REVIEW

Journal of Cachexia, Sarcopenia and Muscle 2018; **9**: 1255–1268
Published online 30 November 2018 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/jcsm.12363

Cellular and molecular mechanisms of sarcopenia: the S100B perspective

Francesca Riuzzi[1,2†], Guglielmo Sorci[1,2†], Cataldo Arcuri[1,2], Ileana Giambanco[1,2], Ilaria Bellezza[1], Alba Minelli[1] & Rosario Donato[1,2,3*]

[1]*Department of Experimental Medicine, University of Perugia, Perugia 06132, Italy,* [2]*Interuniversity Institute of Myology,* [3]*Centro Universitario di Ricerca sulla Genomica Funzionale, University of Perugia, Perugia 06132, Italy*

Abstract

Primary sarcopenia is a condition of reduced skeletal muscle mass and strength, reduced agility, and increased fatigability and risk of bone fractures characteristic of aged, otherwise healthy people. The pathogenesis of primary sarcopenia is not completely understood. Herein, we review the essentials of the cellular and molecular mechanisms of skeletal mass maintenance; the alterations of myofiber metabolism and deranged properties of muscle satellite cells (the adult stem cells of skeletal muscles) that underpin the pathophysiology of primary sarcopenia; the role of the Ca^{2+}-sensor protein, S100B, as an intracellular factor and an extracellular signal regulating cell functions; and the functional role of S100B in muscle tissue. Lastly, building on recent results pointing to S100B as to a molecular determinant of myoblast–brown adipocyte transition, we propose S100B as a transducer of the deleterious effects of accumulation of reactive oxygen species in myoblasts and, potentially, myofibers concurring to the pathophysiology of sarcopenia.

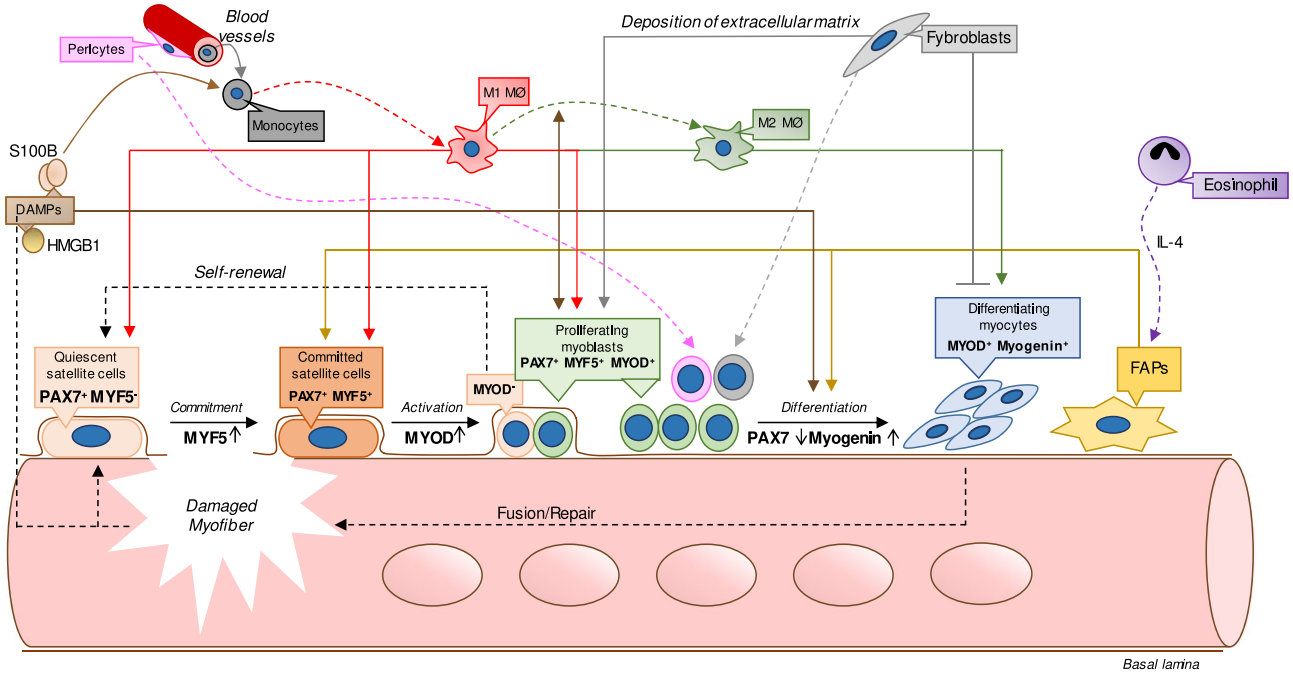
Keywords Sarcopenia; S100B; Oxidative stress; Myoblast; Brown adipocyte; Myofiber

Received: 15 June 2018; Accepted: 27 September 2018
**Correspondence to: Rosario Donato, Department of Experimental Medicine, University of Perugia, Perugia 06132, Italy. Tel.: +39-075-585-8256, Email: rosario.donato@unigp.it*
†The first two authors equally contributed to the present work.

Introduction

Sarcopenia is defined as a condition of reduced skeletal muscle mass and strength, reduced agility, and increased fatigability and risk of bone fractures[1–4] characteristic of aged (≥75-year-old) people and represents a major health problem and an important burden for health systems because of the growing lifespan in advanced countries. Sarcopenic muscles show reduced numbers of myofibers and hypotrophic myofibers (mostly Type II myofibers) and infiltration with adipose and, at later stages, fibrotic tissue, and decreased numbers of satellite cells (SCs),[5] the adult stem cells of skeletal muscles located between the sarcolemma and the basal lamina and essential for the maintenance of muscle mass.[6] Normally quiescent, in case of strain and/or acute muscle injury, SCs become activated, proliferate to expand the population of their progeny (myoblasts), migrate to the sites of damage, and differentiate into myocytes capable of fusing with each other to form new myofibers or with damaged myofiber to repair them (*Figure* 1). During the initial proliferation phase, a minority of myoblasts return to a state of quiescence to replenish the pool of myofiber-associated SCs. This complex of events, known as adult myogenesis, recapitulates embryonic myogenesis and is intended to maintain the skeletal muscle mass constant. It is orchestrated by the sequential activation of essential transcription factors such as Myf5, PAX7, MyoD, and myogenin, which are required for (i) the commitment of SCs to proliferating myoblasts (Myf5, PAX7), (ii) myoblast proliferation and the return of a minority of proliferating myoblasts to the state of quiescent SCs (PAX7), (iii) myoblast proliferation and differentiation (MyoD), and (iv) myoblast differentiation into fusion-competent myocytes (myogenin)[7] (*Figure* 1). A significant extent of cross-regulation occurs among PAX7, MyoD, and myogenin during the myoblast proliferation and early and late myogenic differentiation phases,[8] anticipating that factors and/or conditions capable of altering the relative abundance

Figure 1 Schematic representation of cellular and molecular mechanisms driving adult myogenesis. Following acute (reversible) skeletal muscle injury serum factors (i.e. hormones and growth factors) and molecules passively released from damaged muscle tissue (e.g. DAMPs, among which S100B and HMGB1) or secreted by infiltrating proinflammatory (M1) and anti-inflammatory/reparative (M2) macrophages (e.g. cytokines, S100B, and HMGB1), and components of the extracellular matrix participate in the activation of quiescent muscle SCs and their commitment to myoblasts, proliferation and/or differentiation into fusion-competent myocytes, which form new myofibers and/or repair the damaged ones. It is not known whether S100B and/or HMGB1 can activate quiescent SCs. A minority of proliferating myoblasts return to a state of quiescence to replenish the SC reserve pool. Fibroblasts concur to the repair process by depositing extracellular matrix, but excess extracellular matrix deposition interferes with the regeneration process. Pericytes and IL-4-activated FAPs also participate in the regeneration process. DAMP, damage-associated molecular pattern; FAPs, fibro/adipogenic precursors; IL-4, interleukin-4; SC, satellite cell.



of these transcription factors may disturb the myogenic process or even concur to rhabdomyosarcomagenesis. Infiltrating immune cells, among which macrophages play an essential role,[9–12] vascular pericytes[13,14] and fibro/adipogenic precursors (FAPs)[15,16] participate in muscle regeneration (*Figure* 1). The muscle regeneration process is driven by extracellular factors such as serum factors and molecules passively released from damaged muscle tissue—collectively known as damage-associated molecular patterns (DAMPs)—or secreted by infiltrating immune cells (i.e. hormones, growth factors, and cytokines) and components of the extracellular matrix[17–24] (*Figure* 1) acting via cell surface receptors.

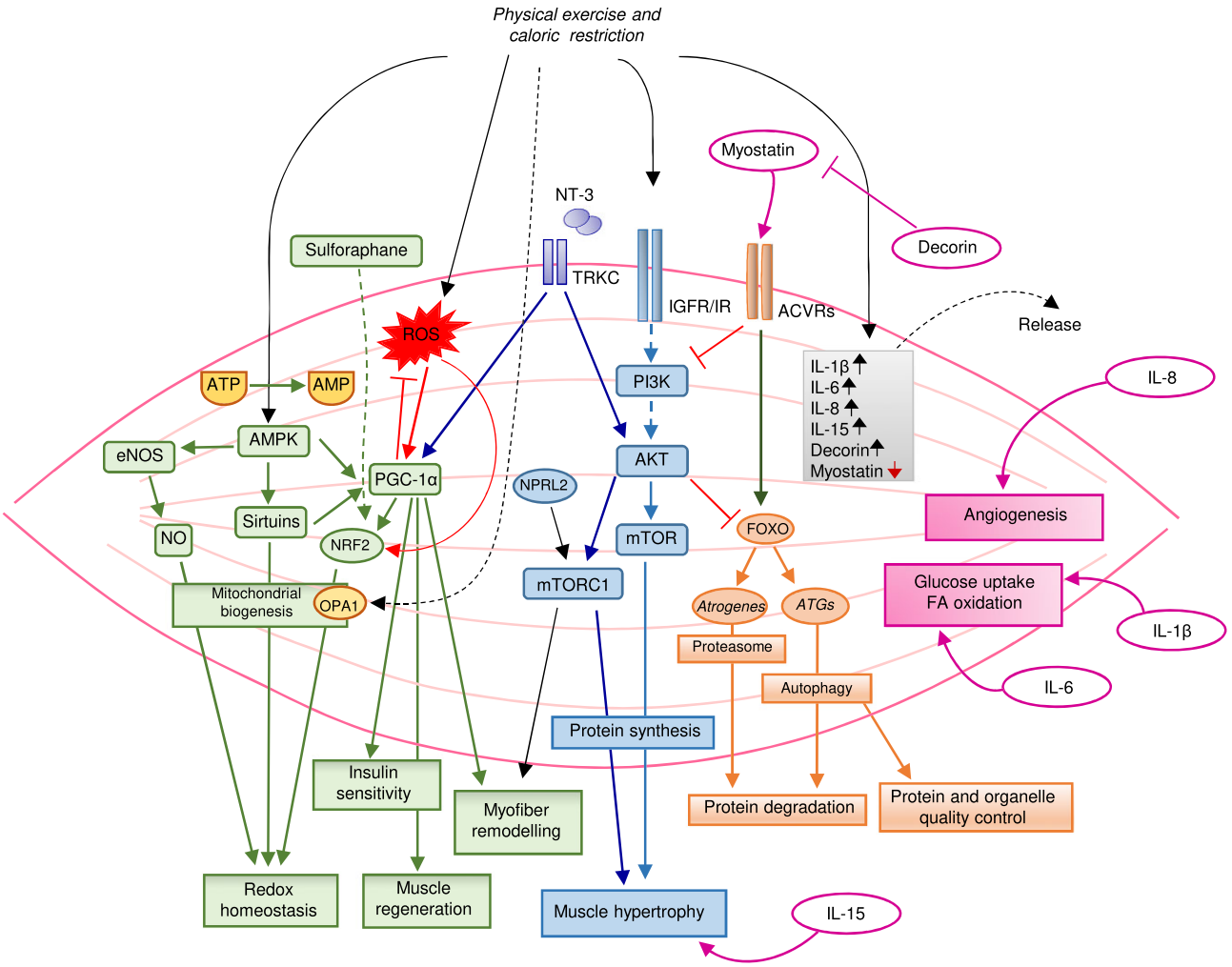
Primary sarcopenia: a matter of altered myofiber metabolism and deranged satellite cell properties

Sarcopenia may be either secondary to chronic inflammatory statuses, diabetes, hormonal alterations, vascular disturbances, renal, respiratory and/or cardiac failure, and immobilization[3] or primary, occurring in otherwise healthy, usually aged persons.[1,2] The pathogenesis of primary sarcopenia is not completely understood. Alterations of myofiber metabolism and deranged SC properties appear to underpin the pathophysiology of primary sarcopenia.

Altered myofiber metabolism

From the side of myofibers, disturbed protein turnover consequent to impairment of protein degradation and intracellular organelle disposal pathways [e.g. the insulin-like growth factor 1/protein kinase B (Akt)/mammalian target of rapamycin (mTOR)/forkhead box O (FoxO), and macroautophagy pathways] leading to a failure of protein and organelle quality control[25,26] might contribute to muscle atrophy (*Figure* 2). Conversely, physical exercise coupled to caloric intake restriction increases mitochondrial calcium uniporter expression levels and affects mitochondria dynamics with improved ultrastructural morphology and performance of aging human

Figure 2 Physical exercise and caloric restriction exert trophic/protective effects on skeletal muscles via several mechanisms. A moderate and balanced production of ROS is beneficial to muscle trophism. Green and light blue boxes/pathways refer to anabolic and/or antioxidant mechanisms leading to muscle structure/function preservation, enhanced insulin sensitivity, myofiber remodelling, and redox homeostasis. Pink boxes/pathways refer to catabolic mechanisms required for protein and organelle quality control and, hence, normal muscle trophism. However, hyperfunction of these pathways leads to muscle hypotrophy/atrophy. Released by exercising muscles, myokines (listed in the grey box; ↑ = increased release, ↓ = decreased release) exert either protective (e.g. IL-1β, IL-6, IL-8, and decorin; magenta boxes and symbols) or detrimental (e.g. myostatin; pink boxes and symbols) effects on muscle trophism. ACVRs, activin A receptors; Akt, protein kinase B; AMP, adenosine monophosphate; AMPK, adenosine monophosphate kinase; *ATGs*, autophagy genes; eNOS, endothelial nitrix oxide synthase; IL-1β and IL-6 promote muscle trophism, stimulating glucose uptake and fatty acid (FA) oxidation. FOXO, forkhead box O; IGFR/IR, insulin growth factor receptor/insulin receptor; IL, interleukin; mTOR, mammalian target of rapamycin; NRF2, NF-E2-related factor 2; NO, nitrix oxide; NPRL2, Nitrogen permease regulator-like 2; NT-3, neutrophin 3; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α; PI3K, phosphoinositol-3-kinase; ROS, reactive oxygen species.



muscles[27] likely mediated by preserved expression levels of mitochondrial fusion protein[28] optic atrophy 1 (*Figure* 2). Peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1α), enriched in tissues with high oxidative capacity, is a key driver of metabolic programming in skeletal muscle in health and disease regulating endurance, fibre-type switching, and insulin sensitivity.[29] PGC1α is induced by oxidative stress, is a powerful regulator of reactive oxygen species (ROS) removal by increasing the expression of numerous ROS-detoxifying enzymes and factors such as NF-E2-related factor 2 (Nrf2),[30] and is directly regulated by the transcription factor Tfe3 in muscles[31] (*Figure* 2). By inducing a moderate level of oxidative stress, physical exercise up-regulates PGC1α essential for mitochondriogenesis promoting oxidative fibre formation at the expense of glycolytic fibre formation,[32] improving exercise performance,[33] increasing muscle mass and strength and resistance to muscle wasting,[34] and augmenting early steps in the activation and proliferation of adult muscle stem cells.[35] Thus, altered PGC1α expression level/activity in muscle tissue likely consequent to age-related enhanced ROS production in a background of altered mitochondria number and properties[36,37] might concur to sarcopenia.

FoxO transcription factors are key effectors in muscle atrophy. They are activated in multiple models of muscle atrophy and are both sufficient and required for muscle atrophy[38] (*Figure* 2). FoxOs co-ordinate a variety of stress-response genes, including autophagy and ROS detoxification, during catabolic conditions.[39] However, sarcopenia was suggested to be not due to FoxO activation or up-regulation of the proteolytic systems as interventions aimed at promoting muscle hypertrophy via Akt overexpression or preventing muscle loss through inactivation of the ubiquitin ligase atrogin-1 were found to paradoxically cause muscle pathology.[25] Yet a ROS–FoxO cross-talk occurs in cells whereby ROS and FoxO reciprocally regulate their levels,[40,41] and old sedentary rat muscles show elevated levels of the atrophy genes (atrogenes), atrogin-1 and MuRF-1.[42]

Defective activity or abundance of Nrf2, an antioxidant factor playing a fundamental role in the maintenance of intracellular redox homeostasis, characterizes the aging muscle tissue (reviewed by Bellezza *et al*.[43]), an event counteracted by a regular exercise that attenuates the age-related changes in Nrf2-mediated pathways and potentially restores the redox homeostasis[28,44–46] (*Figure* 2). Supporting this possibility is the observation that the Nrf2 activator, sulforaphane, enhances running capacity in rats by up-regulation of Nrf2 signalling and downstream genes and attenuates muscle fatigue via reduction of oxidative stress caused by exhaustive exercise.[47] Also, there is evidence that neurotrophin 3 directly influences the protein synthesis and metabolic remodelling in neurogenic muscle via activation of a TrkC/Akt/mTORC1 pathway in myofibers but not myoblasts,[48] and that nitrogen permease regulator like-2, a proposed tumour suppressor gene, activates mTORC1 causing muscle hypertrophy with increased numbers of fast-twitch, Type II glycolytic muscle fibres[49] (*Figure* 2). However, constitutive activation of mTORC1 can affect global metabolic changes and elicit late-onset myopathies.[50–52]

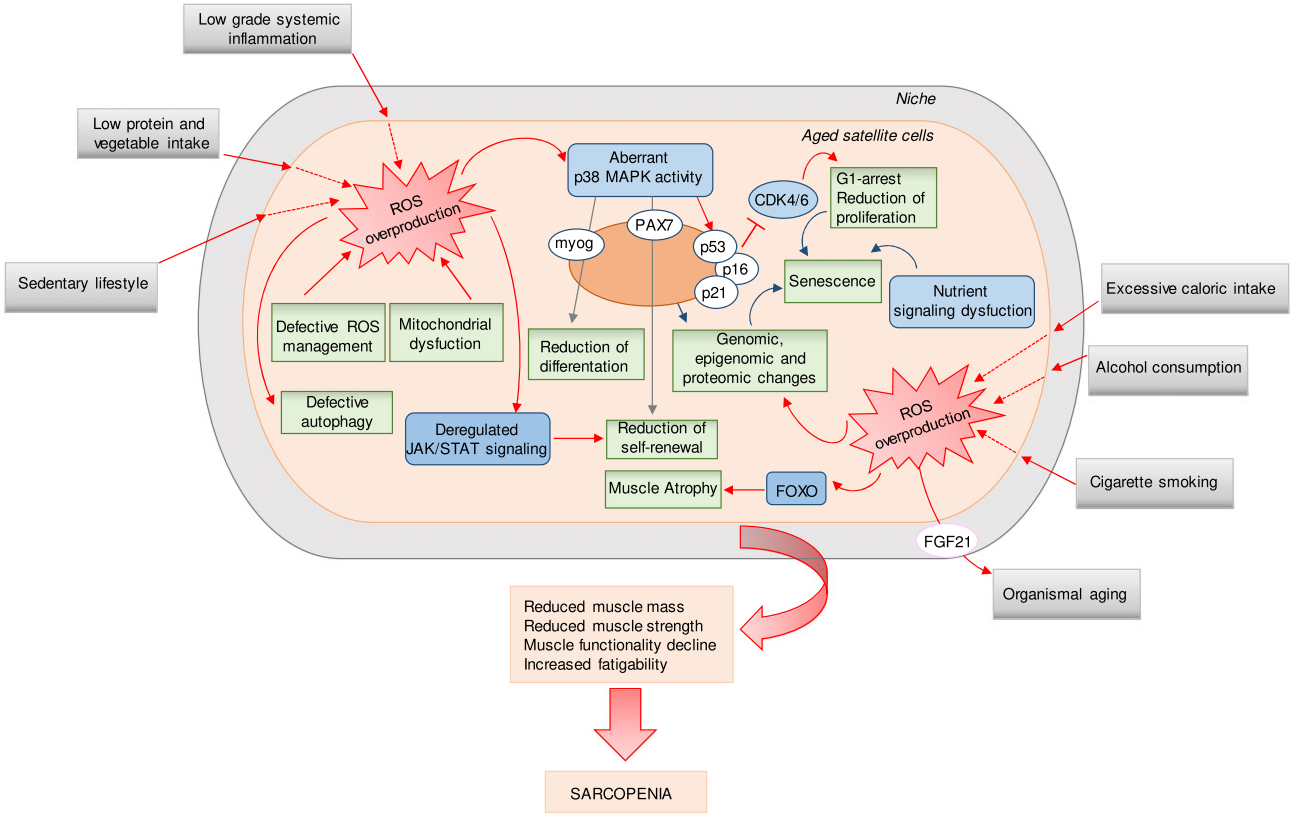
Elevated levels of myogenin, an essential transcription factor in the regulation of myoblast differentiation, were found to induce muscle wasting in different conditions including denervation, spinal muscular atrophy, starvation, and tumor necrosis factor-α (TNF-α)-induced atrophy.[53–56] In these atrophying conditions, myogenin was up-regulated and was required for the maximal activation of atrogin-1 and MuRF-1 by binding their promoters. Recent work in humans has confirmed the elevated levels of myogenin in healthy elderly sarcopenic subjects along with a less vigorous response in terms of Nrf2 levels and, importantly, with higher levels of HSPA1A, a stress-induced chaperone acting upon misfolded proteins.[57] These results suggest that reduced cellular defence factors, increased level of misfolded proteins, and disturbed mitochondria function/dynamics in healthy elderly sarcopenic muscle may contribute to intracellular accumulation of ROS, which may negatively impact muscle trophism and function.[37] Collectively, these observations support the conclusion that metabolic events occurring within myofibers condition muscle histology and performance.

Importantly, skeletal myofibers can produce a plethora of secreted factors collectively called myokines, with such secretory capacity increasing during muscle contractions, myogenesis, and muscle remodelling, or after exercise training.[58–63] Myokines exert autocrine, paracrine, or endocrine effects, thereby not only regulating energy demand but also contributing to the broad beneficial effects of exercise on cardiovascular, metabolic, and mental health.[62] Among the various myokines, interleukin (IL)-15 stimulates myofiber hypertrophy, IL-8 stimulates angiogenesis, IL-6 stimulates glucose uptake and fatty acid oxidation, and decorin binds to and blocks myostatin, another myokine, thus preventing myostatin's inhibitory effects on muscle trophism. Overall, physical exercise reduces myostatin expression/secretion[62] (*Figure* 2). Also, physical exercise recruits neutrophils to the working muscles and activates muscle resident neutrophils, which secrete IL-1β that in turn positively supports muscle performance by priming exercise-dependent glucose transporter 4 translocation.[63] In addition, voluntary exercise was shown to elicit an acute oxidation-induced increase in Ca^{2+} sensitivity in Type II fibres, and specific force production was found to be 10–25% lower in muscle fibres of sedentary compared with active rats with accompanying decrease in excitability of skinned fibres.[64] Thus, physical exercise appears to be the most efficient means to contrast sarcopenia.[65]

Deranged satellite cell properties

From the side of SCs, extrinsic, niche-related factors and intrinsic, cell-autonomous factors concur to determine changes in SCs with aging ultimately leading to reduced SCs' ability to maintain muscle mass[5,18,66–73] (*Figure* 3). Activated aged SCs/proliferating aged myoblasts accumulate ROS; possibly, ROS overproduction, owing to altered mitochondrial function and/or defective ROS management, might be one cause of sarcopenia.[41,57,74,75] ROS imbalance might determine the aberrant p38 mitogen-activated protein kinase (MAPK) activity,[67,68] deregulated expression of the cell proliferation inhibitor, $p16^{INK4a}$,[69] deregulated janus kinase/signal transducer and activator of transcription (JAK-STAT) signalling,[70] and defective autophagy[73] detected in aged SCs and suggested to be responsible for their altered proliferation and differentiation properties. If so, the question is: What determines the accumulation of ROS in aged myofibers and SCs/myoblasts? A sedentary lifestyle, in turn promoted by the reduced muscle strength and agility and increased fatigability inherent to sarcopenic subjects, coupled to a relatively excessive caloric intake, is likely to be one main cause especially if associated with cigarette smoking, alcohol consumption, and low protein and vegetable intake[76] (*Figure* 3). Mechanistically, myostatin was shown to increase ROS and TNF-α production in myoblasts, and the elevated TNF-α in turn stimulates myostatin expression with resultant

Figure 3 Extrinsic and intrinsic, cell-autonomous factors concurring to determine changes in SCs with aging ultimately leading to reduced SCs' ability to maintain muscle mass (sarcopenia). Redox imbalance, due to either ROS overproduction or consequent to defective ROS removal, is a common trait of factors/lifestyle leading to cell senescence, reduction of SC self-renewal, and muscle atrophy (refer to text). CDK, cyclin-dependent kinase; FGF21, fibroblast growth factor 21; FOXO, forkhead box O; JAK/SAT, janus kinase/signal transducer and activator of transcription; MAPK, mitogen activated protein kinase; ROS, reactive oxygen species; SC, satellite cell.



proteasomal-mediated catabolism of intracellular proteins.[77] Also, levels of angiopoietin-like 2 (ANGPTL2), a senescence-associated secretory phenotype factor with proinflammatory properties associated with age-related diseases, increase in skeletal muscle of aging mice; however, mice with a skeletal myocyte-specific *Angptl2* knockout have attenuated inflammation and ROS accumulation in animal models of muscle atrophy, accompanied by increased SC activity and inhibition of muscular atrophy, than have wild-type mice.[78]

In addition, cell senescence caused by telomere erosion; DNA damage; mitochondrial, proteostatic, and nutrient signalling dysfunction; and epigenetic factors is a central hallmark of aging: a combination of these conditions can induce senescence manifesting as the cell's inability to proliferate in response to appropriate stimuli owing to the hyperactivity of two main signalling pathways, $p16^{INK4a}$/Rb and $p53/p21^{CIP1}$, which concur to repress cyclin-dependent kinase 4/6 (CDK4/6) activity ultimately leading to stem cell exhaustion[79] (*Figure* 3). The initial enthusiasm about the finding that growth differentiation factor 11 (GDF11), a myostatin homologue, might support muscle trophism and regeneration and contrast muscle aging[80] was dampened by reports showing that, on the contrary, GDF11 levels increases with age and inhibits muscle regeneration, behaving like transforming growth factor-β and sharing with it intracellular signalling pathways,[81] and is not a rejuvenator of aged skeletal muscle SCs.[82] Instead, GDF11 is risk factor for age-related frailty and disease in humans.[83]

However, there is evidence that exhaustion of the SC pool might not concur to sarcopenia while leading to defective regeneration following acute muscle injury and contributing to age-related muscle fibrosis.[84,85] This would imply that primary sarcopenia might only be a matter of altered myofiber metabolism as discussed earlier. Yet the greater extent of age-related muscle fibrosis, a hallmark of sarcopenia, in a background of genetic ablation of SCs[84] suggests that altered SC properties might concur to sarcopenia given the accepted role of SCs in regulating the extracellular matrix.[86] In addition, as pointed out recently,[87] the daily life of aged mice in which SCs had been genetically ablated is not comparable with that of aged humans in terms of physical activity and occasional muscle injury in the course of bouts of intense physical strain, which calls for caution in the interpretation of results obtained with animal models of sarcopenia as

described by Fry *et al*.[84] and Keefe *et al*.[85] Besides, building on a number of experimental observations, SCs have been recently proposed to 'act as a central node for intercellular communication across a spectrum of cell types within and outside of muscle to coordinate adaptation' including fibroblasts and capillary endothelial cells.[86] In this perspective, the decrease in the SC number associated with sedentary aging individuals as well as altered SC properties as mentioned earlier might impact muscle mass and performance as seen in sarcopenia. Yet the studies by Fry *et al*.[84] and Keefe *et al*.[85] provide critical insight into the potential importance (or lack) of SCs in the development/progression of primary sarcopenia.

The S100B perspective

S100B functions as an intracellular regulator and an extracellular signal

S100B belongs to a family of Ca^{2+}-binding proteins of the EF-hand type and is endowed with intracellular and extracellular regulatory functions.[88] S100B is a Ca^{2+}-sensor protein expressed in a restricted number of vertebrate cell types, where it has been implicated in the regulation of cell proliferation, migration, and differentiation and apoptosis by affecting enzyme activities, the dynamics of cytoskeleton constituents (e.g. microtubules, and Type III intermediate filaments and microfilaments), protein degradation, p53 activity, K^+ channels, Ca^{2+} homeostasis, and the activity of toll-like receptors 3 and 9.[88–90] The high abundance of S100B in the cell types in which it is expressed, its cytoplasmic localization, and its becoming activated on the occasion of Ca^{2+} transients (especially at intracellular sites where free Ca^{2+} attains relatively high concentrations such as sub-plasma membrane domains, Ca^{2+} stores and mitochondria) make it possible that S100B might regulate such a variety of intracellular activities. S100B is constitutively expressed in quiescent SCs, proliferating myoblasts, myotubes, and myofibers.[91–93] S100B was found to be associated with Type III intermediate filaments in myoblasts and myotubes in part[91] and to exert an inhibitory effect on desmin assembly *in vitro*.[94] Functional correlates of S100B association with intermediate filaments and inhibitory effects on assembly state of desmin filaments are not known, yet. Interestingly, S100B expression is induced in certain cell types upon their activation or neoplastic transformation. Cardiomyocytes do not express S100B in normal physiological conditions, but S100B becomes induced by catecholamines in surviving cardiomyocytes of the so-called penumbra (i.e. the peri-infarct heart tissue) and inhibits cardiomyocyte hypertrophic response.[95] Also, bronchial epithelial cells and macrophages do not express the protein normally but express it in case of airway infection (bronchial epithelial cells)[96] or sterile inflammation (macrophages infiltrating muscle tissue upon acute injury).[22] Further, S100B becomes expressed in activated $CD3^+$ T lymphocytes infiltrating pancreatic islets in an animal model of diabetes.[97] Moreover, S100B becomes expressed in breast tumour epithelial cells under the combined action of homeobox C1 and steroid receptor coactivator, and strong associations between S100B tissue expression and reduced disease-free survival in breast cancer were reported, with elevated serum levels of S100B strongly predicting poor survival.[98] Lastly, there is evidence that at least a fraction of intracellular S100B in a number of cell types can derive from uptake of extracellular S100B.[99–101]

Indeed, S100B is secreted constitutively by astrocytes[88] and following stimulation with catecholamines by white adipocytes,[102] and by activated macrophages.[22] Also, S100B is passively released from injured brain[88,89] and heart[103] and from acutely and chronically injured muscle tissue,[104] behaving like a DAMP. Conflicting results have been reported regarding the beneficial or detrimental effects exerted by released S100B in case of acute brain injury (e.g. trauma), whereas at the high levels attained in chronically diseased brain tissue (e.g. Alzheimer's disease), S100B invariably sustains inflammation with resultant direct or indirect killing of neurons and activation of astrocytes and microglia.[88,89] In the infarcted heart, S100B, released by surviving cardiomyocytes, might cause cardiomyocyte apoptosis[103] and might promote vascular endothelial growth factor (VEGF) secretion and VEGF-dependent myofibroblast proliferation potentially contributing to scar formation.[105] In acute muscle injury, a transient release of S100B[104] is required for a rapid and complete regenerative process, whereas the continuous release of the protein by damaged myofibers and infiltrating macrophages as seen in muscular dystrophy fuels inflammation and dampens the reparative process.[22] Notably, elevated levels of S100B are detected in human serum following intense physical exercise (e.g. marathon, swimming races, and soccer games).[106] These elevated serum S100B levels have been suggested to result from brain concussion known to cause increases in serum S100B and subtle, clinically not relevant brain injury as well as from exercising muscles and/or adipocytes.[106–108] Extracellular effects of S100B are largely brought about by its interaction with RAGE (receptor for advanced glycation end products, encoded by *AGER/Ager*)[109,110]; however, at least in the case of muscle injury, S100B can activate either RAGE or the basic fibroblast growth factor (bFGF)/FGF receptor 1 (FGFR1) complex depending on its own local concentration, myoblast density, and bFGF availability.[22,104,111,112] In standard (high-density) myoblast culture conditions, S100B inhibits myoblast differentiation and myotube formation and stimulates myoblast proliferation via bFGF/FGFR1 activation.[111,113,114] On the other hand, in low-density myoblast cultures and at early phases of muscle regeneration following acute injury (i.e. when the myoblast density is relatively low), S100B activates RAGE to stimulate

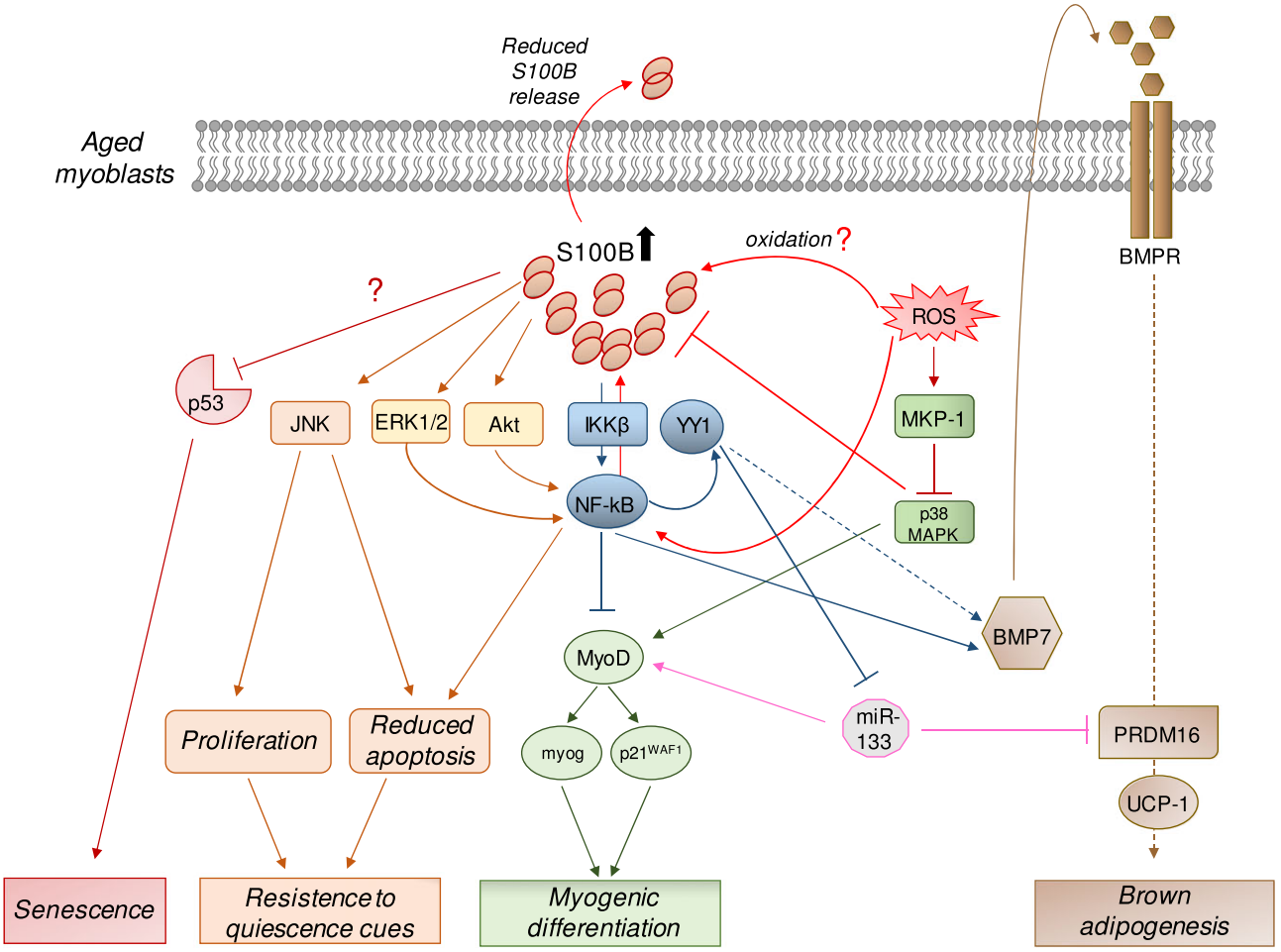
myoblast proliferation, thereby promoting the expansion of the myoblast population and preventing premature myoblast differentiation, attracting macrophages to damage sites, and promoting the transition of infiltrating macrophages from M1 (proinflammatory) to M2 (anti-inflammatory and reparative) phenotype.[22,104] Incidentally, promyogenic effects of RAGE in myoblast cultures and following acute (reversible) injury have been reported; RAGE signalling by HMGB1 promotes myoblast differentiation and myotube formation,[115] and absence of RAGE delays muscle regeneration via altered dynamics of activated SCs, delays macrophage infiltration of damage sites, and prolongs the M1 macrophage inflammatory phase.[20] Yet as observed with cultured astrocytes and neurons,[88,89] at high local concentrations, S100B directly causes myoblast apoptosis[116] and RAGE dependently perpetuates muscle damage by fuelling inflammation as observed during the acute phase of muscular dystrophy.[22] In this latter respect, it is interesting that compared with *mdx* mice, a mouse model of Duchenne muscular dystrophy, muscles of *mdx*/*Ager*$^{-/-}$ mice show restrained inflammation, unaffected fibrosis, and higher muscle strength and that *in vivo* treatment of dystrophic muscles with a RAGE blocking antibody results in reduced necrosis and inflammatory infiltrate.[117] However, extracellular S100B has been reported to impair glycolysis in cultured muscle cells independently of insulin action via inhibition of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity by enhanced poly(ADP-ribosyl)ation of GAPDH with no apparent role for RAGE or FGFR.[116] Notably, muscles of S100B KO mice show reduced levels of poly(ADP-ribosyl)ated GAPDH,[118] but there is no information about the consequences of S100B deletion on muscular performance in terms of muscle strength and resistance to intense physical activity and/or of metabolic rearrangements. While the receptor transducing that S100B's effect remains to be determined, the possibility cannot be excluded that S100B exerts it by acting from inside muscle cells following uptake from the cell milieu. Thus, as an extracellular factor, S100B might pertain to the cohort of extrinsic, niche-related molecules determining myoblast fate, and extracellular levels of S100B must be tightly regulated for it to exert beneficial effects in case of injury.

S100B as an intrinsic, cell-autonomous factor concurring to determine changes in satellite cells/myoblasts

S100B also affects myoblast survival, proliferation, and differentiation by acting from inside. Reducing S100B expression levels promotes, whereas increasing its abundance completely blocks, myoblast differentiation and fusion via activation of the IKKβ/NF-κB axis to inhibit the expression of MyoD and the MyoD-downstream effectors, myogenin and p21^{WAF1}, and to up-regulate Yin Yang 1 (YY1)[93] (*Figure* 4), a transcriptional repressor induced by NF-κB with anti-myogenic activity.[119] Interestingly, myoblasts down-regulate S100B expression once transferred from proliferation medium to differentiation medium via a p38 MAPK-driven transcriptional mechanism as well as a post-translational, proteasome-dependent mechanism, and myoblasts that have not been committed to differentiation resume expressing S100B once transferred back to proliferation medium.[118] Also, down-regulation of S100B in myoblasts during the first few hours of culture in differentiation medium is permissive for differentiation as persistence of relatively high S100B levels results in the inhibition of myogenic differentiation.[120] Down-regulation of S100B expression at the beginning of cell differentiation in not unique to myoblasts being observed also in neuronal precursor cells, oligodendrocytes, astrocytes, and chondrocytes.[121–126] Yet differentiated myoblasts (myocytes) resume expressing S100B under the action of myogenin,[120] and S100B is found expressed in mature myofibers as mentioned earlier. Notably, myoblasts down-regulate S100B expression once transferred from proliferation medium to quiescence medium, and interference with S100B down-regulation results in reduced acquisition of quiescence and a faster proliferation upon transfer of the cells from quiescence medium to proliferation medium in a *c*-Jun N-terminal kinase (JNK)-regulated manner.[120] Also, elevated S100B levels make myoblasts resistant to apoptosis in a MEK-ERK1/2-dependent, Akt-dependent, JNK-dependent, and NF-κB-dependent manner,[120] thus interfering with the process of elimination of those myoblasts which neither differentiate nor return to a quiescent state (*Figure* 4). Given the enhanced activity of the p53/p21^{CIP1} in senescent cells, which concurs to stem cell exhaustion[79] and since S100B inhibits p53 phosphorylation and tetramerization, i.e. its activation,[127–130] and reduces p53 levels,[131,132] it is tempting to speculate that high S100B levels might interfere with myoblast senescence by decreasing p53/p21^{CIP1} activity (*Figure* 4). This potential S100B activity, however, might be mitigated or even abolished by the reported ability of S100B to block the ubiquitin E3 ligases, MDM2 and MDM4,[133] that drive proteasomal degradation of p53.[134] Thus, appropriate levels of S100B in myoblasts appear to be required for myoblasts to undergo apoptosis and to timely differentiate or return to a quiescence state, with excess S100B levels interfering with these outcomes. Taken together, these results suggest that intracellular S100B might contribute to certain stemness properties of proliferating myoblasts, with its levels conditioning cell fate.

Of note, myoblasts derived from SCs isolated from sarcopenic humans are proliferation and differentiation defective and express elevated levels of S100B; however, down-regulation of S100B expression restores their proliferation and differentiation capability, thus rejuvenating them in part.[135] Different from myoblast cell lines and primary myoblasts, myoblasts from sarcopenic subjects show properties

Figure 4 Intracellular S100B levels dictate myoblast properties and fate. Oxidative stress leads to abnormally high levels of S100B in myoblasts via stimulation of NF-κB activity. Accumulation of S100B under chronic oxidative conditions might be consequent to reduced p38 MAPK activity and reduced p38 MAPK-driven S100B proteasomal degradation. Aged myoblasts show remarkably reduced ability to secrete S100B. Whether disulfide-linked formation of S100B oligomers under oxidative conditions interferes with S100B release remains to be investigated (?). In turn, S100B activates NF-κB with resultant up-regulation of YY1 and down-regulation of MyoD, and blockade of myogenic differentiation. S100B-mediated NF-κB activation also results in enhanced propensity of myoblasts to proliferate and to resist to apoptotic stimuli leading to reduced myogenic differentiation and reduced acquisition of quiescence. Although intracellular accumulation of S100B leads to defective p53 activity and uncontrolled proliferation in certain cell types (refer to text), no information is available regarding S100B's regulatory effects on p53 or p53-induced cell senescence in myoblasts (?). Lastly, oxidative stress-induced accumulation of S100B converts myoblasts into brown adipocytes via an NF-κB/YY1/miR-133 axis and NF-κB/YY1/BMP7 axis. BMP7, bone morphogenetic protein 7; BMPR, bone morphogenetic factor receptor; ROS, reactive oxygen species; UCP-1, uncoupling protein 1; YY1, Yin Yang 1.



of senescent cells being proliferation and differentiation defective.[69,135,136] It is presently unknown whether the elevated levels of S100B found in senescent myoblasts[135] are a consequence or a cause of senescence. Yet the finding that down-regulation of S100B partly restored their proliferation and differentiation capability[135] suggests that at elevated levels, S100B contributes to myoblast senescence. While S100B stimulates NF-κB activity,[93] NF-κB stimulates S100B expression in myoblasts[137] (*Figure* 4). Given the reported NF-κB/Nrf2 interplay whereby excess NF-κB activity leads to reduced Nrf2 activity,[43] it is possible that up-regulated S100B in myoblasts from sarcopenic subjects reduces Nrf2 levels and/or activity through its stimulatory effect on NF-κB, thereby fostering oxidative stress. Interestingly, myoblasts derived from SCs of young and sarcopenic humans release relatively high and very low amounts of S100B, respectively; however, administered S100B restores the proliferation and differentiation capability of sarcopenic myoblasts in part.[135] Based on previous results,[104,111,112] the receptor transducing these S100B effects likely is FGFR1 because myoblasts from sarcopenic subjects express a truncated, functionally inactive form of RAGE.[135] While the reason(s) why myoblasts from sarcopenic subjects release very low S100B amounts remains elusive, one might hypothesize that the high ROS levels of these myoblasts might alter the mechanism of S100B secretion and/or oxidize S100B, which can no longer be exported

and thus accumulates within cells (*Figure* 4). Whatever the cause of elevated S100B levels in aged SCs/myoblasts, that is high ROS/NF-κB-induced S100B expression and/or reduced S100B release (also Morozzi *et al*.[137]), intracellular S100B might be regarded as intrinsic, cell-autonomous factor concurring to determine changes in SCs ultimately leading to reduced SCs' ability to maintain muscle mass.

Chronic oxidative conditions lead to S100B accumulation in myoblasts, promoting myoblast–brown adipocyte transition

SCs are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation,[138] and myoblasts and adipocytes share a common *Myf5*$^{+}$ progenitor cell that can generate myoblasts and brown adipocytes.[139] Besides inhibiting myoblast differentiation and participating in the process of myoblast aging, elevated S100B levels also promote myoblast–brown adipocyte transition, and myoblasts from sarcopenic subjects show properties of brown (pre)adipocytes.[137] As mentioned earlier, SCs and their progeny (i.e. myoblasts) accumulate ROS owing to redox imbalance,[41,57,74,75] a condition predisposing *per se* to adipogenic differentiation given that ROS promote adipogenesis and antioxidants or ROS scavengers suppress ROS effects on adipogenesis.[140] Chronically high ROS levels induce the phosphatase MKP-1, which inactivates the promyogenic p38 MAPK.[141] Indeed, ROS overproduction in myoblasts causes accumulation of S100B partly owing to MKP-1-dependent inactivation of p38 MAPK and stimulates NF-κB activity, which cause up-regulation of S100B[137] (*Figure* 4). In turn, S100B stimulates NF-κB activity with resultant up-regulation of YY1 and YY1-dependent inhibition of miR-133, a promyogenic and anti-adipogenic microRNA,[142–144] ultimately leading to transition of myoblasts to brown adipocytes[137] (*Figure* 4). Different from myoblasts and white adipocytes, brown adipocytes express uncoupling protein 1 (UCP-1), which targets mitochondria where it uncouples oxidative phosphorylation to produce heat.[145] ROS/S100B-stimulated NF-κB also up-regulates, in a YY1-dependent manner, bone morphogenetic protein 7 (BMP7),[137] which, once secreted, causes myoblast progenitors[146,147] and cultured myoblasts[137] to differentiate into brown adipocytes by repressing the expression of the adipogenic inhibitors, necdin and preadipocyte factor 1, and inducing the key molecular determinant, PRD1-BF1-RIZ1 homologous domain-containing 16 (PRDM16), and UCP-1 (*Figure* 4). Thus, oxidative stress-induced accumulation of S100B converts myoblasts into brown adipocytes via an NF-κB/YY1/miR-133 axis and NF-κB/YY1/BMP7 axis. Supporting such a role for S100B is the observation that (i) myoblasts from sarcopenic humans show high levels of S100B, YY1, and BMP7 and features of brown (pre)adipocytes, (ii) either antioxidants or NF-κB inhibitors dampen ROS-induced up-regulation of S100B and effects consequent to S100B accumulation in myoblasts, (iii) antagonizing extracellular BMP7 blunts ROS-induced up-regulation of S100B, and (iv) C2C12 and primary myoblasts cultured in adipocyte differentiation medium up-regulate S100B and the brown adipocyte markers, PRDM16, BMP7, and UCP-1, in an NF-κB-mediated manner, and silencing of S100B in myoblast-derived brown adipocytes reconverts them to fusion-competent myoblasts[135] (*Figure* 4). Thus, in aged sedentary subjects—mimicked by geriatric mice[135]—S100B might increase, likely under the action of elevated ROS,[41,57,74,75] to such levels as to make myoblasts incapable of efficiently proliferating/differentiating[93,133] and participating in the maintenance of muscle mass, and to cause myoblast transition to brown adipocytes.[135]

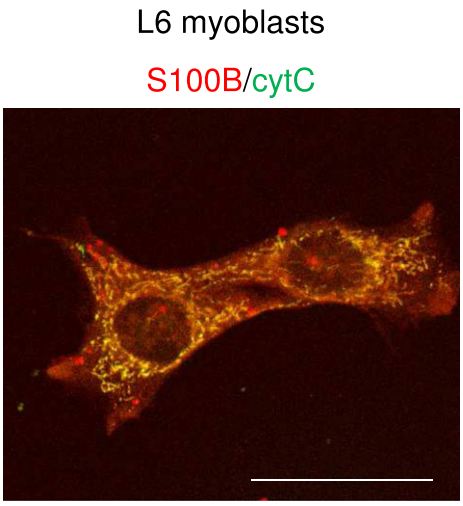
Geriatric muscles show elevated S100B levels and express uncoupling protein 1

The functional role of S100B in myofibers is not known. Given the S100B's localization to the sarcoplasmic reticulum and transverse tubules similar to S100A1,[92,93] a member of the S100 family of Ca^{2+}-binding proteins that is 56% identical to S100B,[89] one may hypothesize that S100B might regulate ryanodine receptor 1 and/or sarco(endo)plasmic reticulum calcium-ATPase activity and/or the phosphorylation state and mechanical properties of the giant sarcomeric protein, titin, as does S100A1.[148] However, there is no experimental evidence that this might be the case, while there is experimental evidence that S100B does not activate protein kinase A, differently from S100A1.[149] Also, S100B was shown to participate in the regulation of energy metabolism via stimulation of fructose-1,6-bisphosphate aldolase and phosphoglucomutase *in vitro* (reviewed by Donato[150]), but information is lacking whether S100B actually affects energy metabolism within myofibers and/or SCs/myoblasts. However, muscles of geriatric mice show highly abundant S100B, expression of UCP-1, and S100B^{+}/UCP-1^{+} interstitial cells and regenerating myofibers as opposed to muscles of young mice, which express lower S100B levels, are S100B^{+}/UCP-1^{-}, and do not show interstitial cells or regenerating myofibers.[137] S100B^{+}/UCP-1^{+} interstitial cells unlikely are fusion competent having the stigmata of brown adipocytes. Thus, the co-expression and co-localization of S100B and UCP-1 in mature and regenerating myofibers might hardly result from fusion of S100B^{+}/UCP-1^{+} interstitial cells. If so, it is tempting to speculate that geriatric myofibers up-regulate S100B in a ROS/NF-κB-dependent manner and acquire the ability to express UCP-1 probably under the action of the ROS/NF-κB/S100B axis. Future studies should validate or negate this possibility.

However, the question arises: What is the functional meaning of UCP-1 expression in geriatric myofibers? The punctuate appearance of the S100B/UCP-1 positivity points to co-localization of the two proteins to mitochondria given the

DOI: 10.1002/jcsm.12363

Figure 5 S100B is found to be associated with mitochondria in myoblasts. Proliferating L6 myoblasts[91] show co-localization of S100B (red signal) with the mitochondrial marker, cytochrome *c* (cytC; green signal). Shown is a merged image. Bar = 100 μm.



mitochondrial localization of UCP-1.[143] While a physical and functional association of S100B with mitochondria was excluded in a previous study,[151] we found that a fraction of S100B co-localizes with the mitochondrial marker, cytochrome *c*, in proliferating myoblasts[113] (*Figure* 5). Whether S100B and UCP-1 are subject to some kind of physical and/or functional interaction remains to be investigated. However, one might speculate that (i) ROS-induced accumulation of S100B in myoblasts of sarcopenic subjects hampers muscle regenerative ability blocking myoblast differentiation and driving myoblast–brown adipocyte transition and (ii) ROS-induced induction of UCP-1 consequent to elevated S100B—shown in myoblasts[137] but not formally proven in myofibers—might be instrumental to heat production in the sedentary sarcopenic subjects. While this latter event might be beneficial in assisting thermoregulation, the consequent reduction of ATP production might be detrimental, subtracting an energetic source for muscle contraction. Future research is warranted to elucidate the functional significance of UCP-1 expression and S100B/UCP-1 relationships in geriatric myofibers.

Conclusions

While the pathophysiology of primary sarcopenia has not been completely elucidated, there is consensus that ROS imbalance caused by cell senescence, defective quality control of mitochondria, reduced physical activity, and/or excess caloric intake is one main cause. ROS imbalance in turn might lead to reduced proliferation and differentiation capability of muscle SCs responsible for skeletal muscle mass maintenance, and exhaustion of the SC reserve pool. ROS imbalance also occurs in myofibers, eliciting metabolic events leading to myofibrillary protein breakdown, that is, muscle atrophy. Several extrinsic and intrinsic factors have been shown to concur to cause changes in SCs ultimately leading to altered SC/myoblasts dynamics and capability to proliferate and repair damaged myofibers. Recent studies using mice in which SCs had been genetically ablated have challenged the possibility that alterations in SC properties might play a fundamental role in the pathogenesis of primary sarcopenia—although SCs were required for reducing sarcopenia-associated muscle fibrosis and for muscle regeneration following acute injury in aged mice. Thus, SCs might not have a role in the pathophysiology of sarcopenia in aging sedentary individuals in which muscle atrophy might be due to disturbed myofiber metabolism only, yet SCs might be important players in aging non-sedentary subjects to mitigate sarcopenia progression by virtue of their ability to regenerate exercise-damaged muscles. This issue needs further investigation. S100B is emerging as an extrinsic factor acting on at least macrophages and myoblasts to regulate the timing of muscle regeneration following acute (reversible) muscle injury and during the acute phase of muscular dystrophy as well as an intrinsic factor accumulating in SCs/myoblasts and, likely, myofibers in chronic oxidative conditions under the action of a ROS/NF-κB axis, dampening myogenic differentiation and promoting myoblast–brown adipocyte transition. While the potential role of S100B in protein and organelle quality control remains to be investigated, we propose S100B as a transducer of the deleterious effects of accumulation of ROS in myoblasts and myofibers concurring to the pathophysiology of sarcopenia.

Acknowledgements

The authors were supported by Association Française contre les Myopathies (projects 12992 and 16812), Associazione Italiana per la Ricerca sul Cancro (project 17581), Ministero dell'Istruzione, dell'Università e della Ricerca, Italy (PRIN 2009WBFZYM_002, PRIN 2010R8JK2X_004, PRIN 2012N8YJC3, and FIRB RBFR12BUMH), and Fondazione Cassa di Risparmio di Perugia (projects 2012.0241.021, 2015.0325.021 and 2016-0136.021).

The authors certify that they comply with the ethical guidelines for publishing in the *Journal of Cachexia, Sarcopenia and Muscle*: update 2017.[152]

Conflict of interest

All authors declare that they have no conflict of interest.

References

1. Mitchell WK, Williams J, Atherton P, Larvin M, Lund J, Narici M. Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review. *Front Physiol* 2012;**3**:260.
2. Sakuma K, Aoi W, Yamaguchi A. Current understanding of sarcopenia: possible candidates modulating muscle mass. *Pflugers Arch - Eur J Physiol* 2015;**467**:213–229.
3. Cohen S, Nathan JA, Goldberg AL. Muscle wasting in disease: molecular mechanisms and promising therapies. *Nat Rev Drug Discov* 2015;**14**:58–74.
4. Fuggle N, Shaw S, Dennison E, Cooper C. Sarcopenia. *Best Pract Res Clin Rheumatol* 2017;**31**:218–242.
5. Verdijk LB, Snijders T, Drost M, Delhaas T, Kadi F, van Loon LJ. Satellite cells in human skeletal muscle; from birth to old age. *Age (Dordr)* 2014;**36**:545–547.
6. Dumont NA, Wang YX, Rudnicki MA. Intrinsic and extrinsic mechanisms regulating satellite cell function. *Development* 2015;**142**:1572–1581.
7. Wang YX, Rudnicki MA. Satellite cells, the engines of muscle repair. *Nat Rev Mol Cell Biol* 2012;**13**:127–133.
8. Riuzzi F, Sorci G, Sagheddu R, Sidoni A, Alaggio R, Ninfo V, et al. RAGE signaling deficiency in rhabdomyosarcoma cells causes upregulation of PAX7 and uncontrolled proliferation. *J Cell Sci* 2014;**127**:1699–1711.
9. Tidball JG, Villalta SA. Regulatory interactions between muscle and the immune system during muscle regeneration. *Am J Physiol Regul Integr Comp Physiol* 2010;**298**:R1173–R1187.
10. Arnold L, Henry A, Poron F, Baba-Amer Y, van Rooijen N, Plonquet A, et al. Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. *J Exp Med* 2007;**204**:1057–1069.
11. Chazaud B. Macrophages: supportive cells for tissue repair and regeneration. *Immunobiology* 2014;**219**:172–178.
12. Tidball JG. Regulation of muscle growth and regeneration by the immune system. *Nat Rev Immunol* 2017;**17**:165–178.
13. Dellavalle A, Sampaolesi M, Tonlorenzi R, Tagliafico E, Sacchetti B, Perani L, et al. Pericytes of human skeletal muscle are myogenic precursors distinct from satellite cells. *Nat Cell Biol* 2007;**9**:255–267.
14. Pannérec A, Marazzi G, Sassoon D. Stem cells in the hood: the skeletal muscle niche. *Trends Mol Med* 2012;**18**:599–606.
15. Joe AW, Yi L, Natarajan A, Le Grand F, So L, Wang J, et al. Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. *Nat Cell Biol* 2010;**12**:153–163.
16. Heredia JE, Mukundan L, Chen FM, Mueller AA, Deo RC, Locksley RM, et al. Type 2 innate signals stimulate fibro/adipogenic progenitors to facilitate muscle regeneration. *Cell* 2013;**153**:376–388.
17. Chargé SB, Rudnicki MA. Cellular and molecular regulation of muscle regeneration. *Physiol Rev* 2004;**84**:209–238.
18. Brack AS, Conboy MJ, Roy S, Lee M, Kuo CJ, Keller C, et al. Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. *Science* 2007;**317**:807–810.
19. Bentzinger CF, Wang YX, Dumont NA, Rudnicki MA. Cellular dynamics in the muscle satellite cell niche. *EMBO Rep* 2013;**14**:1062–1072.
20. Riuzzi F, Sorci G, Sagheddu R, Donato R. HMGB1-RAGE regulates muscle satellite cell homeostasis through p38-MAPK-and myogenin-dependent repression of Pax7 transcription. *J Cell Sci* 2012;**125**:1440–1454.
21. Dormoy-Raclet V, Cammas A, Celona B, Lian XJ, van der Giessen K, Zivojnovic M, et al. Hur and miR-1192 regulate myogenesis by modulating the translation of HMGB1 mRNA. *Nat Commun* 2013;**4**:2388.
22. Riuzzi F, Beccafico S, Sagheddu R, Chiappalupi S, Giambanco I, Bereshchenko O, et al. Levels of S100B protein drive the reparative process in acute muscle injury and muscular dystrophy. *Sci Rep* 2017;**7**:12537.
23. Rozo M, Li L, Fan CM. Targeting β1-integrin signaling enhances regeneration in aged and dystrophic muscle in mice. *Nat Med* 2016;**22**:889–896.
24. Lukjanenko L, Jung MJ, Hegde N, Perruisseau-Carrier C, Migliavacca E, Rozo M, et al. Loss of fibronectin from the aged stem cell niche affects the regenerative capacity of skeletal muscle in mice. *Nat Med* 2016;**22**:897–905.
25. Sandri M, Barberi L, Bijlsma AY, Blaauw B, Dyar KA, Milan G, et al. Signalling pathways regulating muscle mass in ageing skeletal muscle: the role of the IGF1-Akt-mTOR-FoxO pathway. *Biogerontology* 2013;**2013**:303–323.
26. Romanello V, Sandri M. Mitochondrial quality control and muscle mass maintenance. *Front Physiol* 2016;**6**:422.
27. Zampieri S, Mammucari C, Romanello V, Barberi L, Pietrangelo L, Fusella A, et al. Physical exercise in aging human skeletal muscle increases mitochondrial calcium uniporter expression levels and affects mitochondria dynamics. *Physiol Rep* 2016;**4**(). pii: e13005.
28. Tezze C, Romanello V, Desbats MA, Fadini GP, Albiero M, Favaro G, et al. Age-associated loss of OPA1 in muscle impacts muscle mass, metabolic homeostasis, systemic inflammation, and epithelial senescence. *Cell Metab* 2017;**25**:1374–1389.
29. Chan MC, Arany Z. The many roles of PGC-1α in muscle—recent developments. *Metabolism* 2014;**63**:441–451.
30. Austin S, St-Pierre J. PGC1α and mitochondrial metabolism—emerging concepts and relevance in ageing and neurodegenerative disorders. *J Cell Sci* 2012;**125**:4963–4971.
31. Salma N, Song JS, Arany Z, Fisher DE. Transcription factor Tfe3 directly regulates PGC-1alpha in muscle. *J Cell Physiol* 2015;**230**:2330–2336.
32. Lin J, Wu H, Tarr PT, Zhang CY, Wu Z, Boss O, et al. Transcriptional coactivator PGC-1α drives the formation of slow-twitch muscle fibres. *Nature* 2002;**418**:797–801.
33. Calvo JA, Daniels TG, Wang X, Paul A, Lin J, Spiegelman BM, et al. Muscle-specific expression of PPARγ coactivator-1α improves exercise performance and increases peak oxygen uptake. *J Appl Physiol* 2008;**104**:1304–1312.
34. Ruas JL, White JP, Rao RR, Kleiner S, Brannan KT, Harrison BC, et al. A PGC-1α isoform induced by resistance training regulates skeletal muscle hypertrophy. *Cell* 2012;**151**:1319–1331.
35. Dinulovic I, Furrer R, Beer M, Ferry A, Cardel B, Handschin C. Muscle PGC-1α modulates satellite cell number and proliferation by remodeling the stem cell niche. *Skelet Muscle* 2016;**6**:39.
36. Chabi B, Ljubicic V, Menzies KJ, Huang JH, Saleem A, Hood DA. Mitochondrial function and apoptotic susceptibility in aging skeletal muscle. *Aging Cell* 2008;**7**:2–12.
37. Boengler K, Kosiol M, Mayr M, Schulz R, Rohrbach S. Mitochondria and ageing: role in heart, skeletal muscle and adipose tissue. *J Cachexia Sarcopenia Muscle* 2017;**8**:349–369.
38. Sandri M, Sandri C, Gilbert A, Skurk C, Calabria E, Picard A, et al. Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell* 2004;**117**:399–412.
39. Milan G, Romanello V, Pescatore F, Armani A, Paik JH, Frasson L, et al. Regulation of autophagy and the ubiquitin–proteasome system by the FoxO transcriptional network during muscle atrophy. *Nat Commun* 2015;**6**:6670.
40. Guan L, Zhang L, Gong Z, Hou X, Xu Y, Feng X, et al. FoxO3 inactivation promotes human cholangiocarcinoma tumorigenesis and chemoresistance through Keap1-Nrf2 signaling. *Hepatology* 2016;**63**:1914–1927.
41. Klotz LO, Steinbrenner H. Cellular adaptation to xenobiotics: interplay between xenosensors, reactive oxygen species and FOXO transcription factors. *Redox Biol* 2017;**13**:646–654.
42. Ribeiro MBT, Guzzoni V, Hord JM, Lopes GN, Marqueti RC, de Andrade RV, et al. Resistance training regulates gene expression of molecules associated with intramyocellular lipids, glucose signaling

and fiber size in old rats. *Sci Rep* 2017;**7**:8593.
43. Bellezza I, Giambanco I, Minelli A, Donato R. Nrf2-Keap1 signaling in oxidative and reductive stress. *Biochim Biophys Acta – Mol Cell Res* 2018;**1865**:721–733.
44. Cobley JN, Sakellariou GK, Owens DJ, Murray S, Waldron S, Gregson W, et al. Lifelong training preserves some redox-regulated adaptive responses after an acute exercise stimulus in aged human skeletal muscle. *Free Radic Biol Med* 2014;**70**:23–32.
45. Zampieri S, Pietrangelo L, Loefler S, Fruhmann H, Vogelauer M, Burggraf S, et al. Lifelong physical exercise delays age-associated skeletal muscle decline. *J Gerontol A Biol Sci Med Sci* 2015;**70**:163–173.
46. Done AJ, Traustadóttir T. Nrf2 mediates redox adaptations to exercise. *Redox Biol* 2016;**10**:191–199.
47. Oh S, Komine S, Warabi E, Akiyama K, Ishii A, Ishige K, et al. Nuclear factor (erythroid derived 2)-like 2 activation increases exercise endurance capacity via redox modulation in skeletal muscles. *Sci Rep* 2017;**7**:12902.
48. Yalvac ME, Amornvit J, Chen L, Shontz KM, Lewis S, Sahenk Z. AAV1.NT-3 gene therapy increases muscle fiber diameter through activation of mTOR pathway and metabolic remodeling in a CMT mouse model. *Gene Ther* 2018;**25**:129–138.
49. Dutchak PA, Estill-Terpack SJ, Plec AA, Zhao X, Yang C, Chen J, et al. Loss of a negative regulator of mTORC1 induces aerobic glycolysis and altered fiber composition in skeletal muscle. *Cell Rep* 2018;**23**:1907–1914.
50. Bentzinger CF, Lin S, Romanino K, Castets P, Guridi M, Summermatter S, et al. Differential response of skeletal muscles to mTORC1 signaling during atrophy and hypertrophy. *Skelet Muscle* 2013;**3**:6.
51. Castets P, Lin S, Rion N, Di Fulvio S, Romanino K, Guridi M, et al. Sustained activation of mTORC1 in skeletal muscle inhibits constitutive and starvation-induced autophagy and causes a severe, late-onset myopathy. *Cell Metab* 2013;**17**:731–744.
52. Guridi M, Tintignac LA, Lin S, Kupr B, Castets P, Rüegg MA. Activation of mTORC1 in skeletal muscle regulates whole-body metabolism through FGF21. *Sci Signal* 2015;**8**:ra113.
53. Moresi V, Williams AH, Meadows E, Flynn JM, Potthoff MJ, McAnally J, et al. Myogenin and class II HDACs control neurogenic muscle atrophy by inducing E3 ubiquitin ligases. *Cell* 2010;**143**:35–34.
54. Minetti GC, Feige JN, Rosenstiel A, Bombard F, Meier V, Werner A, et al. Gαi2 signaling promotes skeletal muscle hypertrophy, myoblast differentiation, and muscle regeneration. *Sci Signal* 2011;**4**:ra80.
55. Bricceno KV, Sampognaro PJ, Van Meerbeke JP, Sumner CJ, Fischbeck KH, Burnett BG. Histone deacetylase inhibition suppresses myogenin-dependent atrogene activation in spinal muscular atrophy mice. *Hum Mol Genet* 2012;**21**:4448–4459.
56. Beharry AW, Sandesara PB, Roberts BM, Ferreira LF, Senf SM, Judge AR. HDAC1 activates FoxO and is both sufficient and required for skeletal muscle atrophy. *J Cell Sci* 2014;**127**:1441–1453.
57. Brzeszczyńska J, Meyer A, McGregor R, Schilb A, Degen S, Tadini V, et al. Alterations in the in vitro and in vivo regulation of muscle regeneration in healthy ageing and the influence of sarcopenia. *J Cachexia Sarcopenia Muscle* 2018;**9**:93–105.
58. Penkowa M, Keller C, Keller P, Jauffred S, Pedersen BK. Immunohistochemical detection of interleukin-6 in human skeletal muscle fibers following exercise. *FASEB J* 2003;**17**:2166–2168.
59. Pedersen BK, Akerstrom TC, Nielsen AR, Fischer CP. Role of myokines in exercise and metabolism. *J Appl Physiol* 2007;**103**:1093–1098.
60. Pedersen BK, Febbraio MA. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol Rev* 2008;**88**:1379–1406.
61. Fiuza-Luces C, Garatachea N, Berger NA, Lucia A. Exercise is the real polypill. *Physiology (Bethesda)* 2013;**2013**:330–358.
62. Giudice J, Taylor JM. Muscle as a paracrine and endocrine organ. *Curr Opin Pharmacol* 2017;**34**:49–55.
63. Tsuchiya M, Sekiai S, Hatakeyama H, Koide M, Chaweewannakorn C, Yaoita F, et al. Neutrophils provide a favorable IL-1-mediated immunometabolic niche that primes GLUT4 translocation and performance in skeletal muscles. *Cell Rep* 2018;**23**:2354–2364.
64. Xu H, Ren X, Lamb GD, Murphy RM. Physiological and biochemical characteristics of skeletal muscles in sedentary and active rats. *J Muscle Res Cell Motil* 2018. https://doi.org/10.1007/s10974-018-9493-0.
65. Garatachea N, Pareja-Galeano H, Sanchis-Gomar F, Santos-Lozano A, Fiuza-Luces C, Morán M, et al. Exercise attenuates the major hallmarks of aging. *Rejuvenation Res* 2015;**18**:57–89.
66. Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature* 2005;**433**:760–764.
67. Cosgrove BD, Gilbert PM, Porpiglia E, Mourkioti F, Lee SP, Corbel SY, et al. Rejuvenation of the muscle stem cell population restores strength to injured aged muscles. *Nat Med* 2014;**20**:255–264.
68. Bernet JD, Doles JD, Hall JK, Kelly Tanaka K, Carter TA, Olwin BB. p38 MAPK signaling underlies a cell-autonomous loss of stem cell self-renewal in skeletal muscle of aged mice. *Nat Med* 2014;**20**:265–271.
69. Sousa-Victor P, Gutarra S, García-Prat L, Rodriguez-Ubreva J, Ortet L, Ruiz-Bonilla V, et al. Geriatric muscle stem cells switch reversible quiescence into senescence. *Nature* 2014;**506**:316–321.
70. Price FD, von Maltzahn J, Bentzinger CF, Dumont NA, Yin H, Chang NC, et al. Inhibition of JAK-STAT signaling stimulates adult satellite cell function. *Nat Med* 2014;**20**:1174–1181, Erratum in: Nat Med 2015; 21: 414, 2014. *Nat Med* Oct;(10): 1217, 2014, *Nat Med* 21: 414, 2015.
71. Brancaccio A, Palacios D. Chromatin signaling in muscle stem cells: interpreting the regenerative microenvironment. *Front Aging Neurosci* 2015;**7**:36.
72. Blau HM, Cosgrove BD, Ho AT. The central role of muscle stem cells in regenerative failure with aging. *Nat Med* 2015;**21**:854–862.
73. García-Prat L, Martínez-Vicente M, Perdiguero E, Ortet L, Rodríguez-Ubreva J, Rebollo E, et al. Autophagy maintains stemness by preventing senescence. *Nature* 2016;**529**:37–42.
74. Marzetti E, Calvani R, Cesari M, Buford TW, Lorenzi M, Behnke BJ, et al. Mitochondrial dysfunction and sarcopenia of aging: from signaling pathways to clinical trials. *Int J Biochem Cell Biol* 2013;**45**:2288–2301.
75. Vasilaki A, Jackson MJ. Role of reactive oxygen species in the defective regeneration seen in aging muscle. *Free Radic Biol Med* 2013;**65**:317–323.
76. Dennison EM, Avan A, Sayer AA, Cooper C. Epidemiology of sarcopenia and insight into possible therapeutic targets. *Nat Rev Rheumatol* 2017;**13**:340–347.
77. Sriram S, Subramanian S, Sathiakumar D, Venkatesh R, Salerno MS, McFarlane CD, Kambadur R, Sharma M. Modulation of reactive oxygen species in skeletal muscle by myostatin is mediated through NF-κB. *Aging Cell* **10**: 931-948, 2011. Erratum in: Aging Cell 15: 981–982, 2016
78. Zhao J, Tian Z, Kadomatsu T, Xie P, Miyata K, Sugizaki T, et al. Age-dependent increase in angiopoietin-like protein 2 accelerates skeletal muscle loss in mice. *J Biol Chem* 2018;**293**:1596–1609.
79. McHugh D, Gil J. Senescence and aging: causes, consequences, and therapeutic avenues. *J Cell Biol* 2018;**217**:65–77.
80. Sinha M, Jang YC, Oh J, Khong D, Wu EY, Manohar R, et al. Restoring systemic GDF11 levels reverses age-related dysfunction in mouse skeletal muscle. *Science* 2014;**344**:649–652.
81. Egerman MA, Cadena SM, Gilbert JA, Meyer A, Nelson HN, Swalley SE, et al. GDF11 increases with age and inhibits skeletal muscle regeneration. *Cell Metab* 2015;**22**:164–1674.
82. Hinken AC, Powers JM, Luo G, Holt JA, Billin AN, Russell AJ. Lack of evidence for GDF11 as a rejuvenator of aged skeletal muscle satellite cells. *Aging Cell* 2016;**15**:582–584.
83. Schafer MJ, Atkinson EJ, Vanderboom PM, Kotajarvi B, White TA, Moore MM, et al. Quantification of GDF11 and myostatin in human aging and cardiovascular disease. *Cell Metab* 2016;**23**:1207–1215.

84. Fry CS, Lee JD, Mula J, Kirby TJ, Jackson JR, Liu F, et al. Inducible depletion of satellite cells in adult, sedentary mice impairs muscle regenerative capacity without affecting sarcopenia. *Nat Med* 2015;**21**:76–80.
85. Keefe AC, Lawson JA, Flygare SD, Fox ZD, Colasanto MP, Mathew SJ, et al. Muscle stem cells contribute to myofibres in sedentary adult mice. *Nat Commun* 2015;**6**:7087.
86. Murach KA, Fry CS, Kirby TJ, Jackson JR, Lee JD, White SH, et al. Starring or supporting role? Satellite cells and skeletal muscle fiber size regulation. *Physiology (Bethesda)* 2018;**33**:26–38.
87. Snijders T, Parise G. Role of muscle stem cells in sarcopenia. *Curr Opin Clin Nutr Metab Care* 2017;**20**:186–190.
88. Donato R, Sorci G, Riuzzi F, Arcuri C, Bianchi R, Brozzi F, et al. S100B's double life: intracellular regulator and extracellular signal. *Biochim Biophys Acta* 2009;**1793**:1008–1022.
89. Donato R, Cannon BR, Sorci G, Riuzzi F, Hsu K, Weber DJ, et al. Functions of S100 proteins. *Curr Mol Med* 2013;**13**:24–57.
90. Hermann A, Donato R, Weiger T, Chazin WJ. S100 calcium-binding proteins and ion channels. *Front Pharmacol* 2012;**3**:67.
91. Sorci G, Bianchi R, Giambanco I, Rambotti MG, Donato R. Replicating myoblasts and fused myotubes express the calcium-regulated proteins, S100A1 and S100B. *Cell Calcium* 1999;**25**:1999.
92. Arcuri C, Giambanco I, Bianchi R, Donato R. Annexin V, annexin VI, S100A1 and S100B in developing and adult avian skeletal muscles. *Neuroscience* 2002;**109**:371–388.
93. Tubaro C, Arcuri C, Giambanco I, Donato R. S100B protein in myoblasts modulates myogenic differentiation via NF-κB-dependent inhibition of MyoD expression. *J Cell Physiol* 2010;**223**:270–282.
94. Garbuglia M, Verzini M, Giambanco I, Spreca A, Donato R. Effects of calcium-binding proteins (S100a0, S100a, S100b) on desmin assembly in vitro. *FASEB J* 1996;**10**:317–324.
95. Tsoporis JN, Marks A, Haddad A, Dawood F, Liu PP, Parker TG. S100B expression modulates left ventricular remodeling after myocardial infarction in mice. *Circulation* 2005;**111**:598–606.
96. Sorci G, Giovannini G, Riuzzi F, Bonifazi P, Zelante T, Zagarella S, et al. The danger signal S100B integrates pathogen- and danger-sensing pathways to restrain inflammation. *PLoS Pathog* 2011;**7**: e1001315–e1001315.
97. Mohammadzadeh F, Tsoporis JN, Izhar S, Desjardins JF, Parker TG. Deficiency of S100B confers resistance to experimental diabetes in mice. *Exp Cell Res* 2018;**365**:129–137.
98. McIlroy M, McCartan D, Early S, O Gaora P, Pennington S, Hill AD, et al. Interaction of developmental transcription factor HOXC11 with steroid receptor coactivator SRC-1 mediates resistance to endocrine therapy in breast cancer [corrected]. *Cancer Res* 2010;**70**:1585–1594, Erratum in: *Cancer Res* 70: 3413, 2010.
99. Kubista H, Donato R, Hermann A. S100 calcium binding protein affects neuronal eletrical discharge activity by modulation of potassium currents. *Neuroscience* 1998;**90**:1998.
100. Bargerstock E, Puvenna V, Iffland P, Falcone T, Hossain M, Vetter S, et al. Is peripheral immunity regulated by blood–brain barrier permeability changes? *PLoS One* 2014;**9**:e101477.
101. Hagmeyer S, Cristóvão JS, Mulvihill JJE, Boeckers TM, Gomes CM, Grabrucker AM. Zinc binding to S100B affords regulation of trace metal homeostasis and excitotoxicity in the brain. *Front Mol Neurosci* 2018;**10**:456.
102. Netto CB, Conte S, Leite MC, Pires C, Martins TL, Vidal P, et al. Serum S100B protein is increased in fasting rats. *Arch Med Res* 2006;**37**:683–686.
103. Tsoporis JN, Izhar S, Leong-Poi H, Desjardins JF, Huttunen HJ, Parker TG. S100B interaction with the receptor for advanced glycation end products (RAGE): a novel receptor-mediated mechanism for myocyte apoptosis postinfarction. *Circ Res* 2010;**106**:93–101.
104. Riuzzi F, Sorci G, Beccafico S, Donato R. S100B engages RAGE or bFGF/FGFR1 in myoblasts depending on its own concentration and myoblast density. Implications for muscle regeneration. *PLoS One* 2012;**7**:e28700–e28700.
105. Tsoporis JN, Izhar S, Proteau G, Slaughter G, Parker TG. S100B-RAGE dependent VEGF secretion by cardiac myocytes induces myofibroblast proliferation. *J Mol Cell Cardiol* 2012;**52**:464–473.
106. Schulte S, Podlog LW, Hamson-Utley JJ, Strathmann FG, Strüder HK. A systematic review of the biomarker S100B: implications for sport-related concussion management. *J Athl Train* 2014;**49**:830–850.
107. Donato R, Riuzzi F, Sorci G. Causes of elevated serum levels of S100B protein in athletes. *Eur J Appl Physiol* 2012;**113**:2012.
108. Stocchero CM, Oses JP, Cunha GS, Martins JB, Brum LM, Zimmer ER, et al. Serum S100B level increases after running but not cycling exercise. *Appl Physiol Nutr Metab* 2014;**39**:340–344.
109. Hofmann MA, Drury S, Fu C, Qu W, Taguchi A, Lu Y, et al. RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. *Cell* 1999;**97**:889–901.
110. Huttunen HJ, Kuja-Panula J, Sorci G, Agneletti AL, Donato R, Rauvala H. Coregulation of neurite outgrowth and cell survival by amphoterin and S100 proteins through receptor for advanced glycation end products (RAGE) activation. *J Biol Chem* 2000;**275**:40096–40105.
111. Riuzzi F, Sorci G, Donato R. S100B protein regulates myoblast proliferation and differentiation by activating FGFR1 in a bFGF-dependent manner. *J Cell Sci* 2011;**124**:2011.
112. Sorci G, Riuzzi F, Arcuri C, Bianchi R, Brozzi F, Tubaro C, et al. The many faces of S100B protein: when an extracellular factor inactivates its own receptor and activates another one. *Ital J Anat Embryol* 2010;**115**:147–151.
113. Sorci G, Riuzzi F, Agneletti AL, Marchetti C, Donato R. S100B inhibits myogenic differentiation and myotube formation in a RAGE-independent manner. *Mol Cell Biol* 2003;**23**:4870–4881.
114. Riuzzi F, Sorci G, Donato R. S100B stimulates myoblast proliferation and inhibits myoblast differentiation by independently stimulating ERK1/2 and inhibiting p38 MAPK. *J Cell Physiol* 2006;**207**:461–470.
115. Sorci G, Riuzzi F, Arcuri C, Giambanco I, Donato R. Amphoterin stimulates myogenesis and counteracts the antimyogenic factors basic fibroblast growth factor and S100B via RAGE binding. *Mol Cell Biol* 2004;**24**:4880–4894.
116. Sorci G, Riuzzi F, Agneletti AL, Marchetti C, Donato R. S100B causes apoptosis in a myoblast cell line in a RAGE-independent manner. *J Cell Physiol* 2004;**199**:2004.
117. Sagheddu R, Chiappalupi S, Salvadori L, Riuzzi F, Donato R, Sorci G. Targeting RAGE as a potential therapeutic approach to Duchenne muscular dystrophy. *Hum Mol Genet* 2018. https://doi.org/10.1093/hmg/ddy288.
118. Hosokawa K, Hamada Y, Fujiya A, Murase M, Maekawa R, Niwa Y, et al. S100B impairs glycolysis via enhanced poly (ADP-ribosyl)ation of glyceraldehyde-3-phosphate dehydrogenase in rodent muscle cells. *Am J Physiol Endocrinol Metab* 2017;**312**:E471–E481.
119. Wang H, Hertlein E, Bakkar N, Sun H, Acharyya S, Wang J, et al. NF-κB regulation of YY1 inhibits skeletal myogenesis through transcriptional silencing of myofibrillar genes. *Mol Cell Biol* 2007;**27**:4374–4387.
120. Tubaro C, Arcuri C, Giambanco I, Donato R. S100B in myoblasts regulates the transition from activation to quiescence and from quiescence to activation, and reduces apoptosis. *Biochim Biophys Acta - Mol Cell Res* 2011;**1813**:1092–1104.
121. Arcuri C, Bianchi R, Brozzi F, Donato R. S100B increases proliferation in PC12 neuronal cells and reduces their responsiveness to NGF via Akt activation. *J Biol Chem* 2005;**280**:4402–4414.
122. Hachem S, Laurenson AS, Hugnot JP, Legraverend C. Expression of S100B during embryonic development of the mouse cerebellum. *BMC Dev Biol* 2007;**7**:17.
123. Raponi E, Agenes F, Delphin C, Assard N, Baudier J, Legraverend C, et al. S100B expression defines a state in which GFAP-expressing cells lose their neural stem cell potential and acquire a more mature developmental stage. *Glia* 2007;**55**:165–177.
124. Saito T, Ikeda T, Nakamura K, Chung UI, Kawaguchi H. S100A1 and S100B, transcriptional targets of SOX trio, inhibit

terminal differentiation of chondrocytes. *EMBO Rep* 2007;**8**:504–509.
125. Zhang X, Hamada J, Nishimoto A, Takahashi Y, Murai T, Tada M, et al. HOXC6 and HOXC11 increase transcription of S100β gene in BrdU-induced in vitro differentiation of GOTO neuroblastoma cells into Schwannian cells. *J Cell Mol Med* 2007;**11**:299–306.
126. Brozzi F, Arcuri C, Giambanco I, Donato R. S100B protein regulates astrocyte shape and migration via interaction with Src kinase: implications for astrocyte development, activation and tumor growth. *J Biol Chem* 2009;**284**:8797–8811.
127. Baudier J, Delphin C, Grunwald D, Khochbin S, Lawrence JJ. Characterization of the tumor suppressor protein p53 as a protein kinase C substrate and an S100b-binding protein. *Proc Natl Acad Sci U S A* 1992;**89**:11627–11631.
128. Lin J, Blake M, Tang C, Zimmer D, Rustandi RR, Weber DJ, et al. Inhibition of p53 transcriptional activity by the S100B calcium-binding protein. *J Biol Chem* 2001;**276**:35037–35041.
129. Fernandez-Fernandez MR, Veprintsev DB, Fersht AR. Proteins of the S100 family regulate the oligomerization of p53 tumor suppressor. *Proc Natl Acad Sci U S A* 2005;**102**:4735–4740.
130. van Dieck J, Fernandez-Fernandez MR, Veprintsev DB, Fersht AR. Modulation of the oligomerization state of p53 by differential binding of proteins of the S100 family to p53 monomers and tetramers. *J Biol Chem* 2009;**284**:13804–13811.
131. Lin J, Yang Q, Yan Z, Markowitz J, Wilder PT, Carrier F, et al. Inhibiting S100B restores p53 levels in primary malignant melanoma cancer cells. *J Biol Chem* 2004;**279**:34071–34077.
132. Lin J, Yang Q, Wilder PT, Carrier F, Weber DJ. The calcium binding protein S100B down-regulates p53 and apoptosis in malignant melanoma. *J Biol Chem* 2010;**285**:27487–27498.
133. van Dieck J, Lum JK, Teufel DP, Fersht AR. S100 proteins interact with the N-terminal domain of MDM2. *FEBS Lett* 2010;**584**:3269–3274.
134. Francoz S, Froment P, Bogaerts S, De Clercq S, Maetens M, Doumont G, et al. Mdm4 and Mdm2 cooperate to inhibit p53 activity in proliferating and quiescent cells in vivo. *Proc Natl Acad Sci U S A* 2006;**103**:3232–3237.
135. Beccafico S, Riuzzi F, Puglielli C, Mancinelli R, Fulle S, Sorci G, et al. Human muscle satellite cells show age-related differential expression of S100B protein and RAGE. *Age (Dordr)* 2011;**33**:523–541.
136. Gopinath SD, Rando TA. Stem cell review series: aging of the skeletal muscle stem cell niche. *Aging Cell* 2008;**7**:590–598.
137. Morozzi G, Beccafico S, Bianchi R, Riuzzi F, Bellezza I, Giambanco I, et al. Oxidative stress-induced S100B accumulation converts myoblasts into brown adipocytes via an NF-κB/YY1/miR-133 axis and NF-κB/YY1/BMP-7 axis. *Cell Death Differ* 2017;**24**:2077–2088.
138. Asakura A, Komaki M, Rudnicki M. Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation. *Differentiation* 2001;**68**:245–253.
139. Frühbeck G, Sesma P, Burrell MA. PRDM16: the interconvertible adipomyocyte switch. *Trends Cell Biol* 2009;**19**:141–146.
140. de Villiers D, Potgieter M, Ambele MA, Adam L, Durandt C, Pepper MS. The role of reactive oxygen species in adipogenic differentiation. *Adv Exp Med Biol* 2017; Nov 15. https://doi.org/10.1007/5584_2017_119.
141. Mendelson KG, Contois LR, Tevosian SG, Davis RJ, Paulson KE. Independent regulation of JNK/p38 mitogen-activated protein kinases by metabolic oxidative stress in the liver. *Proc Natl Acad Sci U S A* 1996;**93**:12908–12913.
142. Trajkovski M, Ahmed K, Esau CC, Stoffel M. MyomiR-133 regulates brown fat differentiation through Prdm16. *Nat Cell Biol* 2012;**14**:1330–1335.
143. Lu L, Zhou L, Chen EZ, Sun K, Jiang P, Wang L, et al. A novel YY1-miR-133 regulatory circuit in skeletal myogenesis revealed by genome-wide prediction of YY1–miRNA network. *PLoS One* 2012;**7**:2012.
144. Yin H, Pasut A, Soleimani VD, Bentzinger CF, Antoun G, Thorn S, et al. MicroRNA-133 controls brown adipose determination in skeletal muscle satellite cells by targeting Prdm16. *Cell Metab* 2013;**17**:210–224.
145. Enerbäck S, Jacobsson A, Simpson EM, Guerra C, Yamashita H, Harper ME, et al. Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature* 1997;**387**:90–94.
146. Seale P, Kajimura S, Yang W, Chin S, Rohas LM, Uldry M, et al. Transcriptional control of brown fat determination by PRDM16. *Cell Metab* 2007;**6**:38–54.
147. Tseng YH, Kokkotou E, Schulz TJ, Huang TL, Winnay JN, Taniguchi CM, et al. New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 2008;**454**:1000–1004.
148. Völkers M, Rohde D, Goodman C, Most P. S100A1: a regulator of striated muscle sarcoplasmic reticulum Ca^{2+} handling, sarcomeric, and mitochondrial function. *J Biomed Biotechnol* 2010;**2010**:178614.
149. Melville Z, Hernández-Ochoa EO, Pratt SJP, Liu Y, Pierce AD, Wilder PT, et al. The activation of protein kinase A by the calcium-binding protein S100A1 is independent of cyclic AMP. *Biochemistry* 2017;**2017**:2328–2337.
150. Donato R. S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. *Int J Biochem Cell Biol* 2001;**33**:637–668.
151. Gilquin B, Cannon BR, Hubstenberger A, Moulouel B, Falk E, Merle N, et al. The calcium-dependent interaction between S100B and the mitochondrial AAA ATPase ATAD3A and the role of this complex in the cytoplasmic processing of ATAD3A. *Mol Cell Biol* 2010;**30**:2724–3276.
152. von Haehling S, Morley JE, Coats AJS, Anker SD. Ethical guidelines for publishing in the Journal of Cachexia, Sarcopenia and Muscle: update 2017. *J Cachexia Sarcopenia Muscle* 2017;**8**:1081–1083.