REVIEW

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Cellular interplay in skeletal muscle regeneration and wasting: insights from animal models

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Abstract

Skeletal muscle wasting, whether related to physiological ageing, muscle disuse or to an underlying chronic disease, is a key determinant to quality of life and mortality. However, cellular basis responsible for increased catabolism in myocytes often remains unclear. Although myocytes represent the vast majority of skeletal muscle cellular population, they are surrounded by numerous cells with various functions. Animal models, mostly rodents, can help to decipher the mechanisms behind this highly dynamic process, by allowing access to every muscle as well as time-course studies. Satellite cells (SCs) play a crucial role in muscle regeneration, within a niche also composed of fibroblasts and vascular and immune cells. Their proliferation and differentiation is altered in several models of muscle wasting such as cancer, chronic kidney disease or chronic obstructive pulmonary disease (COPD). Fibro-adipogenic progenitor cells are also responsible for functional muscle growth and repair and are associated in disease to muscle fibrosis such as in chronic kidney disease. Other cells have recently proven to have direct myogenic potential, such as pericytes. Outside their role in angiogenesis, endothelial cells and pericytes also participate to healthy muscle homoeostasis by promoting SC pool maintenance (so-called myogenesis–angiogenesis coupling). Their role in chronic diseases muscle wasting has been less studied. Immune cells are pivotal for muscle repair after injury: Macrophages undergo a transition from the M1 to the M2 state along with the transition between the inflammatory and resolutive phase of muscle repair. T regulatory lymphocytes promote and regulate this transition and are also able to activate SC proliferation and differentiation. Neural cells such as terminal Schwann cells, motor neurons and kranocytes are notably implicated in age-related sarcopenia. Last, newly identified cells in skeletal muscle, such as telocytes or interstitial tenocytes could play a role in tissular homoeostasis. We also put a special focus on cellular alterations occurring in COPD, a chronic and highly prevalent respiratory disease mainly linked to tobacco smoke exposure, where muscle wasting is strongly associated with increased mortality, and discuss the pros and cons of animal models versus human studies in this context. Finally, we discuss resident cells metabolism and present future promising leads for research, including the use of muscle organoids.

Keywords Sarcopenia; Cachexia; Myofibres; Neutrophils; Fibrocytes

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Introduction

Skeletal muscle wasting is a prevalent condition, associated with increased hospitalizations and disability, and is thus a key determinant to quality of life and mortality. It corresponds to a reduction in the size of muscle fibres, mainly linked to an imbalance between anabolism and catabolism in myocytes.[1] In terms of lexical field, it encompasses both

sarcopenia, which is currently defined by a loss of both muscle mass (without body weight loss) and force,[2] and cachexia, which reflects an active state of body weight loss predominantly linked to skeletal muscle loss and associated with an underlying condition.[3] There is indeed a vast panel of conditions associated with muscle wasting, ranging from physiological ageing or muscle disuse to chronic diseases such as cancer (the most well-known cause of cachexia), but also metabolic and cardiovascular diseases (diabetes, chronic kidney disease, heart failure), chronic inflammatory diseases such as rheumatoid arthritis, infectious diseases (acquired immunodeficiency syndrome, sepsis) and respiratory diseases such as chronic obstructive pulmonary disease (COPD).[4] In COPD in particular, a chronic respiratory disease mainly linked to cigarette smoke exposure, muscle wasting affects up to 35% of the patients[5] and is associated with a dramatic increase in mortality even after matching for respiratory function.[6,7] Given the high prevalence of COPD (around 10%) as well as the fact that it currently represents the third cause of death in the world,[8] studying the cellular basis behind COPD muscle alterations is of particular interest.

In addition to being associated with several very different diseases, muscle wasting appears to be heterogeneous in terms of phenotypes or histological and molecular alterations. This might be due to the multifactorial origin of muscle loss. For example, in COPD, muscle wasting can be attributed to both extrinsic factors (deconditioning, repeated corticosteroid use, cigarette smoke exposure, malnutrition) and intrinsic COPD-bound factors (inflammation, hypoxia, oxidative stress).[9] Overall, these factors lead to an imbalance between protein synthesis and degradation in myocytes, in favour of increased catabolism. Affected pathways are well-characterized: Catabolism includes the myostatin pathway (signalling through activin type II receptor), nuclear factor-kappa B (NF-κB) pathway and angiotensin and glucocorticoid receptors, all leading to increased ubiquitin–proteasome system activity (via the transcription factor forkhead box O (*FOXO*)), autophagy and apoptosis.[10] Anabolism mainly includes the insulin-like growth factor 1 (IGF1) receptor, signalling via AKT and mammalian target of rapamycin (mTOR).

However, the initial trigger behind this increased catabolism in myocytes remains unclear. In this context, taking into account the role of tissular resident skeletal muscle cells other than myocytes appears essential. Recent molecular biology techniques such as single-cell RNA sequencing have allowed a complete characterization of the resident cell populations in skeletal muscle, as well as unravelling new populations.[11,12] Indeed, although satellite cells are at the core of myonuclear turnover and muscle regeneration, they interact with many other cells in a finely tuned process. However, muscle wasting is also a highly dynamic process which investigation is hampered by the differential cellular and protein regulation according to the stage of the disease, as illustrated by the shift in macrophages type in muscle repair after injury.[13] As skeletal muscle is not so easily accessible, and repeated muscle biopsies are not always possible in patients already suffering from physical limitations, animal models appear essential to study the dynamics of muscle wasting, which allow access to every muscle as well as time-course studies. Several models are available for muscle wasting, whether primarily devoted to muscle loss/repair or related to the underlying condition.[14]

Here, we will discuss the potential role of tissular resident cells interacting with myocytes within skeletal muscle using data from animal models of muscle wasting. We will discuss the role of and cellular cross-talk between progenitor, immune and vascular cells as well as other constitutional cells such as neural cells and report data concerning their role in homoeostasis and regeneration as well as several sarcopenia models. We also focus our attention on fibrocytes, whose role has recently been highlighted in COPD lung by our team,[15,16] and sum up the current knowledge about the cellular interplay leading to muscle wasting in COPD. Finally, we present future promising leads for research, including the use of muscle organoids.

A summary of the respective role of each cell, as well as the preferential animal models used to study this role, can be found in Table 1.

Progenitor cells

Satellite cells (SCs) are skeletal muscle resident stem cells residing beneath the basal lamina of myofibres, quiescent at the steady state but upon activation, able to proliferate, differentiate and fuse with myofibres. They are thus responsible for both muscle maintenance via myonuclear turnover and functional muscle repair following damage. Their differentiation comes along with successive up-regulation and down-regulation of key transcription factors: The paired box transcription factor *Pax7* and the myogenic regulatory factors (MRFs) *MyoD* and *Myf5* are required for myogenic determination (early stage), whereas the MRF *myogenin* is required for differentiation and *MRF4* for myotube maturation (late stages).[17] This delicate balance is mediated by a cross-talk between the MRFs and the cell cycle regulators. Interestingly, it has recently been shown that a subset of SCs also express *Pax3* and are resistant to environmental stress such as pollution.[18] SCs also participate to physiological muscle regeneration after endurance training, with a metabolic reprogramming favouring lower mitochondrial respiration and oxygen consumption.[19] Indeed, regular exercise increases SC pool and contribution to myofibres.[20]

Concerning muscle regeneration specifically, the crucial and non-redundant role of SCs has been demonstrated by many studies, both in humans and animal models.[21] During regeneration, SCs also benefit from a reciprocal support of resident muscle cells such as fibroblasts[22] or endothelial cells

Table 1 Respective physiological and pathological role of resident tissular cells in skeletal muscle homoeostasis and most common animal models used to study this role. SCs, satellite cells. FAPs, fibro-adipogenic progenitor cells. ECM, extra-cellular matrix.? Indicate putative role

Cell class	Cell type	Studied model(s)	Role (physiological and pathological)	Ref.
Progenitor cells	Satellite cells (SCs)	Acute muscle injury Ageing	Myocyte turnover and regeneration Angiogenesis Muscle reprogramming after tissue injury	17–37; S1–S4
	Fibro-adipogenic progenitor cells (FAPs)	Acute muscle injury Chronic kidney disease Obesity	Cross-talk with immune cells during muscle repair SCs proliferation and differentiation Tissue fibrosis or myosteatosis	38–46; S5,S6
Vascular cells	Endothelial cells	Exercise training	Angiogenesis, nutrient uptake Cross-talk with SCs	47–53; S7-S9
	Pericytes	Exercise training	NG2+/Nestin+: myogenic differentiation SCs pool maintenance Angiogenesis	57–67; S10,S11
Immune cells	Macrophages	Acute muscle injury Ageing	Shift from M1-biased phenotype (debris phagocyting) to M2 (CD163+: connective tissue production) during the transition between the initial inflammatory phase and the resolutive phase	65–75
	Fibrocytes	Duchenne muscular dystrophy	Secretion of pro-inflammatory and pro-fibrotic cytokines upon muscle injury	76–80
	Lymphocytes	Acute muscle injury	$CD8^+$: macrophage recruitment, SCs proliferation $CD4^+$ TRegs: macrophage recruitment and polarization shift, SC activation and differentiation–angiogenesis?	81–86; S12-S15
	Neutrophils	Exercise training	Debris phagocyting, SC proliferation and differentiation, macrophage recruitment; myocyte damage via neutrophil extracellular traps (NETs)	S25–S32
Other	Telocytes Interstitial tenocytes Neural cells	Acute muscle injury Ageing	Cross-talk with SCs and vascular cells? ECM remodelling in a cross-talk with FAPs? Neuromuscular junction degeneration mainly related to SC depletion	S33–S37 S38 S39–S51

ECM, extracellular matrix; FAPs, fibro-adipogenic progenitor cells; SCs, satellite cells.
? indicates putative role.

(see below and Christov *et al*,[23]). Recent data tend to precise the role of SC activation upon injury in the muscle tissue, as well as their plasticity. For example, it has been shown in a mouse model of cardiotoxin-induced injury that the muscle damage enabled in vivo reprogramming of SCs to pluripotency.[24] Very interestingly, muscle repair was dependent on the prior local accumulation of tissular senescent cells and their senescent-associated secretory phenotype, among which interleukin-6 (IL-6) plays a prominent role. This interesting observation counteracts the general idea that senescent cells are deleterious for tissue repair (see below).

In disease, SCs can be altered both in number and activity. Ageing mice models indeed clearly point out a decrease in the SC pool.[25] Whether this is primarily linked to an intrinsic defect in SCs, such as alterations in genomic integrity and metabolic regulation, or alterations in the muscle micro-environment (stem cell niche) remains debated. On the one hand, engraftment of human SCs from both young and old donors in mice led to impaired renewal of the quiescent stem cell population of elderly donors, which was attributed to an increase in global DNA methylation, contributing to stem cell exhaustion.[26] Studies in mice models of ageing also showed intrinsic defects in SCs, such as p16(INK4a)/Rb-driven stem cell senescence.[S1] One the other hand, engraftment of post-mortem human muscle tissue from both young and old deceased donors into immunodeficient mice showed that SCs retained their regenerative capacity.[27] However, contribution of non-myogenic stem cells such as fibro-adipogenic progenitors (see below) cannot be excluded.

Overall, muscle micro-environment seems to play a prominent role in the muscle wasting process. Indeed, SCs isolated from cachectic muscles differentiate faster in vitro than controls.[28] Factors associated with the NF-κB pathway, such as pro-inflammatory cytokines [tumour necrosis alpha (TNF-α), angiotensin II] or myostatin are classically incriminated.[29] One study showed that enhancement of growth differentiation factor 11 (GDF11) circulating content, either by administrating recombinant protein or by parabiosis, could restore genomic integrity of SCs by reducing DNA damage, as well as their myogenic potential assessed by the number of myogenic colonies in vitro.[30] Interestingly, no change in SC proliferation was observed. This study further showed that GDF11 could act through enhancement of mitochondrial biogenesis. The functional improvement opens therapeutic possibilities for GDF11. Oxytocin was also pointed out as a promising candidate improving aged muscle regeneration.[S2]

Data from several other atrophy models such as denervation or cancer cachexia models suggest that impaired regulation of SCs and their ensuing myogenic program is an important contributing factor to the muscle wasting process (for review, see Biressi and Gopinath[31]). The early phase of myogenesis can be affected, with a decreased proliferation

observed in a chronic kidney disease, linked to a reduced insulin growth factor-1 (IGF-1) signalling.[32] Of note, differentiation was also impaired in this study. In addition, observational and experimental data from cancer models support the fact that differentiation is mainly impaired in atrophying muscle,[29] as an initial increase in Pax7$^+$ cells[33] suggests that proliferation is not the limiting factor here. Blockade of the activin type II receptor pathway leads to active proliferation of SCs in vivo in mouse model of cancer-induced cachexia,[34] emphasizing both a preserved proliferative capacity and the mutual cross-talk between myocytes and SCs (*Figure* 1). The increase in Pax7 expression also reflects an increase of assisting non-SC populations, such as fibro-adipogenic progenitors (FAPs) and pericytes, which seem to undergo a lineage switch and adopt myogenic fate[35] (*Figure* 1). Of note, the number of SCs is reduced in late-stage atrophy in both human and rodent models,[36] emphasizing the dynamic process of muscle wasting and the fact that timing is crucial, both to determine underlying mechanisms and as a therapeutic prospect. Finally, another potential cause for muscle atrophy is a defect of SC fusion with myoblasts, as observed in a denervation model.[36,S3] In another similar model, the decreased fusion was attributed to a lack of up-regulation of M-cadherin transcription.[S4] A decreased fusion was also observed in vitro in senescent myoblasts.[37]

Fibro-adipogenic progenitor cells (FAPs) are mesenchymal extra-laminal stromal cells that are usually quiescent unless induced by injury or intense exercise. They can be identified thanks to their expression of stem cell antigen 1 (Sca-1) and platelet-derived growth factor receptor alpha (PDGFRα), and their phenotypical plasticity allows them to differentiate into adipocytes or myofibroblasts according to the environment.[38] Although they are quiescent at the steady state, FAPs are required for homoeostatic muscle maintenance,[39,40] notably by secreting extracellular matrix (ECM) components.

In muscle injury repair, they play a physiological role by promoting first phagocytosis and later regenerative fibrogenesis by producing ECM, in a delicate interplay with macrophages and lymphocytes.[41] Failure of T regulator lymphocytes (Tregs) recruitment has notably been attributed to absence of IL-33 signalling by senescent FAPs.[42] They also sustain SC regenerative potential in a paracrine manner by secreting IL-6 and WNT1-inducible signalling pathway protein 1 (WISP1/CCN4) to promote their proliferation and follistatin

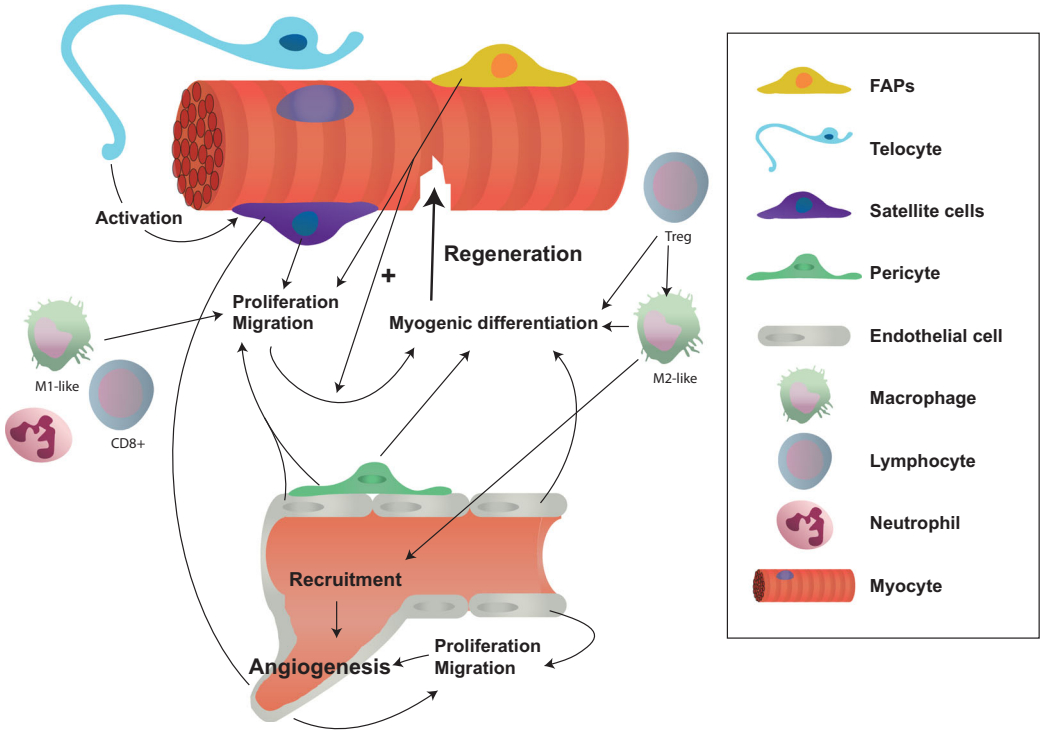

FIGURE 1 Cellular interplay in skeletal muscle homoeostasis. Tissular resident cells interact with myocytes and with each other in a finely tuned process. Immune cells take part in muscle repair, initially by phagocyting debris (M1-like macrophages and neutrophils, coordinated by CD8$^+$ T cells); at a later stage, they activate muscle regeneration through satellite cells proliferation, migration and differentiation [promoted by M2-like macrophages as well as T regulator lymphocytes (TRegs)]. The vascular compartment also plays an important role by regulating muscle nutrient uptake. Moreover, endothelial cells and pericytes can promote both angiogenesis and muscle regeneration, as well as recruit inflammatory cells. In addition, pericytes have been reported to undergo myogenic differentiation. Telocytes, which have been identified close to satellite cells, could activate their differentiation into myoblasts. Fibro-adipogenic progenitor cells (FAPs) interact with immune cells and satellite cells to promote skeletal muscle regeneration. Finally, satellite cells are essential to muscle regeneration, by proliferating and differentiating into myotubes, which later fuse and give rise to functional myofibres.

and IL-10 to promote their differentiation.[38] In turn, they are controlled by several surrounding cells, including macrophages via transforming growth factor-β (TGF-β) production (promoting their differentiation towards fibroblasts), myocytes via IL-15 secretion (inhibiting their adipogenic differentiation) and eosinophils producing IL-4 and IL-13 (also inhibiting adipogenic differentiation).[38] IL-4 even directly promotes fibrogenic differentiation, supporting myogenesis by clearing out necrotic debris.[43] In acute muscle injury, FAPs are thought to be activated at least partly by IL-4 produced by recruited eosinophils. IL-4/IL-13 signalling further promotes proliferation of FAPs to support myogenesis while inhibiting their differentiation into adipocytes.[43] This physiological response is disrupted in the case of chronic trauma or disease such as Duchenne muscular dystrophy, where FAPs are responsible for prolonged connective tissue production and muscle fibrosis.[44] Moreover, $CD34^{-/-}$ mice suffer from more severe muscle atrophy induced by hypoxia than wild-type mice, and this seems to be at least partly mediated by a lower number of FAPs.[45]

Overall, the role of FAPs in skeletal muscle tissue has mainly been characterized in regeneration, where they take part in both phases by secreting growth factors and cytokines. In other diseases, they are mainly associated with muscle fibrosis, for example, in models of chronic kidney disease, upon stimulation by myostatin.[S5] In this study, myostatin was responsible for the transition of FAPs to a fibroblast-like phenotype. Importantly, specific deletion of FAPs leads to a premature ageing phenotype with sarcopenia features.[40] They have also been associated with skeletal muscle wasting in the context of obese mice models by promoting fibro-adipogenic tissue replacement in the diaphragm.[S6] Quite interestingly, transplanting intra-peritoneally senescent FAPs into mice is sufficient to induce a sarcopenia-like functional phenotype using functional outcomes such as decreased grip strength and walking speed. At the molecular level, the authors observed an increase in quadriceps muscle TNF-α and IL-6.[46] Of note, senolytics 'cocktail' using dasatinib and quercetin improved significantly the functional parameters and the mice lifespan.

Vascular cells

Endothelial cells

Endothelial cells (ECs) in healthy muscle act as sensors for metabolic demand to induce upstream arterial dilation, providing a blood flow perfectly matched to oxygen and nutrient requirements. This active hyperaemia accounts for a large part of the oxygen convective supply capacities and is as such a major determinant of muscle oxygen uptake.[47] Moreover, muscle glucose uptake has also been strongly related to the endothelial insulin sensitivity.[48] ECs are thus critical in muscle adaptation to exercise training, with a pivotal role in muscle angiogenesis. However, besides their role in substrate delivery, few studies investigate ECs in skeletal muscle homoeostasis. In contrast, the role of skeletal muscle microvasculature has been well described, notably physiological angiogenesis such as exercise and altitude induced.[49] Indeed, there is a complex interplay between the muscle hypertrophic process and angiogenesis (*Figure* 1), also referred to as the myogenic-endothelial cross-talk. The acute and transient hypoxia generated by exercise seems to be responsible for vascular endothelial growth factor (VEGF) secretion, notably within myofibre-derived extracellular vesicles,[50] which stimulates angiogenesis in a HIF-1α-dependent manner.[S7] Moreover, exercise induces PGC-1α expression, which induces indirectly MCP-1 secretion by macrophages, which in turn activates ECs, pericytes and smooth muscle cells.[51] Other pro-angiogenic stimuli induced by exercise include shear stress and oxidative stress.[52]

During the regenerative process, SCs can produce pro-angiogenic stimuli that induce EC proliferation and migration.[53,S8] Conversely, an abnormal endothelial cell response could compromise the muscle repair. Endothelial progenitor cells, mobilized at the site of muscle injury, may promote both angiogenesis and muscle regeneration.[54,S9] ECs could also play a role in muscle mass regulation.[55] Indeed, they appear closely juxtaposed to SCs in the muscle compartment,[23] and an increase of the endothelial cell number during the myogenesis process appeared as a strong stimulant of myogenic progenitor cells migration and proliferation.[53] Another study demonstrated the close vicinity between endothelial cells and SCs using muscle tissue clearing.[56] They also showed that both type of cells interact through VEGF-A and Notch signalling. This cross-talk results on the one hand in resulting in capillaries patterning by SCs and on the other hand in promoting SC self-renewal. M2-biased macrophages (or restorative) also take part in the myogenesis–angiogenesis coupling: Indeed, adding M2-like macrophages in a 3D model composed of SCs and endothelial cells stimulated both capillaries and myotube formation.[53] This effect could be inhibited by blocking oncostatin M secreted by macrophages.

Pericytes

Pericytes are perivascular cells wrapped around capillaries, responsible for various physiological functions in skeletal muscle, including regulation of vessel growth, stabilization and permeability.[57] Few data are available concerning their precise role in skeletal muscle homoeostasis. However, two points are to be considered: their myogenic potential and their role in exercise-induced angiogenesis. Indeed, a myogenic potential for pericytes has been demonstrated in skeletal muscle, both in animal (including murine and canine models of dystrophy) and human studies.[58,59,S10] A particular subtype of pericytes, the nerve/glial antigen-2 $(NG2)^{+}$/$Nestin^{+}$ subtype, is responsible for this myogenic role.[60] Pericytes are

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responsive to skeletal muscle contraction and provide significant variation of gene expression, notably up-regulation of genes involved in immunomodulation and ECM remodelling.[61] Moreover, pericyte transplantation in skeletal muscle associated with exercise training have been associated with better improvements in muscle maximal contraction force.[61] Regarding the role of skeletal muscle angiogenesis in the muscle perfusion and fatigability, pericytes appear as essential to obtain viable and functional vasculature with angiogenesis[62] (*Figure* 1).

Pericytes also participate to muscle regeneration independently of SCs, due to their myogenic capacity.[59] Moreover, pericytes are able to induce re-entry into quiescence on completion of regeneration through angiopoietin-1 (Ang1) secretion.[63]

In a context of muscle damage, consecutive to muscle dystrophy or after eccentric exercise, the activity of skeletal muscle pericytes is increased and participate to muscle repair.[63, S11] Moreover, they could contribute to muscular dystrophy, both by participating to fat accumulation due to the adipogenic potential of PDFGRα^+ type 1 pericytes and contributing to fibrosis via a possible differentiation to myofibroblasts.[64]

Immune cells

Macrophages

Resident macrophages are a heterogeneous cell population, quiescent at the steady state, appearing to maintain tissue homoeostasis and promoting muscle growth and regeneration, although little is known about their precise role.[65] The latter has been more extensively characterized in acute muscle injury experimental models.

The regeneration process of acute muscle injury models is classically biphasic, with an initial inflammatory phase followed by a secondary resolutive phase (for review, see Tidball[13]). During the initial phase, resident macrophages differentiate into pro-inflammatory M1-biased phenotype (CD163$^-$), aiming to phagocyte debris. This is coordinated by interferon-γ (IFN-γ), a major component of muscle inflammatory response, acting through the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway. Indeed, IFN-γ levels are dramatically increased immediately after the injury, and blocking IFN-γ reduces M1-type macrophage activation.[13] TNF-α is the second major mediator of the inflammatory phase by activating the NF-κB pathway in macrophages. In addition to resident macrophages, circulating monocytes are also recruited during this initial inflammatory phase.[66] A study performed in a cardiotoxin-induced acute injury model in mice showed that secretion of the IGF-1 propeptide IGF-1Ea by M1-biased macrophages promoted SC proliferation in the acute inflammatory phase.[67] IGF-1 secretion was later on sustained mainly by a population of non-myeloid, non-myogenic cells, which were probably fibroblasts.[68] Interestingly, IGF-1 also had effects on macrophages themselves, via an autocrine feedback loop limiting inflammation and promoting muscle reconstruction. The inflammatory phase appears essential for muscle homoeostasis, as doxorubicin treatment in exercise-challenged mice provokes muscle loss and accelerated death specifically by depleting the M1-macrophage population.[69]

The transition towards the regenerative phase is accompanied by a progressive shift from M1 to M2-biased phenotype (CD163$^+$) within the muscle tissue, after 4–7 days, in order to support the production of connective tissue and promote regenerative fibrosis. This polarization shift is likely to occur in the same cells as in the inflammatory phase[66,70] and is possibly functionally coupled to the transition in stages of myogenesis (from proliferation to differentiation). This transition is paralleled by a shift in secreted factors, from IFN-γ and TNF-α to IL-10, amphiregulin, IGF-1 and TGF-β.[71] Tregs produce IL-10 and amphiregulin, whereas macrophages produce IL-10 and IGF-1. Apart from secreted factors, the phenotypic transmembrane marker CD163 also actively takes part in the regeneration process by binding to haemoglobin–haptoglobin complexes and enhancing IL-10 secretion.[72] The polarization shift is also accompanied by 5′-adenosine monophosphate-activated protein kinase (AMPK) activation (at least partly via IL-10), which further enhances phagocytosis and promotes M2 polarization. Differentiated, M2-biased macrophages stimulate myogenesis and myotube fusion.[66,71]

Thus, the sequence of events leading to muscle repair has been well identified. However, the specific reason for the polarization shift remains unclear, although phagocytosis of debris has been recognized as one direct mechanism, both by creating space for repair and by activating the secretion of myokines implicated in the later stages, such as TGF-β, through the activation of AMPK pathway. Perhaps most interesting is the hypothesis that phagocytosis of debris could generate a form of immunological memory in macrophages that would facilitate their ability to recognize and respond to tissue damage, as suggested by an elegant study performed in drosophila.[73]

This physiological response to injury is disturbed in several sarcopenia models. For example, a recent study showed that in mice, ageing leads to reduced macrophage density in the muscle and impaired IGF-1 production. Furthermore, ageing mice do not display the classical polarization shift contrary to young mice.[74] Another ageing mouse model recently allowed the identification of a novel subset of skeletal muscle macrophages, named interferon-responsive macrophages (IFNRMs), which promote the proliferation and differentiation of SCs via CXCL-10 secretion; such cells were less abundant in older mice muscle.[75]

Of note, a particular subset of macrophages called fibrocytes, originating from the bone marrow and bearing features of both leukocytes and fibroblasts,[76] could also play

a role in muscle homoeostasis. Fibrocytes are defined as monocytes-derived, double-positive for CD45 and collagen,[77] which allows to distinguish them from FAPs. Although they have initially been described as pro-fibrotic, matrix-producing cells, more recent data pointed towards an extended regulatory role of these cells, which are notably able to regulate ECM turnover by collagen endocytosis.[77] They also have an immune regulatory role, as they are able to secrete pro-inflammatory cytokines as well as interacting with immune cells.[78] Fibrocyte differentiation is associated with chronic inflammation, ECM remodelling,[79] and metabolic reprogramming towards increased oxidative phosphorylation capacity,[80] suggesting that intramuscular fibrocytes could play both physiological and pathological roles in skeletal muscle function. One study reports their presence and role in the early phase of muscle regeneration after acute injury, mainly through the secretion of pro-fibrotic and pro-inflammatory factors, in the *mdx* murine model of Duchenne's myopathy.[79] Although the *mdx* model does not involve muscle wasting per se, our recent identification of fibrocytes in the quadriceps of a murine model of COPD (unpublished data) suggests that fibrocytes might play a role in skeletal muscle regeneration and wasting.

Lymphocytes

Lymphocytes take part in skeletal muscle homoeostasis notably by removing necrotic cells and secreting growth factors necessary for SC proliferation and differentiation, although they are virtually absent from the muscle tissue at the steady state.[S12] Interestingly, a recent study has shown a positive correlation between the number of $CD8^+$ T cells in the muscle rectus abdominis (as well as the total T lymphocyte number) and muscle mass, as evaluated, respectively, by immunohistochemistry and fibres cross-sectional area/computed tomography.[81] In microarray analysis, $CD8^+$ T-cell genes were also negatively associated with genes from catabolic pathways in this study (notably ubiquitin–proteasome and autophagy pathways).

In muscle repair, lymphocytes mainly coordinate the immune response in order to restore muscle homoeostasis. T lymphocytes might also directly promote myogenesis, as they support migration and proliferation of muscle SCs in vitro notably via secretion of IL-1α, TNF-α, IFN-γ and IL-13.[82] Specifically, $CD8^+$ T-cell infiltration helps in repairing the damage by promoting monocyte recruitment through the enhancement of C–C motif chemokine ligand 2 (CCL2)/monocyte chemoattractant protein-1 (MCP-1) production by resident macrophages.[82] $CD4^+$ lymphocytes are notably represented by regulators (Tregs) which account for around 50% of $CD4^+$ T cells. Tregs accumulate in injured muscles and reach a peak at approximately 4 days post-injury, where they promote the polarization shift from M1 to M2 macrophages[83] by secreting IL-10 (cf supra). Upon IL-33 signalling, they are also able to activate SCs through the growth factor amphiregulin and play a critical role in skeletal muscle regeneration. Altogether, their depletion prevents muscle repair.[42,83] They also highly express C–C chemokine receptor type 2 (CCR2), the receptor for the chemokine CCL2/MCP-1, which attracts monocytes into damaged muscle. Thus, the same chemotactic axis could also be of importance for Tregs recruitment.

Few data are available regarding the role of lymphocytes in other muscular diseases. Historically, T lymphocytes are rather associated with a deleterious effect on skeletal muscle, such as dystrophic muscles as in the mdx mouse model[84] or idiopathic inflammatory myopathies.[S13] However, given their important role in regeneration of healthy muscle, it could be that this negative effect would only occur in case of prolonged presence in the tissue[85] as in the dystrophic models. In an ageing model, a failure to recruit enough TRegs has been observed, which partly accounts for defective repair and was improved by IL-33 administration.[42] Other types of $CD4^+$ T cells have been less investigated. However, conditioned media from the Th2 and Th17 subsets was shown to enhance angiogenesis in vitro and in vivo in a mouse model of skeletal muscle ischaemia.[S14]

A current theory is that T cells are able to take part in a 'muscle damage memory' mechanism, by analogy with the antimicrobial immune response. Supporting this theory is that an identical T-cell receptor arrangement (indicating peptide specificity) was repeatedly found among different animals in separate experiments of repeated contraction-induced damages.[83] This phenomenon could at least partly be driven by IL-15, as later demonstrated in a mouse model of autoimmune myositis.[S15] Altogether, T lymphocytes represent a growing research interest for the future, due to their recently highlighted therapeutic potential.[86]

Other potential cells of interest, such as neutrophils, telocytes, interstitial tenocytes and neural cells (terminal Schwann cells, motor neurons, kranocytes), are discussed in *Data* S1.

Cellular interplay in COPD muscle wasting: An unmet need

COPD, a chronic respiratory disease mainly linked to cigarette smoke exposure, is a global burden and is currently the third cause of death worldwide.[8] Its pathophysiology is still partly unknown and combines genetic predisposition, epigenetic modifications and environmental factors.[87] Skeletal muscle dysfunction in COPD affects 4–35% of the patients[5] is responsible of a significant alteration of the quality of life and is associated with higher mortality. Notably, a mid-thigh muscle cross-sectional area of less than 70 cm^2, a low fat-free mass index and a reduced quadriceps strength are all independent predictors of mortality, with odds ratio up to 13-fold.[6,7,88] Muscular wasting could appear only after a few days of hospitalization according to a recent study.[89] However, no spe-

cific and efficient pharmaceutical therapy is available to date, whereas pulmonary rehabilitation remains the only validated treatment.[5]

As for many other diseases, the respective role of each cell in skeletal muscle wasting in COPD patients remains unclear. To our knowledge, no data have been reported concerning the role of immune cells, except for neutrophils, which have been found elevated in the quadriceps of patients compared with controls[90] and hypoxaemic patients versus non-hypoxaemic.[91] Vascular cells drew more interest in this field. In COPD patients, both convective and diffusive oxygen supply are impaired, contributing to the reduced $V'O_2max$. We and others have found reduced indices of muscle capillarization, which was correlated to the patients $V'O_2max$.[92,S16,S17] In addition, the training-induced muscle angiogenesis was blunted in COPD patients, when compared with healthy age and sex-matched sedentary subjects.[92] Thus, targeting capillary rarefaction and angiogenic impairment in COPD patients could be an interesting strategy to reduce muscle fatigability and improve maximal exercise capacity. We have also observed evidences of an impairment of the capillary maturation, with disturbed pericyte/endothelial interactions during exercise training.[93] In contrast with healthy sedentary subjects, the pericyte coverage of muscle capillaries in COPD patients during exercise training has been found defective.[94] This capillary maturation impairment occurs early in the disease time course, as a consequence of deleterious muscle micro-environment.[94]

Concerning progenitor cells, it has been shown that SCs exhibit alterations of their own in COPD muscle, notably a senescent phenotype and increased oxidative stress, although their density seems unchanged.[95,96] Furthermore, primary SCs isolated from COPD patients display~~ed~~ a delayed activation in culture, a decreased expression of myosin heavy chain during myotube formation[97] and a reduced myotube diameter[95] compared with controls, pointing towards impaired differentiation. In vitro proliferation capacity was unchanged in these reports.[95,97] Interestingly, a recent study suggested that a deleterious micro-environment to SCs brought by serums from COPD patients could contribute to the atrophy of healthy human myotubes in culture.[98] A study also showed evidences of activated SCs as well as increased apoptotic rates in the quadriceps of sarcopenic patients compared with non-sarcopenic, as well as in COPD patients compared with control subjects.[99] These data pointed towards a lower regenerative potential. Last, recent data point towards impaired autophagy specifically in satellite cells from a mouse model of emphysema exhibiting sarcopenic features.[100] Of note, the muscle hypertrophic response to exercise training has been found impaired specifically in hypoxaemic patients compared with non-hypoxaemic ones,[101] pointing out altered muscle adaptive response in this specific subset. Finally, as some studies have found evidences of intra-muscular fat increase in COPD patients, notably at the tissular level evaluated by CT,[S18] studying the role of FAPs could be of interest in COPD muscle, considering their adipogenic potential.

Evidences for neuromuscular junction degeneration also exist in the quadriceps of COPD patients, and mice exposed to CS extract during 4 months exhibit early neuromuscular junction degeneration preceding fibre-type shift and muscle atrophy.[102] Interestingly, the transcript muscle specific kinase (MuSK), regulating neuromuscular transmission, has been found to be more than twofold increase in COPD quadriceps compared with healthy subjects.[103] Moreover, neuromuscular junction degeneration following ageing has been shown to be accompanied by increased cholinergic activity, and increased cholinergic environment is a well-known key feature of COPD. Reduction in acetylcholine level in mice showed larger neuromuscular junction but also reduced expression of the pro-atrophic *FoxO1* transcription factor and increase in satellite cell number.[S19] Altogether, increased cholinergic signalling should be investigated as a possible contributor for muscle wasting in COPD.

Modelling COPD to study its complex pathophysiology is a relevant challenge. Several animal models (mouse, rat, guinea pig, hamster) with various modalities (exposure to cigarette smoke extract, elastase, lipopolysaccharide or transgenic animals) are reported in the literature.[104] Most of these models are primarily designed to investigate the pulmonary condition[105]; however, some of these models are used to study muscle wasting in COPD and are able to recapitulate features of the human disease, whether phenotypic (decreased muscle mass and strength), histological (decrease in myofibres cross-sectional area, fibre-type switch from type I to II, decreased capillarity) or molecular (increased catabolism, decreased mitochondrial metabolism).[14] To our knowledge, only one recent study investigated the role of resident skeletal muscle cells in muscle wasting and found a reduced proliferative capacity of SCs, linked to a deaccelerated autophagy.[100] Overall, no animal model perfectly recapitulates all features of the disease, as results are often heterogeneous between studies, and within a same model, between the different muscles. Moreover, none of the models accurately reflect the complexity and the different phenotypes of the human disease. Table 2 recapitulates the pros and cons of animal versus human experimentation in addressing the question of peripheral skeletal muscle wasting in COPD. Finally and importantly, neither animal models nor human studies currently address the specific question of the cellular interplay in muscle wasting.

Discussion and perspectives

Skeletal muscle wasting is a prevalent condition, whose diagnosis is hampered by the heterogeneity of clinical entities as well as under-diagnosis, and management suffers from lack

Table 2 Pros and cons of animal versus human experimentation in addressing the question of peripheral muscle wasting in COPD

			Animal model (rodents)	Human	Ref.
Biological concerns	Recapitulation of disease complexity/ diversity of COPD phenotypes		V	V	105
	Model relevance	Mechanical concerns	V (quadrupedal walking)	V	
		Myofibre types	V (I, IIa, IIb, IIx)	V (I, IIa, IIx)	S21
		Fibre-type repartition	V (less mixed muscles)	V	
		Mitochondrial respiration	V (only one different respiration state)	V	S22
		Sex	V/V (mostly males)	V	S23
	Functional assessment		V (difficult to control)	V	S24
	Availability of samples		V	V	
	Acute muscle injury experiments		V	X	13
	Time-course experiments		V	V	
	Access to several muscles		V	V	
	Primary cell culture		V	V	
	Genome-wide screening		X	V	
	Therapeutic interventions		V (ethically possible, but difficulty to translate results to human)	V (need to pass pre-clinical studies)	34, 111
Ethical concerns	Level of ethical committee requirements		V/V (existing limitations)	V	
Practical concerns	Duration of experiments		V (some models require several months of CS exposure)	V	
	Relative cost		V	V	

CS, cigarette smoke; V, best; V, partly suitable; X, not suitable.

of targeted therapeutics. In this review, we address the respective roles of resident tissular cells graviting around myocytes in skeletal muscle homoeostasis, regeneration and sarcopenia, thanks to data obtained from various animal models, such as COPD, cancer, acute muscle injury or ageing. Progenitor cells such as satellite cells and fibro-adipogenic progenitors are primarily implicated in maintaining the myocyte pool, but vascular, immune and nervous cells all play a role in progenitor cells proliferation and differentiation, in addition to having a potential myogenic fate for vascular cells. Overall, few mechanisms are common between homoeostasis and regeneration, as most resident cells are quiescent at the steady state but become activated upon disruption of homoeostatic condition, whether subtle (e.g. exercise) or substantial (muscle injury or disease). Such activation comes along with a metabolic switch from predominant oxidative phosphorylation to glycolysis, as seen in FAPs[41] or SCs.[19] This highlights the sensor role for nutrients and redox state for these cells, as well as the requirement of rapid energy production upon proliferation, as described in other cell types.[106] Interestingly, fibrocytes switch their metabolism towards predominant glycolysis upon differentiation.[80] Furthermore, inhibiting mitochondrial respiration in cultured SCs from control mice promoted the expression of stemness, self-renewal and return-to-quiescence markers.[19] In contrast, stimulation of mitochondrial protein synthesis via the metabolic modulator trimetazidine promoted myogenesis markers expression in vivo in aged mice.[107] This discrepancy might come from the fact that metabolic modulators act indifferently on every skeletal muscle resident cell in vivo. Nevertheless, these data open new therapeutic avenues in the field of metabolic reprogramming in order to enhance skeletal muscle regeneration capacities. Such approach has already been successfully tested in a mouse model of amyotrophic lateral sclerosis.[S20] Should this be translated to sarcopenia, studies should aim at identifying regulators of the different metabolic pathways involved in every resident muscle cell type, in order to develop a targeted therapy, which should be tested in vivo in order to examine the effect on the whole tissue. Finally, promoting regeneration should be handled with caution as it is possible that repeated events of micro-regeneration (e.g. due to hypoxia or toxicity of CS exposure, which could be incriminated in COPD) lead to premature SCs exhaustion. Current treatments for muscle wasting depend on the underlying condition. Efforts to safely increase both muscle mass and force tend to be unsuccessful. The anabolic hormone testosterone has shown to increase both muscle mass and function,[108]; however, it is associated with important side effects such as increased cardiovascular events in older individuals.[109] Although blocking the activin type II receptor (ActRIIB) pathway has been shown to be very promising in animal cancer cachexia models, in terms of not only muscle mass gain but also lifespan,[34] such treatment has not been implemented successfully yet in clinical practice. Its use in patients with COPD in particular did not improve skeletal muscle function.[110] Moreover, blockade of ActRIIB in wild-type mice led to severe fatigue, and even to decreased muscle force via decreased capillarization and oxidative metabolism in the *mdx* mouse model.[111] Overall, there is currently no FDA-approved drug to treat muscle wasting. However, promising leads are still emerging, such as the use of IL-4 in a pre-clinical cancer model, allowing to rescue muscle function and prolong lifespan.[112] Overall, precising the role of tissular resident cells can be useful in order to develop specific ther-

apies for diseases associated with muscle wasting, for example, by targeting cell recruitment or differentiation.

Pre-clinical models do not completely reflect the complexity of human disease and are often quite heterogeneous in their ability to reproduce every characteristic of disease-induced muscle wasting, as illustrated in the field of COPD. Moreover, current research tends to replace animal models with models avoiding the use of living organisms, such as the use of organoids, whether animal or human derived (also referred to as 'BAM': bio-artificial muscles). Although the field of muscle organoids is not as advanced as in other organs such as lung organoids,[113] it is clearly expanding and a very promising lead to study the dynamics of cellular regeneration. Several models have been published in the last decade, ranging from mono-cellular to a complex functional multi-cellular organoid, vascularized, innervated and able to contract.[114–116] Such models have been used successfully to model exercise training, showing an increase in IL-6 secretion and an AKT signalling pathway activation.[117] Of note, secretome of tumour organoids can also exhibit characteristic features of cachexia, as shown by a recent study performed in pancreatic tumour organoids.[118] However, to our knowledge, there is currently no study investigating the molecular and cellular mechanisms of muscle wasting using such constructs. Nevertheless, there is no doubt that muscle organoids will be at the core of basic research in the future years.

To conclude, skeletal muscle wasting is a crucial issue for patients suffering from chronic diseases, but also ageing healthy individuals. Better characterizing the role of each cell population within skeletal muscle is of interest in order to develop specific therapies for muscle regeneration and growth.[119]

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Conflict of interest

Pauline Henrot, Léo Blervaque, Isabelle Dupin, Maéva Zysman, Pauline Esteves, Fares Gouzi, Maurice Hayot, Pascal Pomiès and Patrick Berger declare that they have no conflict of interest regarding this work.

Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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