Stem Cells in Adult Skeletal Muscle Tissue: More than a Working Hypothesis

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Abstract

It has been assumed that a stem cell derived from adult tissues can give rise only to progeny specific to that tissue type. However, this dogma has been challenged recently by a series of studies that suggest that adult tissue-derived stem cells may have the potential to differentiate into disparate cell types. In adult skeletal muscle, a particular cell type named satellite cells are believed to form a stable, self-renewing pool of stem cells. However, this tissue contains also at least two other types of muscle-derived stem cells: i) a cell population so-called MDSC, which may represent a predecessor of the satellite cell and ii) a novel stem cell population purified as a side population (SP), which possess the ability to differentiate also into hematopoietic cells.

Key words: muscle regeneration, satellite cells, stem cells.

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Adult stem cells perform two major functions: self-renewal and multi-lineage differentiation. Stem cells are functionally responsible for the development and regeneration of tissue and organs; developmental signals, both biochemical and biomechanical, trigger the proliferative action of the stem cells in early development.

Traditionally, it has been assumed that stem cells derived from adult tissues can give rise only to progeny specific to that tissue type. However, recently this dogma has been challenged by a series of studies that suggest that adult tissue-derived stem cells may have the potential to differentiate into disparate cell types. For example, purified hematopoietic stem cells (HSC), derived from whole bone marrow, have been shown to contribute to the regeneration of skeletal muscle, cardiac muscle, liver tissue, and multiple epithelial tissues. It has also been proposed that stem cells from other tissues may differentiate outside their tissue of origin, as well. Thus, a few donor bone-marrow cells, when transfused into immuno-deficient mice, were recovered as glial cells in the host brain or as cells expressing neuronal antigens. Alternatively, neural stem cells can differentiate into myeloid and lymphoid cell lineages upon transplantation into the hematopoietic system of irradiated hosts. Although these studies have provoked new critical thinking concerning the capacity of stem cell differentiation, definitive proof of trans-differentiation remains to be established at a clonal level [8].

The mature functional skeletal muscle cell, the multinucleated myofiber, is surrounded by special stem cells known as satellite cells, which lie outside the sarcolemma but within the basal lamina. These cells, which appear to be committed precursor cells, were first described based on their location and morphology by Mauro in 1961 [7]. Satellite cells in adult muscle remain quiescent until external stimuli trigger re-entry into the cell cycle. Their progeny, myoblasts, fuse to form new multinucleated myofibers. Cell surface markers associated with the satellite cell phenotype, either in the quiescent or activated state, have been identified and include: M-cadherin, c-met, and CD34. Thus, satellite cells are believed to form a stable, self-renewing pool of stem cells in adult muscle where they function in tissue growth and repair

Moreover, skeletal muscle also contains at least two other types of muscle-derived stem cells: i) the so-called MDSC, a cell population which possesses several differentiative abilities in addition to the characteristic function of a myogenic precursor, capable of regenerating skeletal muscle and demonstrating self-renewal properties and thus may represent a predecessor of the satellite cell [6]; ii) a novel stem cell population purified as a side population (SP) which actively excludes Hoechst 33342 dye. Muscle SP cells that express the hematopoietic stem

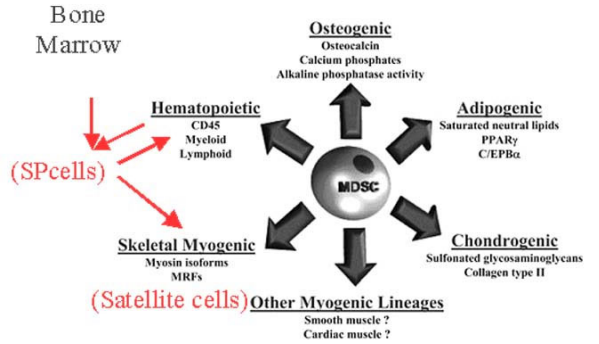

Fig 1. Differentiative capacity and typology of skeletal muscle stem cells

cell marker Sca-1 possess the ability to differentiate into either hematopoietic cells, skeletal muscle, or satellite cells following transplantation. The muscle SP fraction also contains cells expressing the hematopoietic marker CD45 that are capable of differentiation into hematopoietic cells and muscle cells [1]. Thus, these novel muscle stem cells appear to have characteristics similar to those of hematopoietic stem cells, and can participate in muscle regeneration.

This possibility was also demonstrated by Goodell MA *et al* [5] through experiments in which cells prepared from muscle by enzymatic digestion and a 5 day *in vitro* culture were harvested and introduced into each of six lethally irradiated recipients together with distinguishable whole bone marrow cells. Six and twelve weeks later, all recipients showed high-level engraftment of muscle-derived cells representing all major adult blood lineages. The mean total contribution of muscle cell progeny to peripheral blood was 56%, indicating that the cultured muscle cells generated approximately 10- to 14-fold more hematopoietic activity than whole bone marrow. Although the identity of the muscle-derived hematopoietic stem cells is still unknown, they may be identical to muscle satellite cells, some of which lack myogenic regulators and could possibly respond to hematopoietic signals. The authors also found that stem cells in the bone marrow could contribute to cardiac muscle repair and neovascularization after ischemic injury. Recent studies in mice, however, have revealed the potential for highly purified hematopoietic stem cells from bone marrow to participate in muscle regeneration. Perhaps more significantly, a population of putative stem cells isolated directly from skeletal muscle efficiently reconstitutes the hematopoietic compartment and participates in muscle regeneration following intravenous injection in mice [9]. In fact, in regenerating muscle, the number of myogenic precursors exceeds that of resident satellite cells, implying the migration or recruitment of undifferentiated progenitors from other sources. Transplantation of genetically marked bone marrow into immunodeficient mice revealed that marrow-derived cells migrate into areas of induced muscle degeneration, undergo myogenic differentiation, and participate in the regeneration of the damaged fibers [4].

Furthermore, the ability of hematopoietic cells to undergo myogenesis has prompted new investigations into the embryonic origin of satellite cells. Recent developmental studies suggest that a population of satellite cells is derived from progenitors in the embryonic vasculature [5].

Taken together, these studies provide the first evidence that pluripotential stem cells are present within adult skeletal muscle. Tissue-specific stem cells, including satellite cells, may share a common embryonic origin and possess the capacity to activate diverse genetic programs in response to environmental stimuli. Manipulation of such tissue-specific stem cells may eventually revolutionize therapies for degenerative diseases, including post-traumatic muscle repair and muscular dystrophy.

Culture methods have also been used to separate distinct muscle-derived cell populations [2]. Based upon the variable adherence of cells obtained from freshly dissociated muscle, pre-plating has been used as a purification technique in obtaining different stem cell populations. Using such techniques to isolate cells that demonstrate slow adhesion characteristics, it was shown that greater transplantation efficiency could be achieved, even without an anti-inflammatory strategy. In a subsequent study, this purification technique was used to establish a clonal cell line that was shown to be highly efficient in regenerating dystrophin-positive myofibers upon direct injection and also demonstrated, although to a lesser extent, the ability to regenerate muscle following intravenous delivery.

A strategy to compensate for this poor capacity to produce a number of cells sufficient for valid cell therapy consists of the preparation of engineered muscle fibres, which are able to avoid the problems associated with the use of cultured stem cells [3]. The potential applications for functional engineered skeletal muscle extend from that of basic research to drug discovery to surgical transplantation, hybrid prosthetics, and robotics. The adult human body is approximately 40% skeletal muscle by mass. Diseases of skeletal muscle range from the debilitating to the crippling to the lethal. The ability to take a few cells from an adult mammal (or human) and produce a large mass of functional skeletal muscle would be of incalculable benefit to mankind.

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