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Review

Muscle stem cells: what's new in orthopedics?

Carlo Biz[1], Alberto Crimì[1], Ilaria Fantoni[1], Assunta Pozzuoli[1, 2], Pietro Ruggieri[1]

[1] Orthopedic Clinic, Department of Surgery, Oncology and Gastroenterology DiSCOG, University of Padova, Padova, Italy; [2] Musculoskeletal Pathology and Oncology Laboratory, Department of Orthopedics and Orthopedic Oncology, University of Padova, Padova, Italy

Summary. *Background and aim of the work:* Adult stem cells were studied as a source of potentially useful development for tissue engineering and repair techniques. The aim of this review is to clarify the actual and possible uses of muscle stem cells in orthopedics. *Methods:* A selection of studies was made to obtain a homogeneous and up to date overview on the muscle stem cells applications. *Results:* In recent years muscle was studied as a good source of adult stem cells that can differentiate into different cell lineages. Muscle stem cells are a heterogeneous population of cells, which demonstrated in vitro a great potential for the regeneration and repair of muscle, bone and cartilage tissue. Among muscle stem cells, satellite stem cells are the most known progenitor cells: they can differentiate in osteoblasts, adipocytes, chondrocytes and myocytes. *Conclusions:* Although muscle stem cells are a promising field of research, more pre-clinical studies in animal models are still needed to determine the safety and efficiency of the transplant procedures in humans. (www.actabiomedica.it)

Key words: muscle stem cells, satellite cells, muscle repair, bone healing, cartilage healing

Introduction

Stem cells can be adult stem cells or embryonic stem cells. They can be totipotent (cells capable of becoming an entire organism), pluripotent (cells capable of generating the three germ layers) and multipotent (cells of a specific germ layer becoming organ-specific progenitors). The adult stem cells have two characteristics: self-renewal and multi-lineage differentiation (1). Stem cells give tissues and organs the possibility to develop and regenerate. Biochemical and bio-mechanical signals regulate proliferation and differentiation of stem cells, typical of early development and tissue regeneration (2). There is considerable heterogeneity in the classification of Muscle Stem Cells (MSCs). The International Society of Cell and Gene Therapy (ISCT) system is still the current classifying system for MSCs (3). After birth, muscle regeneration is mediated mostly by Satellite Cells (SCs): these cells are flattened cells, located between the sarcolemma and the basal lamina of myofibers (4). They represent a heterogeneous population of self-renewable stem cells. They are quiescent in vivo, but they can be activated by increased muscle work such as after-load-induced hypertrophy, prolonged exercise, and in some pathological conditions such as myotraumas. When activated, SCs proliferate, migrate from the myofibers, and express specific myogenic markers, thus becoming muscle precursor cells (MPCs). Recent studies on Muscle Stem Cells (MSC) highlighted their possible use in repair of muscles and regeneration of tissues like bone and cartilage. MSCs can be separated in 2 subtypes CD45+ and CD45-. The first ones, if isolated by the muscle, have a limited myogenic potential but a high hematopoietic potential. The CD45- cells have a high myogenic potential and a low hematopoietic potential (5-7). Environmental signals like Wingless/Integrated 8 (Wnt8) can modify the differentiation potential of the MSCs (8).

MSCs demonstrated good transplantation behavior in animal models and resistance to in vitro manipu-

lation, becoming in this way very useful in the repair and regeneration of musculoskeletal tissues (9).

The aim of this review is to investigate the actual and possible use of muscle stem cells in musculoskeletal diseases.

MSCs and factors that regulate stem cell self-renewal and differentiation

MSCs are related with endothelial cells of the capillaries or with pericytes; some myogenic-endothelial progenitor cells are in fact CD34+ and CD45- (10). These cells can differentiate in vascular endothelial cells or musculoskeletal cells (11). Some studies demonstrated that MSCs are associated with vascular structures, particularly with the myofibers surrounding capillaries (6, 12). The hypothesis is that repair of the local skeletal muscle is made by resident stem cells (8). MDSCs cell cycle is modified and enhanced in vitro by growth factors: insulin-like growth factor-1 (IGF-1), epithelial growth factor (EGF), stem cell factor (SCF) and fibroblast growth factor-2 (FGF-2) (13).

Harvesting technique

One of the major limitations in the use of satellite cells is the low number of extracted cells due to the small size of biopsies and the difficult separation from other cellular components, it is still a challenge to obtain enough muscle stem cells in vitro. The first effort to obtain a method for dissociating mammalian muscle into intact, living single fibers was introduced by Bekoff and Betz in 1977 (14). Afterwards, Bischoff modified the Bekoff and Betz method to permit the study of SC proliferation on rat flexor digitorum brevis muscle fibers in vitro (15). Rosenblatt et al. (16) proposed a method for isolating myogenic cells based on the previous method described by Bischoff (15). This allows isolation of SCs from single muscle fibers. Cells can easily be removed from culture and analyzed. In this way, differences in myogenic cell behavior can be detected with greater sensitivity and reliability, both within and between muscles (16). Muscle stem cells can be obtained with two different approaches: single fiber isolation and whole muscle enzymatic digestion. There are different protocols to obtain these cells. An efficient protocol to isolate and expand in culture human muscle precursor cells from different skeletal muscles was described by Franzin et al. (17).

Muscle regeneration and repair

Muscle injuries usually imply a mechanism of shearing, with torn connective tissue and myofibers, or a punctiform damage. In this case only the myofibers are damaged while connective tissue does not present damage. Immediately after the trauma there is hematoma formation, muscle degeneration, necrosis and infiltration of inflammatory cells (18). After this phase there is a reparative phase, with phagocytosis of necrotic or damaged tissue, muscle fiber regeneration, formation of scar tissue and neovascularization (19). In the following remodeling phase there is muscle regeneration and reorganization of scar tissue. The MDSCs (CD45+) are involved in muscle regeneration (7). MDSCs can differentiate in myofibroblast-like cells in vitro and so can contribute to scar formation after muscle injury in vivo, mainly if stimulated with Tumor Growth Factor β-1 (TGF- beta1) (20, 21). The activation of SCs induces fibroblasts to produce extracellular matrix and proliferate (22). This extracellular matrix production in some traumas can lead to excessive scar formation with insufficient muscle regeneration (21). In these cases, some studies demonstrated that some signals can prevent formation of an over-fibrotic scar (gamma interferon, decorin) and others (IGF-1) can improve muscle healing (23-25). In any case, MSC transplantation techniques still have bad results (26). Recent studies highlighted that only a small part of the satellite cells are true muscle stem cells. This subpopulation proliferates slower than the main one (27, 28), but it is in charge of the long-term survival of implanted cells (29). Rossi et al. demonstrated how hydrogel technology can be applied to skeletal muscle for the reconstruction of damaged muscles, designing the delivery of either stem cells or muscle progenitor cells (30).

Bone healing

Fracture repair involves: acute response to damage, intra-membraneous bone formation, endochon-

dral bone formation, cartilage formation and bone remodeling (18). Different techniques were studied to repair bone defects, in particular biologically enhanced allografts, gene- or cell-based tissue engineering (31, 32). MSCs can be induced to have osteogenic differentiation and can heal bone defects in animals (18). A subpopulation of MSCs in skeletal muscle can be induced by osteogenic proteins. It was shown that murine MDSCs genetically modified to express bone morphogenetic protein- 2 (BMP-2) and BMP-4, a group of proteins of the TGF family with a pivotal role in bone remodeling, can differentiate into an osteogenic lineage, determining, in these studies, bone healing in long bones in mice models (33-38). Moreover, vascular endothelial growth factor (VEGF) modulates bone formation, improving bone healing after implantation of MDSCs with expression of BMP2 and BMP4 in animal models (39, 40). There are ongoing Clinical Trials on humans.

Articular cartilage repair

Cartilage is known to have poor healing capacity. Adult articular cartilage has no vascularization or innervation, and defects with a diameter larger than 2-4 mm usually do not heal (41, 42). Nowadays, the main operative treatments of articular cartilage defects are: total joint replacement, transplantation and articular surface debridement. The tissue repaired with transplantation does not integrate and degenerate over time (43).

Cartilage repair via chondrocyte transplantation

There are different articular cartilage repair techniques, all of them with unproven long-term efficacy in animal models (44, 45). Investigated procedures are: transplantation of cartilage plugs (46), autologous chondrocytes transplantation (44), allogenic chondrocytes transplantation (47) and fetal chondrocytes transplantation (48, 49).

Muscle-derived cells for cartilage repair

A satisfactory result was obtained in cartilage healing using muscle-derived stem cells. MSCs showed if transplanted in cartilage articular defects artificially created in rabbits a result comparable to chondrocytes transplantation (50), with the production of type-II collagen (51).

Other future promising techniques for cartilage repair

Furthermore, genetic engineering can have an important role in regenerative medicine. An adenoviral vector (with IGF-1 expression) was used to transduce and enhance equine mesenchymal stem cells (53). Cells so enhanced secreted IGF-1 stimulating changes in cartilage matrix gene expression (54), inducing cartilage healing. Other growth factors can stimulate stem cells proliferation, migration and differentiation: BMPs bone morphogenetic proteins (BMP), Transforming growth factor (TGF)-beta1, beta2 and beta3 and fibroblast growth factors (54). A better understanding of these factors could lead to a combined use of stem cells and growth factor in articular defects.

Conclusions

There are still many obstacles in the use of MSCs in regenerative medicine. Their transplantation as clinical therapy is far from being efficient (55). Some clinical studies reported the use of MSCs to treat pathologies like rotator cuff tears (56) and articular cartilage damage (57). Other fields of application were clinical trials on human cardiac disease, stress incontinence of the bladder and muscular dystrophies (58). The biological properties and effects of MSCs in vivo on musculoskeletal tissue healing remains overall not satisfactory. An obstacle to the success of myogenic stem cell therapy in humans is to obtain a sufficient number of freshly isolated satellite cells (59). Basic science studies and preclinical works are needed before the use in clinical practice in orthopedics of these techniques with an acceptable level of efficiency and safety. Recent research is focused on the clinical use of reconstructive techniques to obtain repair of tissue loss in murine models (60). The increasing knowledge of molecular mechanisms at the basis of the activation, differentiation, and phenotypic switch of the MSCs is the first step towards the comprehension of their role in muscular pathologies. The promising combination of adult

stem cell use, gene therapy techniques and tissue engineering will obtain new and effective therapies for the healing of tissues with low regenerative capacity.

Authors' contribution:
C.B. and A.C.: study concept and design; drafting of the paper;
A.C. and I.F.: literature research and data collection;
C.B. and A.P.: analysis and interpretation of data;
P.R.: final approval of the version to be published.

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Correspondence:
Carlo Biz MD, Assistant Professor,
Orthopaedic Clinic, Department of Surgery,
Oncology and Gastroenterology DiSCOG,
University of Padua, via Giustiniani 2 - 35128 Padova, Italy
Tel. 0039 049 8213239
E-mail: carlo.biz@unipd.it