Experimental Gerontology 103 (2018) 35–46

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

The impact of mechanically stimulated muscle-derived stromal cells on aged skeletal muscle

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ARTICLE INFO

Editor: Christiaan Leeuwenburgh

Keywords:
Aging
Skeletal muscle
Perivascular stromal cells
Mesenchymal stem cells
Exercise
Vascular perfusion
Neurogenesis
Cognition

ABSTRACT

Perivascular stromal cells, including mesenchymal stem/stromal cells (MSCs), secrete paracrine factor in response to exercise training that can facilitate improvements in muscle remodeling. This study was designed to test the capacity for muscle-resident MSCs (mMSCs) isolated from young mice to release regenerative proteins in response to mechanical strain *in vitro*, and subsequently determine the extent to which strain-stimulated mMSCs can enhance skeletal muscle and cognitive performance in a mouse model of uncomplicated aging. Protein arrays confirmed a robust increase in protein release at 24 h following an acute bout of mechanical strain *in vitro* (10%, 1 Hz, 5 h) compared to non-strain controls. Aged (24 month old), C57BL/6 mice were provided bilateral intramuscular injection of saline, non-strain control mMSCs, or mMSCs subjected to a single bout of mechanical strain *in vitro* (4×10^4). No significant changes were observed in muscle weight, myofiber size, maximal force, or satellite cell quantity at 1 or 4 wks between groups. Peripheral perfusion was significantly increased in muscle at 4 wks post-mMSC injection ($p < 0.05$), yet no difference was noted between control and preconditioned mMSCs. Intramuscular injection of preconditioned mMSCs increased the number of new neurons and astrocytes in the dentate gyrus of the hippocampus compared to both control groups ($p < 0.05$), with a trend toward an increase in water maze performance noted ($p = 0.07$). Results from this study demonstrate that acute injection of exogenously stimulated muscle-resident stromal cells do not robustly impact aged muscle structure and function, yet increase the survival of new neurons in the hippocampus.

1. Introduction

Mesenchymal stem/stromal cells (MSCs) reside in close proximity to vessels in nearly all tissues, directly replacing injured tissue or indirectly facilitating repair and remodeling *via* secretion of growth factors and other small regenerative molecules [Augello et al., 2010; Meirelles Lda et al., 2009; Murray et al., 2014]. We recently demonstrated that perivascular, muscle-resident MSCs (mMSCs), isolated based on positive selection for stem cell antigen-1 (Sca-1) and negative selection for the hematopoietic cell marker CD45, can promote Pax7$^+$ progenitor cell (satellite cell) quantity, new fiber formation, myonuclear accumulation, arteriogenesis, and myofiber size when injected into skeletal muscle immediately prior to an eccentric exercise training program in a mouse model [Huntsman et al., 2013; Valero et al., 2012; Zou et al., 2015]. Transplantation in the absence of exercise training did not confer any benefit, highlighting the importance of mechanical strain or a similar exercise-specific cue in mMSC-mediated skeletal muscle adaptation [Huntsman et al., 2013; Valero et al., 2012; Zou et al., 2015]. Our prior studies also demonstrate that mMSCs are non-myogenic, as mMSCs do not form myotubes in culture and do not

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https://doi.org/10.1016/j.exger.2017.12.012
Received 30 August 2017; Received in revised form 16 November 2017; Accepted 14 December 2017
Available online 18 December 2017
0531-5565/

directly fuse with muscle fibers *in vivo* [Valero et al., 2012; Zou et al., 2015]. Thus, perivascular stromal cells contribute to exercise-induced structural and functional gains in a manner that does not require myogenesis.

Aging can significantly reduce the potential for skeletal muscle to grow in response to anabolic stimuli, including exercise and/or amino acid consumption in humans and mice [Katsanos et al., 2006; Kumar et al., 2009; Lee et al., 2016]. The molecular basis for anabolic resistance and subsequent declines in mass and function is controversial, yet inadequate progenitor cell activation and muscle protein turnover likely contribute [Carnio et al., 2014; Conboy et al., 2003; Fan et al., 2016]. Perivascular stromal cells, including mMSCs, may also underlie anabolic resistance in the context of aging. We recently isolated and evaluated perivascular stromal cell function in sedentary (non-exercised) aged mice compared to young adult controls [Munroe et al., 2017]. The results from this study demonstrated that mMSC gene expression is robustly compromised in aged muscle, as evidenced by reduced transcription of several regenerative growth [epidermal growth factor (EGF), leukemia inhibitory factor (LIF), hepatocyte growth factor (HGF), insulin-like growth factor 1 (IGF-1), vascular endothelial growth factor alpha (VEGFα)], neurotrophic [brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), fibronectin type II domain 5 (Fndc5)], and ECM remodeling proteins [matrix metalloproteinases 2 and 14 (MMP2/14), collagen 1α1] that are collectively important for muscle repair and remodeling. Tumor necrosis alpha (TNFα) gene expression was also elevated in aged mMSCs compared to young. The aged mMSC transcriptional signature suggests that aberrant stromal cell function may contribute to anabolic resistance and the subsequent loss of muscle structure and function during the natural process of aging [Munroe et al., 2017]. This information, as well as the unique immune-privileged status of MSCs [Meirelles Lda et al., 2009; Ranganath et al., 2012] and limitations associated with satellite cell transplantation, justify the development of mMSC-focused strategies for the prevention and treatment of age-related disability.

A majority of older adults possess some capacity to exercise, and engagement in a physical activity program may be sufficient to maintain mMSC function and strength throughout the lifespan. However, disability can occur with extended bedrest due to disease or injury, and an alternative cell-based therapy may be desirable. One of the challenges of creating a stromal cell-based therapy is the requirement of promoting stimulation and release of regenerative factors upon transplantation. Our *in vitro* experiments to date have demonstrated specific sensitivity of mMSCs to force, as a single bout of mechanical strain (10% biaxial, 1 Hz, 5 h) can significantly enhance the capacity for freshly isolated mMSCs to secrete EGF (4-fold), granulocyte macrophage colony-stimulating factor (GM-CSF) (3-fold), and HGF (1.5-fold), among other proteins [Huntsman et al., 2013]. The full data set from our protein array is now reported. Based on the results, we speculated that mechanical strain preconditioning of mMSCs prior to transplantation could potentially promote the release of regenerative factors necessary to initiate adaptation in aged skeletal muscle in the absence of exercise, including progenitor cell activation and myofiber growth. In addition, consistent with 1) recent studies that suggest a role for systemic factors in exercise-mediated neuroplasticity [Gu et al., 2012; Moon et al., 2016; Wrann et al., 2013] and 2) evidence of a decline in mMSC neurogenic factor secretion (BDNF, NGF, FNDC5) in aged muscle [Munroe et al., 2017], we secondarily hypothesized that intramuscular transplantation of preconditioned mMSCs could confer beneficial outcomes in the distal central nervous system.

Here we report that a single intramuscular injection of young mMSCs preconditioned with mechanical strain did not robustly initiate skeletal muscle adaptation in the absence of overt injury and disease, yet significantly increased the quantity of new neurons in the hippocampus of aged mice.

2. Methods

2.1. Animals

Protocols for animal use were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Illinois at Urbana-Champaign. Animals were fed standard laboratory chow and had access to water *ad libitum*. Mice were housed in a temperature-controlled specific pathogen-free animal room maintained on a 12:12 light-dark cycle at the animal facility at the Beckman Institute. Five wk. old C57BL/6 mice were used for all mMSC isolation and transplantation experiments. Aged mice (C57BL/6, 24 months, female) were acquired from National Institute of Aging (NIA) colonies and used as recipients for mMSC transplantation. Mice were acclimated to the housing room for at least 1 wk prior to each experiment.

2.2. Isolation of MSCs from skeletal muscle (mMSCs)

Five wk. old C57BL/6 mice were subjected to a single bout of eccentric exercise (−20° treadmill run, 17 m/min, 30 min) prior to isolation of stem cells to increase yield necessary for transplantation. At 24 h post-exercise, gastrocnemius-soleus complexes were excised and Sca-1$^+$CD45$^-$ cells were isolated as previously reported [Huntsman et al., 2013]. Briefly, after mechanical disruption and enzymatic digestion of the dissected muscle tissue, filtered samples were incubated in anti-mouse CD16/CD32 (1 μg/10^6 cells) (eBioscience, San Diego, CA) to block non-specific Fc-mediated interactions and then stained in a cocktail of monoclonal anti-mouse antibodies Sca-1-PE (600 ng/10^6 cells) and CD45-APC (300 ng/10^6 cells) (eBioscience) diluted in 2% FBS in phosphate buffered saline (PBS). Fluorescence activated cell sorting (FACS) was performed using an iCyt Reflection System (Champaign, IL), located at Carle Hospital (Urbana, IL). Sca-1$^+$CD45$^-$ cells were collected in uncoated tissue culture dishes in medium incubated at 37 °C and 5% CO_2 for 8 to 10 days to allow for recovery and short-term expansion up to 80–90% confluency.

2.3. mMSC preconditioning

FACS recovered, unpassaged Sca-1$^+$CD45$^-$ cells were seeded at subconfluent levels onto pre-manufactured laminin peptide (YIGSR)-coated flexible silicone elastomer membrane plates (9.62 cm^2) at 1.0×10^5 cells/well (10.4×10^3 cells/cm^2) (Flexcell International, McKeesport, PA). Cells were incubated in high glucose DMEM with 10% fetal bovine serum (FBS) at 37 °C and 5% CO_2 for 24 h to allow for sufficient cell attachment. Prior to mechanical strain, cells were washed with warm PBS and switched to serum-free high glucose DMEM. Equibiaxial mechanical strain (10%, 1 Hz) was applied to the cells for 5 h using a FX-4000 Flexercell strain unit (Flexcell International, McKeesport, PA). Cells maintained under static conditions were used as non-strained controls. Three hours following the completion of mechanical strain, cells were detached using an accutase enzyme solution and placed on gel elastomers for evaluation of gene expression *in vitro* or prepared for *in vivo* transplantation experiments (see sections below). In a separate experiment, conditioned media was collected 24 h following initiation of mechanical strain and analyzed with Proteome Profiler Antibody Arrays (ARY015, ARY013; R&D Systems, Minneapolis, MN) as previously described [Huntsman et al., 2013].

2.4. Evaluation of mMSC function on PDMS elastomer gels

To determine the extent to which mMSCs could sustain paracrine factor synthesis in a stiff, collagen-enriched microenvironment that replicated aged muscle, unstrained and strained mMSCs were placed on collagen-coated PDMS elastomer gels of varying stiffness for 1 wk prior to evaluation of gene expression. To create the gels, PDMS reagents (Dow Corning, Midland, MI) were mixed at ratios of 50:1, 40:1, and