Stem cells for skeletal muscle regeneration: therapeutic potential and roadblocks

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Conditions involving muscle wasting, such as muscular dystrophies, cachexia, and sarcopenia, would benefit from approaches that promote skeletal muscle regeneration. Stem cells are particularly attractive because they are able to differentiate into specialized cell types while retaining the ability to self-renew and, thus, provide a long-term response. This review will discuss recent advancements on different types of stem cells that have been attributed to be endowed with muscle regenerative potential. We will discuss the nature of these cells and their advantages and disadvantages in regards to therapy for muscular dystrophies. (Translational Research 2014;163:409–417)

Abbreviations: DMD = Duchenne muscular dystrophy; ES cells = embryonic stem cells; iPS cells = induced pluripotent stem cells; MD = muscular dystrophy; MDSCs = muscle-derived stem cells; MyoD = myogenic differentiation antigen; Pax3 = paired box homeodomain 3; Pax7 = paired box homeodomain 7

Muscular dystrophies (MDs) comprise more than 30 neuromuscular disorders of inherited origin.[1] A common feature of this clinically and genetically heterogeneous group of disorders is progressive muscle weakness in particular subsets of degenerating skeletal muscles, which leads to atrophy, and frequently the confinement of affected patients to a wheelchair. The most common, Duchenne muscular dystrophy (DMD), affects one out of 5000 male live births, and is caused by mutations in the dystrophin gene that result in biochemical defects of the dystrophin-glycoprotein complex in skeletal muscle and other tissues.[2,3] Affected patients lose mobility by their teenager years with eventual death because of respiratory and/or cardiac failure.

Loss of skeletal muscle mass is also observed in the aging population, a process known as sarcopenia, and as a secondary effect in some cancer patients, known as cachexia. These conditions can also cause severe debilitating weakness and metabolic dysfunction.[4-7]

Skeletal muscle comprises about 40% of the human body mass, and it is well recognized for its robust capacity for regeneration. Seminal observations in this regard were first described in 1953 by Russian investigator A.N. Studitsky, who chopped a muscle biopsy into 1-mm^3 pieces and observed remarkable new muscle formation following reimplantation of this minced muscle tissue back into the muscle bed.[8] This rudimentary model was proven to be very useful to understand the initial principles of skeletal muscle regeneration. Subsequently, Carlson and Gutmann[9] repeated these experiments in rats and demonstrated that the new fibers were functionally and morphologically similar to those present in normal muscle. Several years later, this model was used to investigate the impact of age on this regenerative process. Whereas young and aged minced muscle tissue regenerated well in young hosts, this was not the case in aged hosts,[10,11] indicating for the first time that

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the environment plays an important role in skeletal muscle regeneration. Several recent reports have corroborated this observation.[12-15]

Based on the premise of this incredible regenerative capacity, pioneering studies initiated in the late 1980s[16] and extended into the 1990s[17-19] began to explore cell-based therapies to promote muscle regeneration as a potential treatment for MDs. Initially, most of these studies focused on the transplantation of adult myoblasts aiming to regenerate skeletal muscle by the fusion of those cells (donor myoblasts) with recipient's cells to form new or hybrid fibers. Adult myoblasts were isolated from muscle biopsies and expanded *ex vivo* before transplantation.[16-18] Despite the encouraging findings obtained using the dystrophin-deficient *mdx* mouse model, clinical trials performed in a cohort of DMD patients were disheartening due to poor myoblast transfer efficacy and failure to improve strength in treated muscles.[3,20,21] Major factors underlining this poor outcome included low ability of myoblasts to migrate beyond the injection site[22,23] and poor survival of injected cells.[24,25] Several research groups have been working toward the goal of overcoming these issues as well as the immune response observed in the recipient following myoblast transfer.[26-29]

As an alternative to poorly engrafting myoblasts, much recent interest has developed around the idea of therapy with stem cells. These cells have the ability to self-renew and to differentiate into specialized cell types, and can be primarily classified as adult and pluripotent stem cells, which differ significantly in regard to their differentiation potential and *in vitro* expansion capability. Adult stem cells are tissue-specific and have limited capacity to be expanded *ex vivo* whereas pluripotent stem cells have the ability to differentiate into any cell type of the body while possessing unlimited *in vitro* self-renewal. Below we review the literature on some of the most studied stem cell populations that have been ascribed with *in vivo* muscle regenerative potential, pointing out their advantages as well as caveats.

ADULT STEM CELLS

Satellite cells. Studies in the last decade have clearly proven that the regenerative ability of adult skeletal muscle is due to the satellite cell, a quiescent stem cell population of muscle precursors located between the basal lamina and sarcolemma of each myofiber.[30-32] The satellite cell was first described by Mauro in 1961 using electron microscopy,[33] and later by Bishoff [34] in 1986 using phase-contrast microscopy on single myofiber explants. Upon injury, satellite cells become activated, giving rise to proliferating myoblasts, which then fuse to existing muscle fibers or to other myoblasts to form new myofibers to repair muscle damage.[35-39] Meanwhile, a small subset of satellite cells does not undergo differentiation but retains the ability to return to a quiescent state and thus preserve the satellite cell pool.[4,30,40,41] In addition to their typical localization, a hallmark of these cells is the expression of Pax7, a paired box homeodomain-containing transcription factor[32,42] necessary for the maintenance of the muscle stem cell compartment in adult mice[32,42-44] as well proliferation following injury[45] and, consequently, being indispensable for adult skeletal muscle regeneration.[46] There is evidence for heterogeneity within the satellite cell compartment, with a subset of satellite cells having greater potential to engraft the satellite cell compartment.[45,47,48]

It took about 50 years from their initial identification in the early 1960s for pure preparations of mouse satellite cells to be isolated and tested for their regenerative potential.[30,31] One group took the approach of transplanting single muscle fibers, which demonstrated that each myofiber, containing 7 or fewer satellite cells, could generate over 100 new myofibers in engrafted muscles.[30] The other approach made use of a transgenic reporter mouse for Pax3, a paralog of Pax7, which allowed for the direct isolation of $Pax3^{+}$ (green fluorescent $protein^{+}$) muscle satellite cells by flow cytometry.[31] Cells isolated from adult skeletal muscles displayed homogenous expression of Pax7, and contributed to both fiber repair and to the muscle satellite cell compartment following their transplantation into dystrophic mice.[31] Subsequently Sacco et al demonstrated that intramuscular transplantation of a single luciferase-expressing muscle stem cell, isolated from Myf5 reporter mice, resulted in extensive proliferation and contribution to muscle fibers. In addition, these authors showed that $Pax7^{(+)}luciferase^{(+)}$ mononuclear cells could be readily re-isolated, providing evidence for the self-renewal of this cell population.[49]

Satellite cells have also been characterized phenotypically by the expression of several surface markers, such as M-cadherin,[50] CD34,[51] syndecan-3/4,[52] $\alpha 7\beta 1$-integrin,[53,54] and the chemokine receptor CXCR4,[55] among others.[56-59] The first report making use of surface markers to isolate muscle precursor cells was published in 2004, in which the authors used a combination of negative and positive selection to discern muscle activity among different cell fractions.[60] *In vitro* and *in vivo* myogenic potential was found within the $CD45^{-}Sca\text{-}1^{-}Mac\text{-}1^{-}CXCR4^{+}\beta 1\text{-}integrin^{+}CD34^{+}$.[60,61] In 2009, Tanaka et al[62] documented that the $Sca\text{-}1^{+}/ABCG2^{+}/Syndecan\text{-}4^{+}$ cell