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*Review*

# Stem cells for skeletal muscle repair

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Skeletal muscle is a highly specialized tissue composed of non-dividing, multi-nucleated muscle fibres that contract to generate force in a controlled and directed manner. Skeletal muscle is formed during embryogenesis from a subset of muscle precursor cells, which generate both differentiated muscle fibres and specialized muscle-forming stem cells known as satellite cells. Satellite cells remain associated with muscle fibres after birth and are responsible for muscle growth and repair throughout life. Failure in satellite cell function can lead to delayed, impaired or failed recovery after muscle injury, and such failures become increasingly prominent in cases of progressive muscle disease and in old age. Recent progress in the isolation of muscle satellite cells and elucidation of the cellular and molecular mediators controlling their activity indicate that these cells represent promising therapeutic targets. Such satellite cell-based therapies may involve either direct cell replacement or development of drugs that enhance endogenous muscle repair mechanisms. Here, we discuss recent breakthroughs in understanding both the cell intrinsic and extrinsic regulators that determine the formation and function of muscle satellite cells, as well as promising paths forward to realizing their full therapeutic potential.

**Keywords:** satellite cell; muscular dystrophy; sarcopenia; muscle degeneration; myogenesis

## 1. SATELLITE CELLS: MEDIATORS OF SKELETAL MUSCLE GROWTH AND REPAIR

Skeletal muscle is composed predominantly of post-mitotic, multi-nucleated muscle fibres, which account for up to half of the mass of the human body. Skeletal muscle allows for locomotion by producing the contractile forces that move our bodies. Muscle also serves as a primary site for glycogen storage, insulin uptake and amino acid catabolism, and thereby plays a crucial role in regulating the body's overall metabolism. Yet, effective muscle function is both mechanically and energetically demanding, making this tissue particularly susceptible to damage. Such damage to muscle can limit mobility and contribute to metabolic disease. Indeed, there exist more than 100 recognized muscle diseases in humans, and in the progression to type 2 diabetes, skeletal muscle typically manifests the earliest detectable signs of insulin resistance [1]. Thus, successful maintenance of muscle function throughout life represents a critical and important challenge to maintaining a healthy and active lifestyle.

Lifelong maintenance of skeletal muscle function depends in large part on preserving the regenerative capacity of muscle fibres, which may be subjected to a variety of physical and biochemical insults that introduce substantial muscle damage. Repair of injured fibres requires a unique population of tissue-specific muscle stem cells called satellite cells [2]. These cells are marked by expression of the paired box transcription factor Pax7 and reside beneath the basal lamina of mature muscle fibres, maintaining a close physical interaction that enables an exquisite sensitivity to muscle injury (figure 1). In response to fibre damage, the normally quiescent population of muscle satellite cells is induced to proliferate and differentiate to form mature myoblasts. Those myoblasts then rapidly exit the cell cycle to fuse with each other and with surviving fibres to generate new and repaired muscle tissue (reviewed in [3,4]).

A number of cell types have been reported to contribute to skeletal myogenesis under particular physiological conditions [5–10]; however, several lines of evidence, including single myofibre grafts [11] and direct, prospective isolation of cells from mouse skeletal muscle [12–16], suggest that satellite cells represent the predominant reservoir for muscle regeneration in adult animals (reviewed in [17]). In addition, early studies that used tritiated thymidine to mark any cells in the muscle capable of division, in combination with transplantation and damage models [9,10], or during normal muscle growth [11], indicated that proliferative activity in regenerating muscle is restricted to the sub-laminar satellite cell compartment, and that new myonuclei found in regenerated muscle fibres derive from these proliferating satellite cells. More recent studies have confirmed these findings, employing the powerful techniques of mouse transgenesis and cell-specific lineage tracing by Cre-recombination to demonstrate that all new myonuclei incorporated into repaired skeletal muscle indeed originate from satellite cells expressing the canonical satellite cell marker Pax7 (although,

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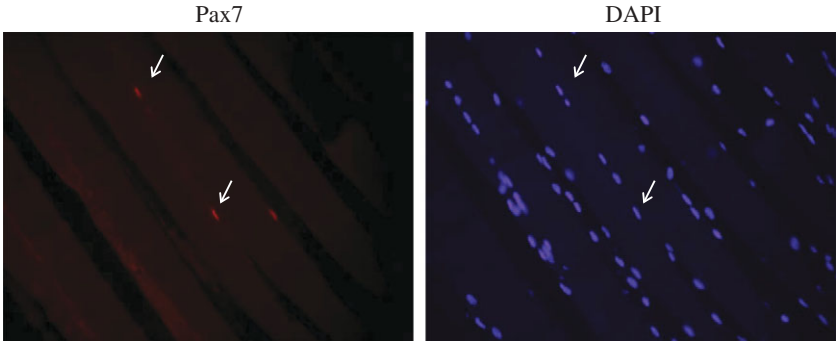


Figure 1. Immunostaining of adult mouse skeletal muscle with antibodies against the satellite cell marker Pax7 (red) highlights the close association of these cells (marked by white arrows) with skeletal myofibres and their relative infrequency in comparison with myonuclei (visible by DAPI staining, blue). Please note that while the majority of DAPI+ nuclei are myonuclei, some represent non-myogenic cells, including infiltrating inflammatory cells and fibrogenic/adipogenic cells (see text for details). Images courtesy of Sara Jurga.

surprisingly, conditional deletion of Pax7 in such Cre-recombination models suggests that adult satellite cells may contribute to muscle regeneration even in the absence of Pax7 [18]).

Given the critical role of satellite cells in maintaining muscle fibre integrity through regenerative myogenesis, these tissue-specific stem cells represent promising candidates for therapeutic intervention in situations of deficient muscle repair. As discussed further below, much progress has been made in delineating the cellular and molecular cues that guide the activity of these cells, and current models point to both cell intrinsic and extrinsic regulators that may modulate muscle repair efficiency.

## 2. CELLULAR AND MOLECULAR REGULATORS OF SATELLITE CELL FUNCTION

Proper control of satellite cell function is critical for effective muscle regeneration, and possibly also to protect against the emergence of muscle tumours [19]. A number of pathways have been implicated in regulating the regenerative function of muscle satellite cells, including the evolutionarily conserved Notch, Wnt and transforming growth factor (TGF)-beta pathways, as well as local and systemic inflammatory mediators such as interleukin (IL)-6 (see below). In addition, non-myogenic mononuclear cells that are found in association with muscle fibres are receiving increased attention in light of their role in regulating satellite cell proliferation and differentiation. The non-myogenic cells implicated in the functional regulation of muscle satellite cells constitute a heterogeneous mix of mesenchymal cell types, including progenitors for fat and fibrous tissue, as well as infiltrating haematopoietic cell types [20–22].

Two recent reports [20,21] identified by cell sorting a particular subset of muscle mesenchymal cells that represent bipotent fibrogenic/adipogenic precursors (FAPs). Intriguingly, these studies also demonstrated a functional cross-talk between FAPs and satellite cells in skeletal muscle. In particular, while the presence of FAPs potentiated myogenic differentiation of satellite cells, the presence of satellite cell-derived myofibres inhibited adipogenic differentiation of FAP cells [20,21]. These data suggest that the relative representation of each cell population must be carefully controlled in order to ensure appropriate muscle regeneration. Furthermore, alterations in the frequency of either population, such as occurs with advancing age (see below), may have detrimental effects on the myogenic capacity of skeletal muscle and lead to increased intramuscular fibrosis or adipogenesis upon skeletal muscle injury.

## 3. MUSCLE DISEASE AND DISUSE ALTER SATELLITE CELL FUNCTION

A number of pathological conditions, including congenital myopathy, denervation and muscle atrophy owing to disuse, all of which involve progressive loss of muscle mass and strength, also may exhibit a decrease in the number and proliferative potential of muscle satellite cells. In particular, muscular dystrophies, encompassing a group of related degenerative diseases resulting from specific genetic lesions that impair the structure, function and/or regenerative potential of skeletal muscle, have been reported to show premature loss of satellite cell regenerative activity. In a mouse model of Duchenne muscular dystrophy (DMD) (*mdx* [23]), flow cytometric quantification of the muscle stem cell pool indicated an approximately threefold decrease in the frequency of these cells in young *mdx* animals, when compared with age-matched, wild-type controls [24]. The underlying mechanisms responsible for these changes in the satellite cell pool in diseased muscle have yet to be fully elucidated, but may relate to intrinsic alterations introduced by the proliferative stress associated with the necessity for repeated bouts of muscle regeneration in response to a chronic degenerative condition. Such repeated cycles of satellite cell activation may lead to telomere shortening [25] or accumulation of mutations in key satellite cell regulatory genes, resulting in a loss of satellite cell self-renewal activity and impaired myogenic capacity. Consistent with this notion, a recent report noted that the severity and progression of muscular dystrophy were substantially enhanced in *mdx* mice with short telomeres owing to dysfunctional telomerase activity [26]. This exacerbated dystrophic phenotype, which was associated with impaired proliferation and deficient regenerative potential of satellite cells, could be partially corrected by transplantation of unaffected satellite cells, implying

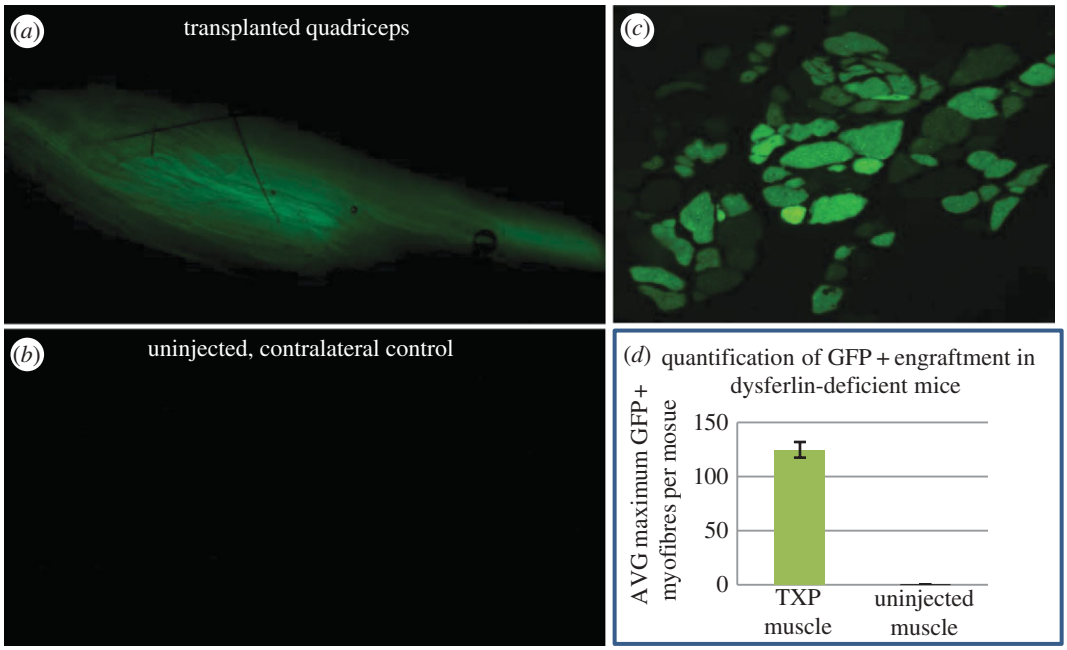


Figure 2. Satellite cell engraftment in dysferlin-deficient muscles. 6000 green fluorescent protein positive (GFP+) satellite cells, isolated from dysferlin-expressing green GFP-transgenic mice, were transplanted into the quadriceps muscle of cardiotoxin pre-injured C57BL/10.SJL-*Dysf* recipient mice aged 3–12 month. (*a*,*b*) Representative, whole mount images acquired by inverted fluorescent microscopy of the transplanted, (*a*) and uninjected control (*b*) muscles of the same recipient animal demonstrate widespread engraftment of GFP+ cells (shown in green) specifically in the transplanted muscle. (*c*) Frozen sections of transplanted muscles showing GFP+ (green) fibres. (*d*) Myofibre engraftment in the transplanted (TXP) and uninjected muscles was quantified by counting the maximum number of GFP+ myofibres in cross section. Data represent the mean ± s.d. for $n = 3$ transplanted mice.

a cell-autonomous contribution of satellite cell dysfunction to muscle degenerative disease [26].

Yet, in addition to intrinsic deficits, disease-associated alterations in muscle satellite cell function also may reflect changes in the dystrophic environment, which may act to suppress the myogenic activity of these cells. Several non-myogenic cell types normally found within the skeletal muscle have been suggested to contribute directly to failed muscle regeneration. For example, fibroblasts in dystrophic patients have been shown to secrete increased levels of insulin-like growth factor (IGF)-1 binding proteins, which may sequester IGF-1 away from myogenic cells [27]. Likewise, secreted factors, including proliferation- and migration-inducing cytokines like the CXCR4 ligand SDF-1α, are induced in injured and regenerating muscle and may help to regulate and topographically organize post-natal skeletal myogenesis [28,29]. Together, these observations suggest a critical role for the satellite cell microenvironment in modulating myogenic precursor cell activity, a hypothesis that could have important implications for muscle therapeutic strategies aimed at stimulating endogenous satellite cell activity, as well as for enhancing muscle fibre engraftment in transplantation-based approaches (see below).

In conclusion, though often unaffected by the primary genetic lesion that gives rise to muscular dystrophy, disease-related effects on satellite cells may nonetheless contribute to progressive muscle degeneration. Reduced satellite cell numbers, arising from chronic proliferative engagement, coupled with a potentially suppressive microenvironment, may hasten failure of muscle homeostasis in diseased or dystrophic tissue.

## 4. THERAPEUTIC AVENUES: TRANSPLANTATION OF MUSCLE SATELLITE CELLS SUPPORTS MUSCLE REPAIR

As noted above, many different forms of degenerative muscle disease exist, many of which are caused by an inherited deficiency or mutation of critical muscle structural or regulatory proteins. DMD, for example, is an X-linked disease that results from the loss of expression of the protein dystrophin, which normally serves to link the myofibre cytoskeleton to the extracellular matrix. DMD affects one in every approximately 3000 male births annually, causes severe muscle wasting and weakness, and uniformly results in premature death. While there are currently no broadly effective treatments for muscular dystrophy, recent progress has been encouraging with respect to interventions aimed at particular sub-types of muscle disease. For example, clinical trials in exon skipping have shown promise for a subset of dystrophic patients [30], and enzyme replacement therapy in patients with Pompe disease (a glycogen storage disease that disproportionately impacts skeletal and cardiac muscle) can significantly improve cardiac function and increase life expectancy [31–34]. However, despite these recent advances, new and innovative approaches still are needed to develop strategies that can combat the multitude of mechanisms by which skeletal muscle can become dysfunctional in human patients.

Given that satellite cells represent the major regenerative cell population in adult skeletal muscle [4,17], and can support multiple rounds of regeneration of mature muscle fibres while still maintaining the satellite cell pool, these cells are attractive candidates for

cell-based therapy in DMD and related disorders. Indeed, when transferred into the muscle of injured or diseased mice (such as the DMD model *mdx* mice or dysferlin-deficient C57BL/10.SJL-*Dysf* animals, which model limb girdle muscular dystrophy type 2B (LGMD2) and Miyoshi myopathy), satellite cells are able to contribute extensively to the formation of new muscle fibres, in some cases engrafting the majority of fibres in the transplanted muscle ([11,16,24,26,35] and figure 2). Moreover, these engrafted fibres exhibit donor-cell-derived production of muscle-specific proteins including dystrophin, which is normally absent in *mdx* mice and DMD patients. Engrafted muscles also show improved histology and contractile activity [24,26]. In addition, unlike differentiated muscle myoblasts, transplanted satellite cells are able to reseed a reserve pool of undifferentiated, donor-derived satellite cells within recipient muscles [15,24]. Because this reserve of muscle stem cells remains within the transplanted tissue, it can be recruited to mediate subsequent rounds of muscle regeneration [24], providing a renewable source of cells for muscle repair.

Taken together, these data confirm that at least a subset of muscle satellite cells exhibits the stem cell properties of self-renewal and differentiation [4], and that transplantation of these cells into injured or dystrophic muscle can yield enduring production of 'wild-type' copies of disease-affected genes in engrafted myofibres, thereby ameliorating disease pathology. However, several previous studies and clinical trials have tested the efficacy of cell therapy for muscle regeneration and, generally speaking, have met with rather disappointing results, including low levels of engraftment and insignificant improvements in muscle function [36]. This outcome could reflect limitations on the receptivity of human muscle tissue to engraftment by transplanted cells; however, an alternative explanation might implicate the source of cells used for transplantation. In fact, most cell therapy trials in skeletal muscle have employed *ex vivo*-expanded myoblasts, rather than purified satellite cells. Thus, the poor performance of transplanted cells in these trials may relate to culture-induced changes or to the advanced differentiation state of the myoblasts employed for transplantation, rather than any intrinsic limitation in engraftment of human skeletal muscle. Consistent with this notion, Montarras *et al.* [13] have reported that *ex vivo* expansion of satellite cells for as little as 3 days results in severe reduction in their ability to contribute donor-derived myofibres upon transplantation *in vivo*. Additionally, clonal assays comparing freshly isolated cells with the progeny of these cells generated by short-term culture indicated that previously cultured cells showed decreased proliferative capacity and an increased fraction of differentiated cells [13], which probably resulted in their limited regenerative capacity upon transplant.

In the light of these data, one may postulate that improved clinical outcomes might be achieved through the use of more primitive satellite cells, freshly purified from donor muscle tissue. However, such an approach introduces a different set of limitations arising from the extreme rarity of satellite cells in normal skeletal muscle (figure 1), the relatively laborious methods currently available for their extraction from the tissue (see [37]), and the inability to expand these cells *ex vivo* without severely decreasing their capacity for self-renewal. Even the most promising methods recently reported for culturing muscle satellite cells on synthetic, elastic hydrogels, which better model the *in vivo* muscle environment, achieved only an approximately fourfold increase in apparent self-renewal of these cells, and have not been tested for maintenance of engraftment capacity for periods longer than one week [38]. Thus, effective exploitation of the regenerative potential of satellite cells in transplantation-based approaches still awaits the development of new strategies that support extensive expansion of these cells while maintaining their functional engraftment potential. Such strategies will be particularly important in considering approaches in which cell and gene therapies are coupled, such that autologous cells harvested from a patient can be corrected *ex vivo*, for instance, through the use of site-specific Zn-finger nucleases [39,40], and then reintroduced into the same patient for therapeutic effect.

Yet, rather than attempting *ex vivo* satellite cell expansion prior to transplant, it also is possible that this issue of small cell numbers could be addressed instead by targeting the satellite cell niche itself. In this regard, a greater molecular understanding of the signals that allow satellite cells to become activated and expand during the normal course of muscle regeneration may yield clues as to how one might encourage this response in the context of cell transplantation. Thus, improved outcomes for therapeutic muscle transplant probably will involve a more refined selection of the cell population used for transplant, improved methods of satellite cell isolation and culture, and transplant-coupled interventions that enhance the regenerative environment to improve the survival and engraftment capacities of the transplanted cells.

## 5. AGEING: INEXORABLE LOSS OF MUSCLE FUNCTION AND REPAIR CAPACITY

Recent changes in the global demographics of the human population are likely to herald the dawn of a new era in human healthcare. For the first time in human history, people over 65 will outnumber children under 5, and this subset of elderly individuals (age 65+) is predicted to grow to greater than 1 billion over the next quarter century [41]. Indeed, people over 85 now represent the fastest growing demographic in many national populations. This change in population distribution places increasing emphasis on the treatment of chronic, non-communicable diseases as the major cause of death and disability worldwide [42], driving an increased impetus to achieve a clearer understanding of the ageing process and better strategies to combat age-related disease.

Among the numerous diseases and disorders associated with advancing age, perhaps one of the most debilitating is the progressive loss of skeletal muscle mass and strength, known as sarcopenia [43]. Sarcopenia affects approximately 25 per cent of individuals

over 70 and 40 per cent of those over 80. Decreased muscle function, as well as deficient muscle regeneration after injury, impedes the performance of normal daily activities in elderly populations, and the accompanying fatty replacement of skeletal muscle can exacerbate age-associated metabolic disease. Importantly, although regular exercise and strength training may help to improve physical performance [44], these interventions alone are insufficient to halt age-related decline in muscle function, or to maintain muscle regenerative potential.

A number of studies have documented specific age-associated deficiencies in muscle maintenance and regeneration, and diminished satellite cell number and function may lie at the root of at least some of these age-associated muscle pathologies [45–50] (figure 3). In newborn mice, satellite cell nuclei comprise approximately 30 per cent of myofibre-associated nuclei, but their number declines with maturity such that only approximately 5 per cent of nuclei in muscles of adult mice represent satellite cells [51]. Numbers of satellite cells associated with muscle fibres decline further with age [45,52], accompanied by a relative increase in the frequency of muscle-resident FAP cells (M.C., K.Y.T. and A.W. 2008–2010 unpublished observations), which normally form fat and scar tissue and also may regulate myogenesis [20,21]. In rats, this age-associated decrement in satellite cells was shown to be ameliorated somewhat by endurance exercise, but even in exercised animals, satellite cell numbers in aged individuals are significantly reduced in comparison with identically treated young animals [53]. Compounding their numeric loss, aged satellite cells that remain in the muscle fail to respond normally to muscle injury. Satellite cells isolated from the skeletal muscle of aged rodents show reduced formation myogenic colonies and a decreased ability to proliferate and/or to produce activated myoblasts [47,49,53]. In addition, the proliferative capacity of activated myoblasts generated by aged satellite cells is significantly impaired, leading to more rapid entry into senescence, as assayed by propagation in culture [54]. These defects in satellite cell maintenance and function most likely underwrite the deficiencies of muscle repair typically seen in older individuals; however, some studies suggest that the potential of aged satellite cells to contribute to myogenesis remains in old age, and under appropriate conditions their proliferative capacity can be restored ([47,49,55] and see below).

## 6. INTRINSIC EFFECTS ON REPAIR ACTIVITY IN AGED SKELETAL MUSCLE

Age-dependent decline in the regenerative properties of many tissues has been attributed to a combination of changes in tissue-specific stem cells and changes in the environmental cues that promote participation of these cells in tissue maintenance and repair. In the skeletal muscle, several published studies in mice have begun to investigate the mechanisms behind age-related deficits in satellite cell regenerative function (figure 3). On a cell-intrinsic level, ageing has been associated with the accumulation of oxidative damage, a reduction in genome maintenance that impairs genomic integrity, and alterations in mitochondrial function that may reduce mitochondrial biogenesis, impair oxidative phosphorylation and enhance the production of damaging reactive oxygen species (ROS) [56–59]. Gene expression studies also have pointed to critical differences in the transcriptional profile of aged versus young satellite cells, noting particularly inappropriate regulation of the myogenic differentiation programme, activation of FOXO-dependent genes associated with muscle atrophy, and changes in genes associated with mitochondrial function and protein folding [54,59]. Additionally, aged satellite cells fail to appropriately upregulate the Notch ligand DII1, a feature that is at least partly responsible for insufficient activation of the Notch signalling pathway in these cells in response to muscle injury [47,55]. Indeed, induced activation of Notch enhances the effective repair of aged muscle [47], while Notch inhibitors can impair the normally robust regenerative capacity of young mouse skeletal muscle. Finally, erosion of telomeric sequences owing to either repeated satellite cell recruitment, regenerative myogenesis or oxidative damage may contribute to the reduced proliferative potential of aged satellite cells and their progeny [26,54,60,61].

## 7. EXTRINSIC EFFECTS ON REPAIR ACTIVITY IN AGED SKELETAL MUSCLE

Extrinsic signals also have been implicated in age-dependent regulation of satellite cell function. In particular, experiments evaluating muscle regeneration in parabiotic mice (animals that are surgically joined and develop a shared blood circulation) indicate a significant influence of the systemic environment on satellite cell function. In this system, heterochronic (young:old) parabiosis appears to provide a 'youthful' systemic milieu that promotes successful muscle repair after injury [46,48]. Importantly, this 'rejuvenating' effect of heterochronic parabiosis does not involve trafficking of myogenic cells from the young partner to the old muscle. Instead, it represents a restoration of efficient activation and function of endogenous, aged satellite cells, which has been associated with restored activation of regeneration-specific Notch signalling [48]. Rejuvenation of aged muscle satellite cells happens surprisingly quickly (within a few weeks of joining), and can be maintained for several weeks after separation of the pair. Furthermore, it can be partially recapitulated *in vitro* by exposing old muscle precursors to serum from young mice [48], providing a useful system for unbiased and candidate-based screening approaches to uncover the systemic factor(s) responsible for age-dependent suppression and enhancement of satellite cell activity.

In a related study, Brack *et al*. [46] employed heterochronic parabiosis to examine the impact of the systemic environment on the balance between muscle fibrosis and myogenesis in aged mice [46]. These authors reported that exposure to factors carried in the blood of old mice may inhibit the proliferation and myogenic function of young muscle satellite cells [46]. Moreover, they found that enhanced signalling in aged satellite cells through the canonical Wnt pathway can reduce myogenic function, while increasing muscle fibrosis following injury [46]. Consistent with

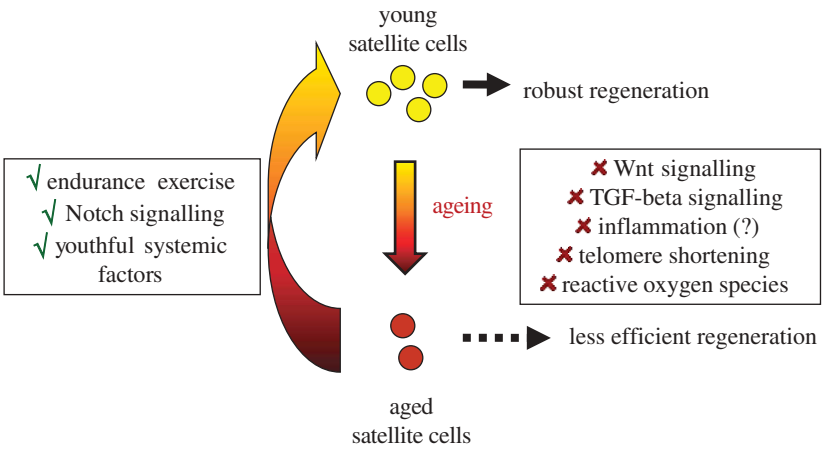


Figure 3. With ageing, the number of satellite cells per myofibre decreases, and those cells that remain exhibit functional deficiencies that limit their regenerative potential. Excessive signalling through the Wnt and TGF-beta signalling pathways, as well as chronic inflammation, telomere shortening and reactive oxygen species (ROS) have been implicated in these age-associated effects. Significantly, however, at least some age-related defects in muscle satellite cells appear to be reversible and activation of Notch signalling, endurance exercise and exposure to a 'youthful' systemic milieu have been reported to restore regenerative potential via changes in satellite cell number or function.

this, suppression of the Wnt pathway *in vivo* by carefully-timed administration of Wnt inhibitors enhanced muscle regeneration in aged mice [46]. Likewise, aged muscle has been reported to accumulate extracellular TGF-beta, which may signal in a paracrine fashion to muscle satellite cells to suppress their myogenic activity [62]. Interestingly, both the Wnt- and TGF-beta-associated signalling pathways in satellite cells appear to intersect at some point with signalling through the Notch pathway [62,63]. These interactions begin to illuminate the complex regulatory network that controls satellite cell activity *in vivo* and how this network may be perturbed in ageing muscle.

In addition to the evolutionarily conserved and developmentally regulated signalling pathways discussed above, local and systemic inflammatory pathways also have been implicated in the altered function of aged muscle satellite cells. While inflammation is fundamentally an important component of the body's defence system against invading pathogens, chronic, non-productive inflammation has been noted in association with many age-associated diseases, including: cancer, diabetes, Parkinson's, Alzheimer's, cardiovascular disease, macular degeneration, rheumatoid arthritis, amyotrophic lateral sclerosis (ALS) and sarcopenia [64–68]. Chronic inflammation is typified by the simultaneous occurrence in a single tissue of active inflammation (characterized by oedema, leucocyte recruitment, and fibroblast and endothelial cell proliferation), tissue destruction and tissue repair. This sub-acute inflammatory state often manifests with increases in tissue-infiltrating inflammatory cells and higher circulating levels of pro-inflammatory cytokines (such as TNF-alpha, IL-6 and CCL2), complement proteins, and cell adhesion molecules [64]. Importantly, the impact of inflammatory mediators on satellite cell function is likely to be quite complex. Accumulating evidence suggests that such factors can either inhibit or enhance muscle repair through direct and indirect means and in a highly concentration and time-dependent manner [52,69–71] (figure 3).

The molecular mechanism underlying the enhanced 'inflammatory tone' in older individuals is still unknown, although cell death induced by accumulated DNA damage, a result of long-term exposure to metabolic by-products, chemical mediators and ionizing radiation has been suggested [72]. In this model, DNA damaging agents generate oxygen-free radicals that react with proteins, lipids, carbohydrates and nucleic acids to cause peroxidation, fragmentation and breakage of cellular building blocks. This cellular damage in turn provokes a pro-inflammatory response, which ironically leads to the generation of additional free radicals, feeding a positive regulatory loop that ultimately results in chronic inflammation [64,72]. Significantly, even individuals without overt manifestation of age-associated disease exhibit signs of chronic, low-grade inflammation. For example, there is a strong correlation of age with increased circulating levels of pro-inflammatory cytokines [73,74], while the absence of serum inflammatory proteins appears to correlate with the maintenance of muscle function, as measured by grip strength [74]. Additionally, based in part on transcriptional profiling studies in whole human muscle [75], a predictive association has been suggested between inflammation and sarcopenia. However, as mentioned previously, the molecular basis for age-related induction of inflammation in the muscle, and whether these inflammatory pathways play a direct or indirect role in the age-associated dysfunction of muscle satellite cells, remains unclear.

Taken together, the data discussed in the last two sections strongly suggest that age-associated deficits in skeletal muscle repair can be attributed to cell-intrinsic changes in genomic integrity and biochemical signalling pathways that regulate satellite cell function, as well as microenvironmental signals that extrinsically modulate the activation and the proliferation of myogenic cells. Importantly, however, these changes appear to be reversible, such that significant muscle-forming activity can be restored to aged satellite cells via modulation of either local or systemic signals

[46,48] (figure 3). Better understanding of the causes and consequences of age-related changes in both intrinsic and extrinsic pathways will be essential to developing strategies to manipulate these processes to enhance healthy muscle function and repair in elderly individuals.

## 8. USING STEM CELLS TO MODEL DISEASE AND DISCOVER NEW DRUGS

As discussed in detail above, muscle satellite cells are potent tissue-specific stem cells that hold significant promise for regenerative medicine, either through rejuvenation or activation of the endogenous myogenic programme or by cell transplantation to repopulate a depleted or dysfunctional satellite cell pool (or a combination of both). Such approaches could be applied in the treatment of a variety of skeletal muscle diseases, including muscular dystrophies, congenital myopathies and sarcopenia. The path forward to realize these promises will be illuminated in part by basic biological studies, employing genetic and genomic analysis of satellite cells in their natural environment (the skeletal muscle). However, drug discovery through *in vitro* modelling and chemical screening will also be likely to play a key role.

In this regard, we are fortunate that muscle satellite cells can be directly isolated and cultured. Such *in vitro* myogenic differentiation systems will allow researchers to perform genetic, pharmacological or small molecule interventions that can lead to the discovery of novel pathways that enhance myogenic function. However, for some human muscle diseases, appropriate animal models may not be available, and patient biopsy tissue will probably be in short supply. In these situations, drug discovery strategies will depend upon the development of conditions that permit extensive *in vitro* propagation of bona fide muscle satellite cells or, alternatively, the adaptation of newly emerged technologies for the establishment of human disease-specific induced pluripotent stem cells (iPSCs) to support *in vitro* differentiation of skeletal myogenic cells for research and drug development [76].

IPSCs are pluripotent stem cells that can be generated from differentiated adult cells by delivery of particular pluripotency-associated transcription factors or by chemical activation of those proteins within the target cells [77]. Introduction or activation of these protein factors alters the gene expression profile of the target cell such that it reverts to a pluripotent stem cell fate [78], with the potential to be differentiated into any cell type in the body. Because iPSCs retain the same genetic make-up as the somatic donor cell targeted for reprogramming, human iPSCs offer exciting, new possibilities for understanding the complex processes that underlie the initiation and pathology of muscle diseases, and that may contribute to the substantial variation in presentation and progression typically observed among patient populations. In addition, the ability to someday derive transplantable muscle stem cells from patient-specific iPS cells [79–82] ultimately could enable the creation of gene-corrected donor cells that would be genetically matched to individual patients. These cells could then be employed therapeutically to correct genetic deficiencies and restore muscle function in affected patients. Of course, the realization of this promise again will require significant scientific advances, particularly in our ability to direct the differentiation of pluripotent cells along the skeletal myogenic pathway. Currently available techniques are still challenged by lengthy and rather inefficient differentiation protocols, and by difficulties in generating muscle precursors that resemble adult satellite cells and can support robust muscle regeneration upon transplantation [83–87]. Nonetheless, studies in this area currently are being pursued by a number of excellent laboratories, whose progress will no doubt enable such approaches in the very near future.

## 9. SUMMARY

Skeletal muscle represents a well-established and robust system for studying the mechanisms the regulate adult tissue regeneration from resident stem cells. Muscle satellite cells play a crucial role in supporting muscle repair activity, and impairment or inhibition of their activity contributes to progressive muscle degeneration in a number of human conditions. Satellite cell function is controlled by a complex and integrated network of cell intrinsic and extrinsic signals, many of which represent potential targets for therapeutic intervention to enhance endogenous regenerative capacity and facilitate myogenic cell transplantation approaches. Future studies aimed at enhancing our ability to propagate purified satellite cells in a manner that retains their undifferentiated state and robust engraftment capacity, as well as novel approaches aimed at deriving satellite cells and their progeny from iPSCs will help to accelerate progress in drug development and cell-based therapy for the treatment of muscle degenerative disease.

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