more closely resembled the transcriptional profiles of FAPs found pathologically in chronic disease (25). These findings suggest that DUX4-mediated dystrophy in FSHD results in the progressive fibrotic and adipogenic differentiation of FAPs. However, further studies are needed to further elucidate the molecular mechanisms through which such sub-population selection and fibro-adipogenic differentiation of FAPs supports the pathology of FSHD.

Limb-Girdle Dystrophy

Limb girdle muscular dystrophy 2B (LGMD2B) is a muscular dystrophy caused by mutations in dysferlin that manifests in weakness and atrophy of proximal muscles surrounding the hips and shoulders or limb girdles of patients (26). The paucity of dysferlin in the myofibers of these patients accounts for their impaired sarcolemmal repair, disrupted calcium homeostasis at t-tubes and immune dysbiosis in muscle tissues (27-32). This persistent myofiber damage positively correlates with accumulation of AnxA2, a dysferlin interacting protein that aids in its repair at the site of plasma membrane injury by inciting muscle inflammation (33, 34). AnxA2 levels progressively increases and correlates with disease severity in the ECM, creating a niche that results in FAP accumulation and subsequent adipogenic differentiation. This process is thought to occur through matrix metalloprotease 14 (MMP-14) signaling in myofibers which subsequently activates C/EBP δ and PPAR γ expression in FAPs (35-37).

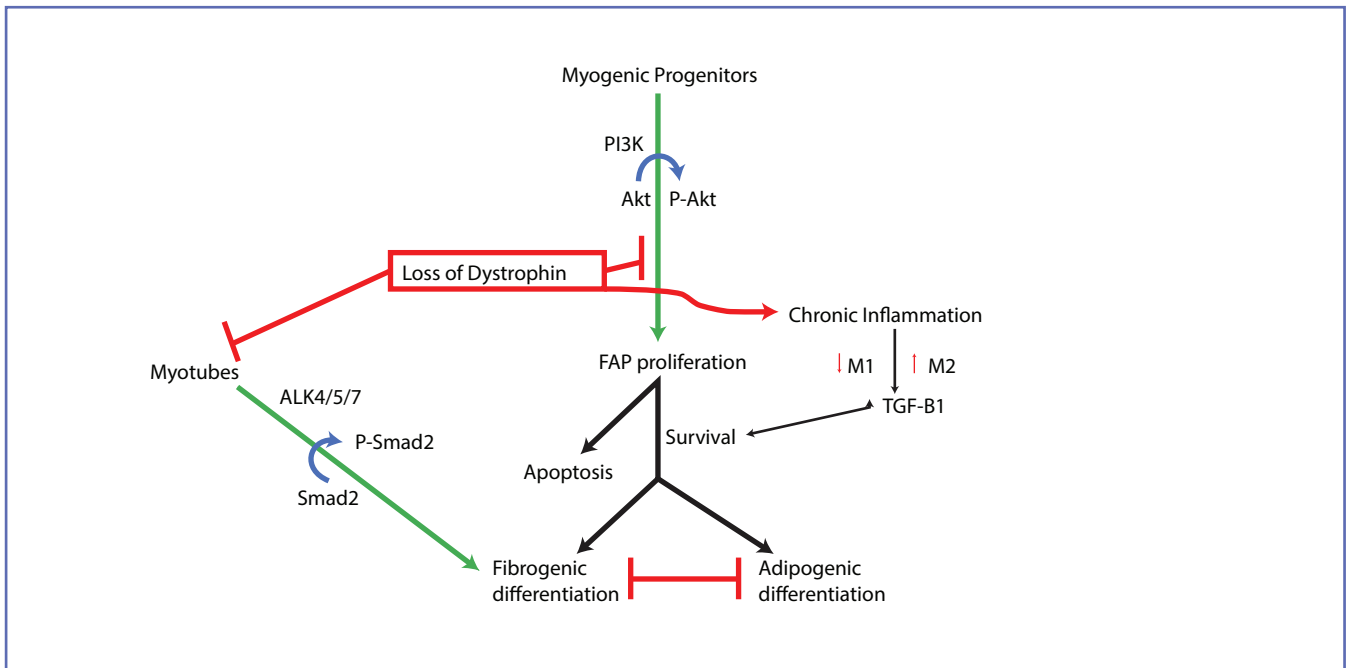


Figure 2. Loss of Dystrophin in DMD alters normal MP and MT secretomes and leads to a chronic inflammatory state resulting in increased adipogenic differentiation of FAPs.

Rotator Cuff tears

Rotator cuff (RC) tears are among the most common musculotendinous injuries and their prevalence greatly increases with age (38, 39). Both the extent of muscle atrophy and fatty infiltration in injured RC muscles are independently predictive of worse outcomes after repair (40, 41). Although the pathophysiology of underlying muscle degeneration following RC tears remains under investigation, our group and others have found pathologic adipogenesis is commonly observed in RC, but no other musculotendinous injuries have been linked primarily to FAPs (42-44). We previously reported that TGF- β 1 signaling is significantly increased in larger RC tendon tears, which not only promotes FAP survival, but also promotes both their fibro-adipogenic differentiation and remodeling of skeletal muscle (45, 46). All of these fibro-adipogenic effects of FAPs and their survival have been demonstrably attenuated with administration of a small molecular inhibitor of TGF- β 1 in our group's and other's murine model of RC injury (43, 45). Additionally, the myokine IL-15 has been shown to activate the JAK-STAT pathway to stimulate FAP proliferation and the desert hedgehog pathway which inhibits FAP adipogenesis in favor of FAP fibrogenesis, such that IL-15 expression correlates with the severity of fibrosis and FAP abundance in chronic RC tears (47). However, a sub-population

of FAPs known as beige FAPs, characterized by their UCP1 expression, have shown therapeutic efficacy in decreasing fibrosis, fatty infiltration, and atrophy and increasing vascularization and muscle function upon transplantation in murine models of RC repair (44, 48). **Figure 3** highlights the heterogeneity of the FAP population in rotator cuff pathology and regeneration. Further investigation is required to elucidate molecular mechanisms that may be modulated to promote beige FAP differentiation *in vivo*.

Chronic disease processes

Muscle fibrosis and sarcopenic obesity in Chronic Kidney Disease

Muscle dysfunction serves as an important cause of morbidity among patients with chronic diseases including chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), cardiovascular disease, and obesity. In patients with CKD, functional decline and loss of mobility, which precipitates falls, hospitalizations and morbidity may be in part due to skeletal muscle fibrosis (48-50). This excess collagen deposition in CKD patients is pathological and contributes to the skeletal muscle functional decline by reducing the transfer of force generated by myofibril contractile units per cross-sectional area of muscle tissue (49, 52-54).

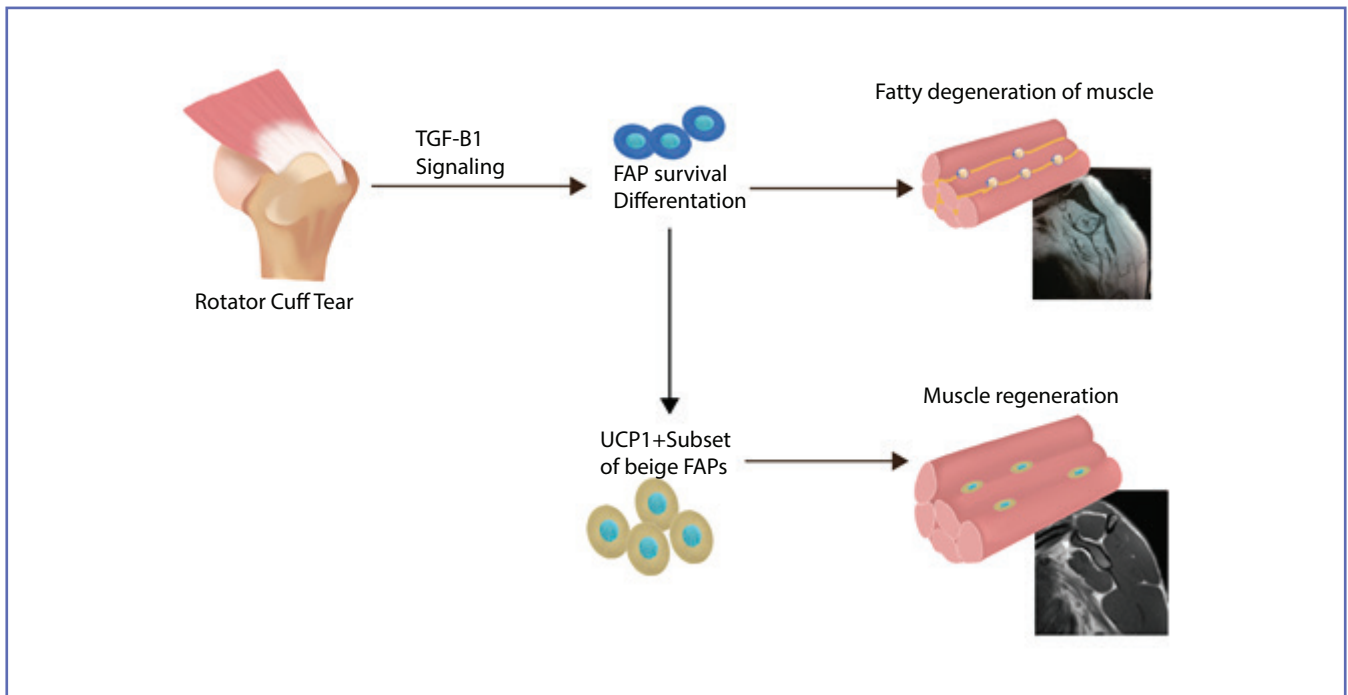


Figure 3. Rotator cuff tears result in sustained TGFβ-mediated survival of FAPs. However, FAPs are a heterogeneous population. One subpopulation contributes to fibrosis and fatty infiltration. The other subpopulation of UCP1⁺ FAPs may play a role in muscle regeneration and reduction of fatty infiltration.

Greater FAP abundance in human CKD muscle compared to controls was positively correlated with intrafibrillar collagen deposition (49). Compared to the muscles of healthy controls, muscles of CKD patients exhibited decreased macrophage abundances and associated decreased expression of TNF- α despite systemic inflammation in CKD patients (49). Therefore, skeletal muscle fibrosis in CKD patients may be linked to increased FAP abundance due to insufficient macrophage-produced TNF- α , which normally functions to promote FAP apoptosis. This results in increased intrafibrillar collagen deposition and subsequent decline in muscle function.

While reduced macrophage TNF- α signaling within skeletal muscle locally results in the aberrant survival of FAPs in muscle, increased systemic TNF- α and downstream effector signaling promotes FAP fibrotic differentiation (49, 55). Systemic elevation in TNF- α signaling in CKD stimulates production of myostatin through NF- κ B at levels inversely proportional to patient's eGFR (56-58). These elevated levels of myostatin are sustained through a signaling loop whereby myostatin signaling amplifies MAPK which in turn promotes IL-6 production followed by Stat3 activation which stimulates transcription factor C/EBP δ which results in production of myostatin (59). This augmented myosta-

tin signaling promotes skeletal muscle atrophy by supporting satellite cell dysfunction and by stimulating Smad2/3 phosphorylation which in turn suppresses Akt phosphorylation, thereby activating the ubiquitin proteasome system, UPS (56, 60). Concomitant activation of UPS and dysfunction of satellite stem cells promotes skeletal muscle atrophy (55). This muscle atrophy has been shown to be suppressed by inhibition of myostatin in murine models of CKD (56). Additionally, elevated myostatin promotes differentiation of FAPs into fibroblasts through Smad3 signaling (55). Because myostatin and FAP-mediated muscle fibrosis and atrophy can be suppressed by myostatin inhibition in murine models of CKD, this pathway may represent a therapeutic target for decreasing FAP-mediated muscle fibrosis in CKD (55).

FAPs are also stimulated to differentiate into adipocytes, resulting in sarcopenic obesity seen in CKD. The extracellular matrix protein CCN1 is a key driver of this fatty degeneration. CCN1 expression is positively correlated with muscle wasting, sarcopenia, and has recently been discovered to be upregulated in both serum and muscle of CKD patients relative to healthy controls (61). This expression increases in a dose dependent manner whereby CKD patients with sarcopenic obesity have higher serum and muscle CCN1 expression relative to CKD patients without sarcopenic obesity

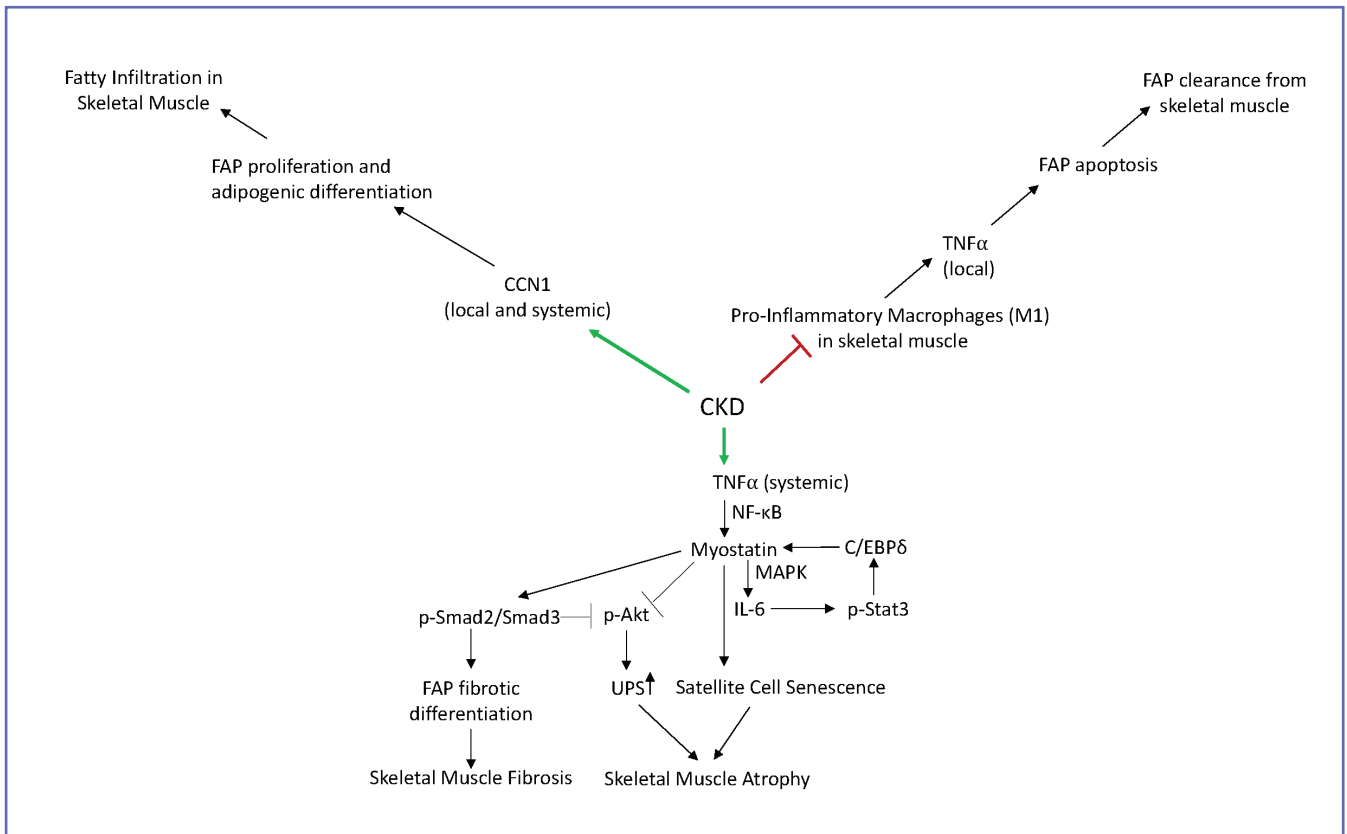


Figure 4. Myostatin and CCN1 stimulated FAP Fibro-Adipogenic skeletal muscle remodeling in chronic Kidney Disease (partially adapted from Dong *et al.* 2017 (54)).

(61). *In vitro* treatment of FAPs with CCN1 promoted the growth of FAPs as well as their differentiation into adipocytes (61). CCN-mediated adipogenesis of FAPs was reversible with administration of a neutralizing CCN1 monoclonal antibody in the same cell culture conditions (61). However, further investigative studies are needed to elucidate the molecular mechanisms through which CCN1 is able to mediate FAP adipogenic differentiation and proliferation as well as satellite cell senescence.

In summary, myostatin and CCN1 inhibition may abrogate FAP-mediated pathogenesis of skeletal muscle fibrosis through differentiation into both adipocytes and fibroblasts, as well as satellite cell senescence contributing to muscle cell atrophy in the context of CKD, as illustrated in **figure 4**.

Muscle atrophy in Chronic Obstructive Pulmonary Disease

Patients with COPD suffer from progressive and irreversible airway obstruction and chronic inflammation of the lungs (62). Skeletal muscle atrophy and dysfunction in these

patients can be correlated with their physical inactivity, chronic inflammation, oxidative stress, and recurrent hypoxic episodes due to COPD exacerbations (63). Hypoxic stress in skeletal muscle due to COPD results in muscle degradation that may be mediated through aberrances in FAP recruitment or retention in skeletal muscle (1). FAPs and muscle progenitors express sialomucin CD34, which has been demonstrated to be necessary for skeletal muscle repair in response to acute-toxin-induced injury (1, 64). One study found that two days of exposure to hypoxic conditions is sufficient to induce muscle wasting and loss of lean mass similar to what is seen in COPD patients in mice and that this muscle wasting was notably worse in CD34 knockout mice (63). While expression of myogenic regulatory factors and protein degradation factor (Atrogin) were similar in both the CD34 knockout mice and wildtypes, the knockout mice's extensor digitorum longus muscle was measured to have decreased maximal strength and recuperation capacity in response to hypoxia compared to that observed in wildtype mice (63). Moreover, FAP abundance in skeletal muscles of

CD34 knockout mice were significantly decreased relative to in wildtype mice (63). Taken together these findings suggest that maintenance of muscle mass and function in response to recurrent hypoxemic insults as seen in COPD may be dependent on CD34-mediated accumulation of FAPs in injured skeletal muscle (63). However, further studies are needed to elucidate the molecular mechanisms that perturb FAP mediated muscle homeostasis in the context of COPD.

Cardiac muscle fibrosis and adipogenesis following acute or chronic injury

Cardiovascular diseases categorically are the leading cause of morbidity and mortality worldwide (65). Cardiac fibrosis and fibro-adipogenic remodeling of the heart in response to acute or chronic ischemic injury that results in reduced ejection fraction, arrhythmogenicity, and heart failure may be mediated in part by FAPs. Post-myocardial infarction fibrogenic remodeling of cardiac tissue by cardiac stromal FAPs can be abrogated by administration of an anti-fibrotic tyrosine kinase inhibitor (66). Perivascular cells that co-express PDGFR α and Gli1 have been observed to differentiate into myofibroblasts and contribute to cardiac fibrosis in murine models following angiotensin-2-induced myocardial fibrosis and ascending aortic constriction (67). Furthermore, genetic ablation of these FAPs ameliorates cardiac fibrosis and preserves ejection fraction in murine heart failure models (67). Additionally, in mice depleted of α -V integrins, which block TGF- β signaling in PDGFR α ⁺/PDGFR β ⁺ cardiac tissue resident FAPs, cardiac fibrosis was significantly reduced (68). Thus, TGF- β signaling mediates FAP fibrogenesis and subsequent fibrotic ECM deposition in both cardiac and skeletal muscle fibrosis (68).

Cardiac resident FAPs have additionally been implicated in the pathogenesis of cardiac fatty infiltration which precipitates arrhythmogenic disorders. In *in vitro* lineage tracing studies of human and mouse cardiac FAPs following genetic ablation of desmosome proteins, such as DSP, adipogenic but not fibrogenic FAPs underwent adipogenesis mediated by Wnt signaling (69). These FAPs account for at least 50% of the pathologically increased cardiac fibro-fatty infiltrate that results in ventricular arrhythmias, heart failure, and even sudden death in arrhythmogenic cardiomyopathy, a genetic disorder due to desmosome protein aberrancies (69, 70). Furthermore, genetic ablation of the quiescence factor HIC1 in cardiac FAPs in an otherwise healthy murine heart also recapitulates the fibro-fatty infiltration and functional arrhythmogenicity observed in patients with arrhythmogenic cardiomyopathy (66).

Finally, cardiac FAPs mirror their skeletal muscle FAP counterparts' multipotency as they contribute to cardiac fibrosis, cardiac fatty infiltration, and vascular calcification. Genetic fate mapping of murine epicardium in concert

with immunostaining analysis of PDGFR α /Sca-1 distribution in fetal heart tissues indicate that cardiac FAPs arise from the epicardium (71). These FAPs primarily contribute to vascular compartments but also assist in cardiac tissue homeostasis (71). Aberrant FAP osteogenesis in the aorta may contribute to the vascular calcification notable in aortic stenosis leading to ventricular hypertrophy, chronic extremity ischemia, and heart failure (71). However, further investigation is needed to elucidate the molecular mechanisms underlying this aberrant osteogenic differentiation that mirrors that of skeletal FAPs in heterotopic ossification.

Obesity-related fatty infiltration of muscle

Obesity is a chronic condition defined by excessive fat accumulation that presents a risk to an individual's health. Increased intramuscular fatty and fibrotic tissue in muscle of obese patients both decreases the force producing capacity of these tissues as well as glucose metabolism thereby contributing to both the pathogenesis of muscle remodeling and decreased sensitivity eventually leading to metabolic syndrome (72). FAP differentiation into fibroblasts or adipocytes in the context of metabolic stress are key contributors to the pathogenesis of obesity-related skeletal muscle fibrosis, degeneration, and metabolic decline.

Several adipokines have been discovered to stimulate FAP-derived adipogenesis in obesity-related myopathies. Thrombospondin 1 (THBS1), a white adipokine secreted from expanded adipose tissue, promoted FAP proliferation in obese mice (73). TGF- β derived from expanded adiposity as well as other organs similarly modulates both FAP proliferation and fibrogenic differentiation *in vitro*. This finding is further substantiated by a reduction in collagen deposition and FAP abundance with TGF- β inhibition (5, 72, 74-77). Increased skeletal muscle adiposity not only contributes to decreased force-generation, but also contributes to insulin resistance of these same tissues.

Additionally, obesity and metabolic disorders such as diabetes are associated with chronic low-grade inflammation wherein macrophages sustain FAP survival, as seen in the other dystrophic states discussed above. In obesity-related fatty infiltration, macrophages secrete TGF- β 1 which abrogates TNF-induced apoptosis of FAPs and instead promotes their fibrogenic differentiation and subsequent ECM deposition (17, 49, 72, 77). As discussed above, sub-populations of polarized M1 and M2 macrophages have opposite effects on FAP differentiation *in vitro*. While IL-1 β polarized macrophages (M1) modulate a reduction in FAP adipogenic differentiation by stimulating Smad2 phosphorylation in FAPs downstream of TGF β -1 signaling as assayed by decreased cellular lipid accumulation and reduced adipogenic gene expression but increased pro-inflammatory cytokines, IL-4

polarized macrophages (M2) enhanced FAP adipogenesis (18). Thus, M1 and M2 macrophages likely sustain fibrogenesis and adipogenesis of FAPs in the pathogenesis of skeletal muscle fibrofatty degeneration in the setting of chronic inflammation associated with obesity and diabetes.

Just as FAPs have been implicated in skeletal muscle fibrosis, degeneration and metabolic decline, they are implicated in the fibro-adipogenic remodeling of the diaphragm contributing to the pathogenesis of respiratory dysfunction of obese patients. This respiratory dysfunction characterized by impaired lung expansion and reduced central response to hypercapnia can contribute to increased cardiovascular morbidity and mortality that necessitates mechanical ventilatory support (73). Six months of high fat diet-induced obesity in mice was sufficient to reproduce shallow, short-interval breaths with increased duty cycle that are notable in obese humans (73). This respiratory dysfunction occurred in parallel with increased diaphragmatic adiposity, fibrosis and contractile dysfunction. Moreover, elevated circulating THBS1 and TGF- β 3 within the diaphragm of obese mice mediated expansion of the FAP pool (73). The progressive adipogenic differentiation of FAPs within the diaphragm predominantly yielded white adipocytes but also produced some beige adipocytes (73). Further research is needed to fully elucidate the molecular mechanisms underlying FAP mediated fibro-adipogenic diaphragm remodeling and respiratory decline.

Sarcopenia and aging

Sarcopenia is the loss of muscle mass and function, frequently associated with normal aging as well as with

chronic disease. Studies examining age-related sarcopenia highlight the important role of FAPs in the maintenance of muscle fiber mass through their crosstalk with MuSCs. Using transcriptome profiling of FAPs, it was shown that FAPs from aged muscle lose expression of WNT1 Inducible Signalling Pathway Protein 1 (WISP1), a matricellular signal that controls the expansion and asymmetric commitment of MuSCs through Akt signalling (78). This study further demonstrated that both transplantation of young FAPs to aged muscle as well as systemic administration of WISP1 to aged mice rescued the sarcopenic phenotype in aged mice (78). An *in vitro* study likewise showed that aged myogenic progenitors (MPs) failed to stimulate proliferation of FAPs through Akt phosphorylation as was observed with MPs from young donors (12). Taken together, these studies suggest that age-related sarcopenia is likely due to a failure of communication between FAPs and myogenic progenitors that occurs due to age-related changes in signaling in both populations of stem cells, as illustrated in **figure 5**.

Heterotopic Ossification

Heterotopic Ossification (HO) is defined as ectopic osteogenesis in non-skeletal tissues including muscle, tendons, and other soft tissues. This debilitating condition can be either a genetic or acquired disease from traumatic injury: soft tissue sports injuries, central nervous system damage, total hip arthroplasty, burns and in combat injuries (79-83). Osteogenic remodeling of muscle in trauma-induced HO precipitates joint ankylosis, impairs rehabilitation and

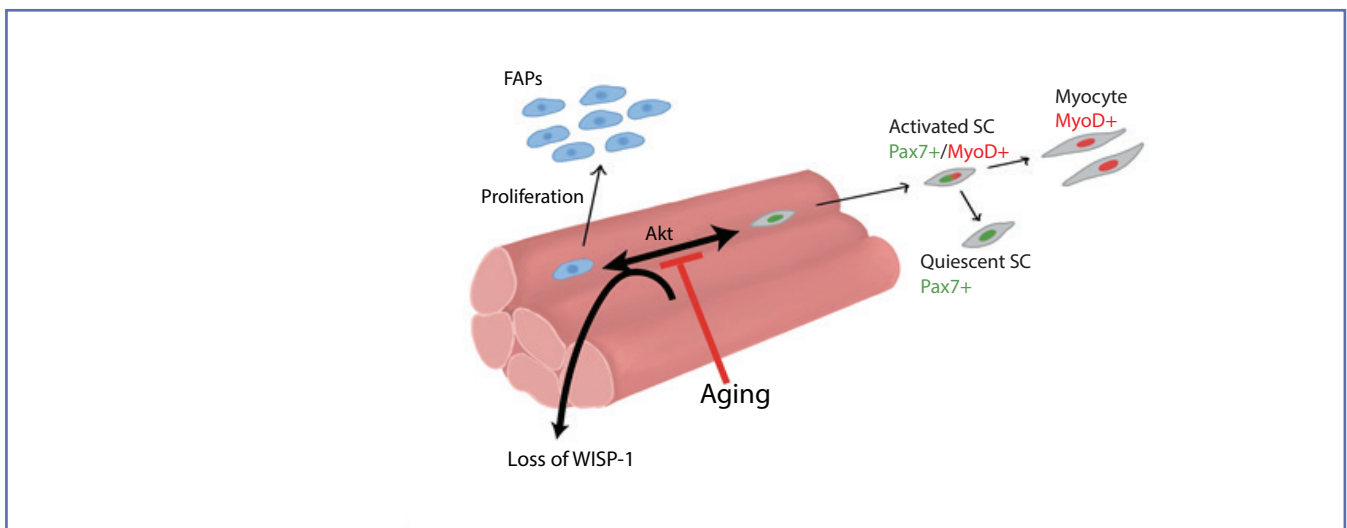


Figure 5. Failed crosstalk between FAPs and MPs promotes sarcopenic degeneration.

prevents maximal functional recovery (79-84). The rare genetic form of HO known as fibrodysplasia ossificans progressiva (FOP) is characterized by spontaneous flares that progressively immobilize patients through ossification of muscle starting in early childhood. Although FAPs were named for their bipotent fibrogenic and adipogenic potential, their recently discovered osteogenic potential implicates them in the pathogenesis of HO.

Both murine models of HO and FOP support excessive BMP signaling from inflammation or injury which stimulates the pathologic osteogenic differentiation of FAPs in these debilitating disease processes. Lineage tracing studies of FAPs in murine models of HO induced by BMP2-matrigel injections of the tibialis anterior muscle demonstrate that FAPs are the predominant source of cells contributing to HO. FAPs are considered to express Tie2, PDGFR α and Sca1 (1, 2, 85). Lineage tracing of FAPs by Tie-2 expression in this model indicated that 50% of heterotopic cartilage and bone could be attributed to FAP differentiation (85). Meanwhile, the more specific lineage tracing of FAPs by PDGFR α expression attributed 80% of osteogenic cells within mature heterotopic ossicles in this HO induction model to FAPs (86). Furthermore, FOP is a result of mutations in the glycine-serine regulatory domain of BMP Type I receptor Alk2 (also referred to as ACVR1 and ActR1), which renders it constitutively active independent of BMP ligands or hypersensitive to BMP ligands (87-91). In murine models genetically engineered to recapitulate the FOP mutation, FAPs are the major contributing cell population to heterotopic cartilage and bone (92). Retinoic acid receptor gamma (RAR γ) agonist, Palovarotene, has shown promising results in clinical trials to inhibit FAP mediated chondrogenic and osteogenic differentiation through reduction of SMAD1/5/8 phosphorylation in the BMP signaling pathway (92). Thus, BMP signaling in HO and FOP likely leads to SMAD1/5/8 phosphorylation which supports pathogenic chondrogenic and osteogenic differentiation of FAPs.

Finally, dysbiosis of skeletal muscle infiltrating inflammatory cells following injury may also support FAP-mediated HO. In fact, prophylactic NSAID or clodronate liposome-mediated macrophage depletion seem to prevent HO (93). Furthermore, murine models of BMP2-mediated HO demonstrated increased ossification volume derived from FAPs in mice genetically engineered to inhibit infiltration of macrophages or deplete these macrophages suggesting that macrophages clear pathogenic FAPs to prevent HO pathogenesis (86, 94). However, further investigation is required to uncover the BMP-independent molecular mechanisms underlying inflammatory dysbiosis mediated HO pathogenesis through osteogenic FAP differentiation.

CONCLUSIONS AND PERSPECTIVE

As FAPs are a ubiquitous source of resident muscle stem cells, it can be expected that they play a diverse role in a variety of musculoskeletal disease processes, ranging from myopathies, to denervation-mediated processes, to chronic musculotendinous injuries. Upon reviewing the disease processes above, it is clear that FAPs play a nuanced role along with myogenic progenitors in maintaining normal muscle homeostasis, and when this balance is disrupted, FAP-mediated muscle degeneration may occur. The pathways highlighted above represent many potential therapeutic targets for the specific disease processes discussed, many of which are under active clinical investigation. However, many of these targeted interventions would likely carry broad systemic effects, in addition to potentially disrupting the nuanced balance between FAP-mediated muscle regeneration and adipogenic or fibrogenic differentiation of FAPs, particularly those that directly affect FAP cell number or viability. Future studies targeting muscle-specific therapeutics to decrease systemic effects may be the next step in ameliorating muscle degeneration.

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CONFLICT OF INTERESTS

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ABBREVIATIONS:

ActR1: Actin related protein 1
ACVR1: Activin receptor type 1
Akt: Protein kinase B
Alk2: Activin receptor-like kinase 2
ALK4/5/7: Activin receptor-like kinase 4/5/7
ALS: Amyotrophic lateral sclerosis
AMPK: Adenosine monophosphate activated protein kinase
BMP: Bone morphogenetic protein
BMP2: Bone morphogenetic protein 2
C/EBP δ : CCAAT enhancer binding protein delta
CCN1: Connective tissue growth factor nephroblastoma overexpressed 1
CD34: Cluster of differentiation 34
CKD: Chronic kidney disease

COPD: Chronic obstructive pulmonary disease
 CTGF: Connective tissue growth factor
 DMD: Duchenne's muscular dystrophy
 ECM: Extracellular matrix
 eGFR: estimated glomerular filtration rate
 FABP4: Fatty acid binding protein 4
 FAPs: Fibro-adipogenic progenitor
 FOP: Fibrodysplasia ossificans progressiva
 GLI1: Glioma associated oncogene 1
 HO: Heterotopic ossification
 IL-15: Interleukin-15
 IL-1 β : Interleukin-1-beta
 IL-4: Interleukin-4
 IL-6: Interleukin-6
 JAK: Janus kinase
 LTBP4: Latent transforming growth factor beta
 binding protein 4
 M1: Macrophage type 1
 M2: Macrophage type 2
 MAPK: Mitogen activated protein kinase
 MP: Myogenic progenitor
 Mrna: Messenger ribonucleic acid
 MT: Myotube
 NF-Kb: Nuclear factor kappa beta
 NMJ: Neuromuscular junction

NSAID: Nonsteroidal anti-inflammatory drug
 p-Akt: phosphorylated protein kinase B
 p-Smad2: phosphorylated smad protein
 PDGFR α : Platelet derived growth factor receptor alpha
 PI3K: Phosphoinositide 3-kinase
 PPAR γ : Peroxisome proliferator-activated receptor gamma
 RAR γ : Retinoic acid receptor gamma
 RC: Rotator cuff
 Sca1: Stem cells antigen 1
 SMA: Spinal muscle atrophy
 Smad1/5/8: Smad protein 1/5/8; main signal transducers
 for receptors of transforming growth factor beta
 Smad2: Smad protein 2; main signal transducers for recep-
 tors of transforming growth factor beta
 Smad3: Smad protein 3; main signal transducers for recep-
 tors of transforming growth factor beta
 STAT3: Signal transducer and activator of transcription 3
 Tcf4: Transcription factor 4
 TGF- β : Transforming growth factor beta
 Tie-2: Angiopoietin-1 receptor
 TNF- α : Tumor necrosis factor alpha
 UCP1: Uncoupling protein 1
 UPS: Ubiquitin proteasome system
 Vcam-1: Vascular cell adhesion protein 1
 α -SMA: alpha smooth muscle actin

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