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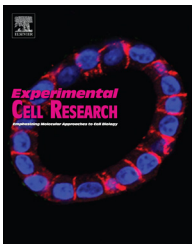

Research Article

Defining the role of mesenchymal stromal cells on the regulation of matrix metalloproteinases in skeletal muscle cells

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ABSTRACT

Recent studies indicate that mesenchymal stromal cell (MSC) transplantation improves healing of injured and diseased skeletal muscle, although the mechanisms of benefit are poorly understood. In the present study, we investigated whether MSCs and/or their trophic factors were able to regulate matrix metalloproteinase (MMP) expression and activity in different cells of the muscle tissue. MSCs in co-culture with C2C12 cells or their conditioned medium (MSC-CM) up-regulated MMP-2 and MMP-9 expression and function in the myoblastic cells; these effects were concomitant with the down-regulation of the tissue inhibitor of metalloproteinases (TIMP)-1 and -2 and with increased cell motility. In the single muscle fiber experiments, MSC-CM administration increased MMP-2/9 expression in Pax-7^{+} satellite cells and stimulated their mobilization, differentiation and fusion. The anti-fibrotic properties of MSC-CM involved also the regulation of MMPs by skeletal fibroblasts and the inhibition of their differentiation into myofibroblasts. The treatment with SB-3CT, a potent MMP inhibitor, prevented in these cells, the decrease of α-smooth actin and type-I collagen expression induced by MSC-CM, suggesting that MSC-CM could attenuate the fibrogenic response through mechanisms mediated by MMPs. Our results indicate that growth factors and cytokines released by these cells may modulate the fibrotic response and improve the endogenous mechanisms of muscle repair/regeneration.

Abbreviations: α-sma, α-smooth muscle actin; bFGF, basal fibroblast growth factor; DIC, differential interference contrast; DM, differentiation medium; ECM, extracellular matrix; EDL, extensor digitorum longus; EDTA, ethylenediaminetetraacetic acid; GFP, green fluorescent protein; HGF, hepatocyte growth factor; IL-1, interleukin -1; MMPs, matrix metalloproteinases; MSC-CM, mesenchymal stromal cell-conditioned medium; MSCs, mesenchymal stromal cells; Pax-7, paired box protein-7; PBS, phosphate buffered saline; PFA, paraformaldehyde; PMSF, phenylmethanesulfonyl fluoride; PVDF, polyvinylidene difluoride; ROI, regions of interest; RT, room temperature; SDS, sodium dodecyl sulfate; TGF-β, transforming growth factor-β; TIMP-1, tissue inhibitor of metalloproteinase-1; TIMP-2, tissue inhibitor of metalloproteinase-2; VEGF, vascular endothelial growth factor

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Introduction

Skeletal muscle has a remarkable ability to regenerate after traumatic injury or disease. This is due to the presence of peculiar stem cells, satellite cells, which lie quiescent under the basal lamina of the muscle fibre until activated in response to injury, when they leave their niche and proliferate before differentiating into myoblasts and fuse into new myofibres [1]. These cells are characterized by the expression of paired box protein (Pax)-7 and myogenic differentiation markers (including Myf5, MyoD, and myogenin) in many muscles [2]. However, the propensity of these cells to repair skeletal muscle is limited in case of extended disease (muscular dystrophies and diabetes), exercise-induced injury [3] or altered use (immobilization, denervation, aging) [4–7]; in these cases, the amount of extracellular matrix (ECM) may increase dramatically relative to muscle fibres, resulting in scarring of the tissue. Muscle fibrosis continues to represent a challenge for clinicians and researchers since it impairs the complete muscle recovery, restricts range of motion, and predisposes to re-injury. Along this line, much attention has been given in the recent years to factors and therapeutic strategies that can improve skeletal muscle healing and regeneration, while reducing scar tissue formation. Studies conducted by our group and others have shown that muscle regeneration can be aided by the administration of growth factors [8–12] and bioactive lipids, including sphingosine 1-phosphate [13–15]. However, these factors are short-lived and more effective methods are required. Emerging evidence suggests that muscle repair can also benefit from stem cell therapy. To this aim, a variety of cell populations have been used, among which bone-marrow-derived mesenchymal stromal cells (MSCs) appear to be attractive candidates for myo-regenerative purposes [16–22]. This because these cells posses some interesting peculiarities for cell therapy, including the relatively easy isolation and expansion in culture, stable phenotype and limited rejection [23]. It is becoming increasingly clear that the MSCs offer benefits beyond their cell replacement potential by providing growth factors and cytokines with multiple effects in the host tissue microenvironment, including neoangiogenesis, the modulation of the endogenous repair mechanisms and prevention of injured cells from the stress response and apoptosis [24–27]. In this context, we have previously demonstrated that MSCs stimulate neonatal cardiomyocyte and skeletal myoblast proliferation [20,28]; these effects are mainly mediated by the secretion of a variety of growth factors and cytokines, including vascular endothelial growth factor (VEGF) [20]. We have also shown that MSC transplantation contributes to skin regeneration by recruiting the local epithelial progenitors to participate in the repair process in the wound [29]. Of interest, the contribution to muscle repair by MSCs and their trophic factors may also involve modulation of fibrosis. Most studies on this field have focused on the role played by MSCs and other stem cells in ameliorating ventricular compliance and improving the cardiac performance after myocardial infarction [19,26,30–35]. However, the role played by these cells in reducing fibrosis in diseased or injured skeletal muscle is less known.

With this in mind, the present study was undertaken to further understand and expand the paracrine activity of MSCs on skeletal muscle repair/regeneration, focusing on the effects of MSCs and their conditioned medium on the expression and activity of matrix metalloproteinases (MMPs), by skeletal C2C12 myoblasts, satellite cells and skeletal fibroblasts derived from mouse skeletal muscle tissue. MMPs are a family of enzymes that selectively digest individual components of ECM; their function is tightly regulated through the action of specific tissue inhibitors of metalloproteinases (TIMPs) and is required for muscle healing, by reducing fibrosis and promoting myogenic cell migration through the ECM to the site of injury [36–41]. We found that factors released by MSCs exert potent anti-fibrotic effects via their ability to regulate the balance of MMP-2 and MMP-9/TIMP-1 and-2 production by the assayed muscle cells and improve satellite cell migration and differentiation, thus providing new insights into the potential role of MSC-cell therapy in muscle regenerative medicine.

Material and methods

Ethics statement

All animals' manipulations were carried out according to the European Community guidelines for animal care (DL 116/92, application of the European Communities Council Directive of 24 November 1986; 86/609/EEC) and approved by the Committee for Animal Care and Experimental Use of the University of Florence. The ethical policy of the University of Florence conforms to the Guide for the care and use of laboratory animals of the U.S. National Institutes of Health (NIH Publication No. 85–23, revised 1996; University of Florence assurance No. A5278-01). The protocols were communicated to local authorities and to Italian Ministry of the Health; according to the Italian law (Art.7/D.lgs 116/92); such procedure doesn't require Ministry authorization. The animals were housed with free access to food and water and maintained on a 12 h light/dark cycle at 22 °C room temperature (RT). All efforts were made to minimize the animal suffering and the number of animals sacrificed. Animals were killed by decapitation.

Cell culture and treatments

Mouse bone marrow mesenchymal stromal cells (m-MSCs) were isolated from femura and tibiae of male C2F1 mice, following the Dobson's procedure [42], expanded *in vitro* and characterized as reported previously [20]. In some experiments, these cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2, in C2C12 myoblast differentiation medium (myoblast DM) or in muscle satellite culture medium or in NIH3T3 cell or primary skeletal fibroblast culture medium for 24 h and the culture medium (MSC-derived conditioned medium, MSC-CM) was harvested and used for culturing C2C12 myoblasts, satellite cells, single muscle fibres, NIH3T3 or primary skeletal fibroblasts to assess MSC paracrine effects.

Transgenic bone marrow green fluorescent protein (GFP)-labeled MSCs (GFP-MSCs) were isolated from male GFP transgenic Lewis rats (RRRC, Missouri, USA), expanded and characterized as described previously [43]. GFP- MSCs were analyzed for green fluorescence intensity at different passages in culture as well as for the expression of particular cell surface molecules using flow cytometry procedures: CD45-CyChromeTM, CD11b-FITC (in order to quantify hemopoietic-monocytic contamination), CD90-PE, CD73-PE, CD44-PE (BD Pharmingen, San Diego, CA, USA).

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