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Stem cell attraction via SDF-1 α expressing fat tissue grafts

Stefan Zwingenberger^{1,2}, Zhenyu Yao¹, Angela Jacobi², Corina Vater², Roberto D. Valladares¹, Chenguang Li¹, Christophe Nich^{1,3,4}, Allison J. Rao¹, Jane E. Christman¹, Joseph K. Antonios¹, Emmanuel Gibon⁵, Axel Schambach⁶, Tobias Mätzig⁶, Klaus-Peter Günther², Stuart B. Goodman¹, and Maik Stiehler²

¹Department of Orthopaedic Surgery, Stanford University, Stanford, California

²Department of Orthopaedics, University Hospital Carl Gustav Carus at Technical University Dresden, Dresden, Germany

³Laboratoire de Biomécanique et Biomatériaux Ostéo-Articulaires, UMR CNRS 7052, Faculté de Médecine, Université Paris 7, Paris, France

⁴Department of Orthopaedic Surgery, European Teaching Hospital, Assistance Publique, Hôpitaux de Paris, Université Paris 5, Paris, France

⁵Department of Orthopaedic Surgery, Bichat Teaching Hospital, Paris School of Medicine, Paris, France

⁶Institute of Experimental Hematology, Hannover Medical School, Germany

Abstract

Mesenchymal stromal cell (MSCs) are key cellular components for site-specific tissue regeneration. The chemokine stromal derived factor 1 alpha (SDF-1 α) is known to attract stem cells via the C-X-C chemokine receptor-4 (CXCR4) receptor. The aim of the study was to develop a model for stem cell attraction using SDF-1 α overexpressing fat tissue grafts. Murine MSCs were lentiviral transduced to express the genes for enhanced green fluorescent protein, firefly luciferase, and human CXCR4 (hCXCR4). Murine fat tissue was adenoviral transduced to express SDF-1 α and red fluorescent protein transgenes. MSCs were cultured on transwells with SDF-1 α containing supernatants from transduced fat tissue. The numbers of migrated MSCs in four groups (with hCXCR4 positive (+) or hCXCR4 negative (–) MSCs with or without SDF-1 α containing supernatant) were investigated. After 36 h of culture, 9025 $\pm$ 925 cells migrated through the membrane of the transwells in group 1 (CXCR4+/SDF-1 α +), 4817 $\pm$ 940 cells in group 2 (CXCR4-/SDF-1 α +), 2050 $\pm$ 766 cells in group 3 (CXCR4+/SDF-1 α -), and 2108 $\pm$ 426 cells in group 4 (CXCR4-/SDF-1 α -). Both, the presence of SDF-1 α and the expression of hCXCR4 significantly increased the migration rates ($p < 0.0001$). MSCs overexpressing the CXCR4 receptor by lentiviral transduction are highly attracted by medium from SDF-1 α expressing fat tissue *in vitro*. Thus, SDF-1 α activated tissue grafts may be a strategy to enhance site-specific musculoskeletal tissue regeneration.

Keywords

MSC migration; SDF-1; CXCR4; adenovirus; lentivirus

Introduction

Systemic recruitment of progenitor cells is a major goal in the field of Regenerative Medicine. Because of their differentiation potential, mesenchymal stromal cells (MSCs) are key cellular components for site-specific tissue regeneration.¹ In addition to their potential to differentiate into specific cells types, MSCs play an important role in directing cell/cell interactions. It has been shown that MSCs can secrete anabolic cytokines and growth factors,² as well as modulate immune responses.^{3,4}

The chemokine stromal derived factor 1 alpha (SDF-1 α), also known as CXCL12, attracts cells expressing C-X-C chemokine receptor-4 (CXCR4). CXCR4 is a G-protein coupled receptor (GPCR) which belongs to the class A, or rhodopsin-like, GPCR family.⁵ The SDF-1 α /CXCR4 axis has been shown to be involved in different processes during tissue regeneration and organogenesis.^{6,7} For instance, SDF-1 α regulates the homing of hematopoietic stem cells toward the bone marrow, and supports their survival and proliferation.^{8,9} Increased levels of SDF-1 α were observed in liver,¹⁰ skeletal muscle,^{10,11} nerve,¹¹ heart,^{12,13} brain,¹⁴ kidney,¹⁵ skin,¹⁶ and bone¹⁷ tissue following injury. An improved healing potential was shown for heart,¹² brain,¹⁴ and skin¹⁹ when SDF-1 α was delivered locally. Therefore, site specific administration of SDF-1 α is a promising approach in tissue engineering.

Sustained local delivery of biologically active proteins over a sufficiently long time period (of days to weeks) usually may be required for adequate biological tissue responses is challenging.¹⁸ For example, prolonged growth factor release is essential for adequate regeneration of musculoskeletal tissue.¹⁸ As SDF-1 α is known as a chemo-attractant, it presents an attractive target-protein for cell-mediated tissue regeneration. As a proof of the chemo-attractive potential of SDF-1 α toward musculoskeletal progenitor cells, Ji et al.¹⁴ demonstrated local MSC recruitment by intracerebral injection of recombinant human SDF-1 α protein in rats. Furthermore, Henderson et al.¹⁹ even showed improved wound healing in rats by use of hydrogel-immobilized SDF-1 α suggesting a potential role of this chemokine to improve tissue regeneration. To produce a more continuous release of SDF-1 α for up to 5 days, SDF-1 α was incorporated into chitosan/poly(γ -glutamic acid) complexes and MSCs were successfully recruited.²⁰ Next to the delayed release of proteins, binding methods to the vehicle can reduce the protein's biological activity after release.²¹ In addition to structural protein immobilization techniques, gene transfer is an appealing concept as cells and tissues can be modified *ex vivo* for prolonged synthesis of specific proteins. As this technique is relatively simple and successfully used with growth factors like BMP-2,²² we suggest, that sufficient local expression of SDF-1 α and thereby attraction of MSCs is possible. In this context, adenoviral transduction has been reported to denote a suitable method for prolonged expression of SDF-1 α .²³

The aim of the current study was to develop a model for stem cell attraction using SDF-1 α expressing fat tissue grafts. Our hypothesis was that by using culture media of adenoviral transduced SDF-1 α expressing fat tissue, MSCs could be strongly attracted. The experiment was designed to be a model for further *in vivo* studies, in which treatment of musculoskeletal diseases using SDF-1 α and MSCs will be investigated.

Methods

Protocol design

Primary murine MSCs were isolated, cultured to passage 4 and characterized according to the protocol of Zhu et al.²⁴ MSCs were transduced using a lentivirus to express the genes for enhanced green fluorescent protein (eGFP), firefly luciferase, and human CXCR4 (hCXCR4). Control groups were transduced to express eGFP and firefly luciferase, without hCXCR4. Primary murine fat tissue was harvested from the proximal hind legs and transduced by an adenovirus to express human SDF-1 α and red fluorescent protein (RFP) transgenes. Transduced fat tissue samples were cultured for 7 days, and supernatants were used for a migration assay. Sixty thousand cells per well were cultured on 8 μ m transwells with or without SDF-1 α containing supernatants for 36 h. The numbers of migrated MSCs in four groups ($n = 6$ replicates in each, with hCXCR4 positive (+) or hCXCR4 negative (–) MSCs and with or without SDF-1 α containing supernatant) were investigated. The experimental design was approved by the Institutional Administration Panel for Laboratory Animal Care (protocol number 26905). University guidelines for care and use of laboratory animals were followed.

Isolation of murine MSCs and harvesting of fat tissue

Primary MSCs were harvested from five 3-week old C57/BL6 mice (Jackson, Bar Harbor, Maine) using the technique described by Zhu et al.²⁴

Primary fat tissue was harvested from two 8-week old C57/BL6 mice (Jackson). The mice were euthanized by cervical dislocation following anaesthesia. Then fat tissue was taken under sterile conditions from the proximal hind legs, rinsed by Dulbecco's modified eagle medium (DMEM) and cut into pieces of 3 mm in diameter.

Fluorescence activated cell sorting (FACS) analysis

Monoclonal antibodies and chemical reagents—For characterization of the murine MSCs, anti-CD11b-FITC, anti-CD14-APC, anti-CD34-FITC, anti-CD45-PE, anti-CD73-PE, anti-CD90.2 APC, anti-CD105-PE, anti-Sca-1-APC and anti-CD16/32 monoclonal antibodies (mAb) were purchased from eBioscience. PE-conjugated anti- hCXCR4 mAb and APC-conjugated anti-mouse CXCR4 mAb were purchased from eBioscience (San Diego, CA) to evaluate the expression rate of the CXCR4-receptor.

Flow cytometry analysis—Single cell suspensions were prepared in phosphate-buffered saline (PBS) with 1% calf serum, pre-incubated with anti-CD16/32 mAb to prevent nonspecific binding via FcR2/3 interactions, and then incubated with the appropriate mAb. Propidium iodide (Sigma, St Louis, MO) was added prior to analysis to exclude any dead

cells. All analyses were performed on a modified four lasers LSR II (BD Immunocytometry Systems, San Diego, CA) in the Shared FACS Facility (Center for Molecular and Genetic Medicine at Stanford University), using FlowJo software (TreeStar, Ashland, OR) for data analysis.

Characterization of the MSCs

Using FACS analysis, cells of passage 4 were tested for the MSC-markers CD73, CD90.2, CD105, and Sca-1 and for the haematopoietic, endothelial, and lymphocyte markers CD11b, CD14, CD34, and CD45.

To prove the differentiation potential of the MSCs, cells were cultured in adipogenic, chondrogenic and osteogenic medium, as described by Zhu et al.²⁴

Adipogenic differentiation: After 14 days of differentiation in adipogenic medium, cells were stained by Oil-Red-O staining (Sigma-Aldrich, St. Louis, MO) for 15 min. Cell culture wells were observed by light microscopy (100 × magnification, Observer.Z1, Carl Zeiss, Jena, Germany).

Osteogenic differentiation: After 28 days differentiation in osteogenic medium, cells were stained by Van Kossa staining. Images were taken by light microscopy (20 × magnification; Observer.Z1).

Chondrogenic differentiation: After 3 weeks of differentiation in chondrogenic medium, the cell pellets were frozen in Tissue-TEK O.C.T. compound (Sakura Finetek, Torrance, CA). Frozen sections of 5 µm were obtained, fixed in acetone and stained by Toluidine Blue staining (Sigma-Aldrich). Sections were analyzed by light microscopy (200 × magnification; Olympus BX 50; Olympus Optical Co., Tokyo, Japan).

Construction of lentiviral vectors

To generate the lentiviral co-expression construct for hCXCR4, a Kozak consensus sequence was put between 5' AgeI and NcoI sites. The latter overlapped with the translational start site of the gene. For co-expression, the natural stop codon was replaced by a glycine-serine-glycine linker followed by a P2A cleavage site. For ease of cloning, the coding sequence of the terminal proline of the P2A overlapped with another NcoI site which was followed by BsrGI, a Stop codon, and XbaI and SalI restriction sites, respectively. The whole sequence was codon-optimized (synthesis by GenScript, NJ) while maintaining restriction sites and the Kozak consensus sequence intact. The human codon-optimized CXCR4-P2A fragment was digested with AgeI and SalI and ligated into the likewise digested lentiviral backbone pRRL.PPT.SF.eGFP.pre*.²⁵ The resulting vector pRRL.PPT.SF.hCXCR4co-P2A.pre* was further equipped with an eGFP cDNA via a three fragment ligation. The parental vector was cut with XhoI/BsrGI and XhoI/NcoI respectively, and eGFP was inserted via NcoI/BsrGI restriction sites resulting in pRRL.PPT.SF.hCXCR4co-P2A-eGFP.pre*. A 5' fusion of T2A with a firefly luciferase (luc2, Promega) mutant devoid of endogenous BsrGI and AgeI restriction sites was amplified with two sets of overlapping primer pairs (Tm_BsrGI-T2A-OL1_FW: 5'-TGT ACA AGG GCT CCG GAG AGG GCC GGG GCT CTC TGC TGA CCT GTG GCG ACG

TGG AGG AGA-3'; Tm_T2A-OL1 luc2_RV: 5'- TAA TGT TTT TGG CAT CTT CGG GGC CGG GGT TCT CCT CCA CGT CGC CAC AG -3'; Tm_luc2_OL2_FW: 5'-GGC CCC GAA GAT GCC AAA AAC ATT AAG-3'; Tm_luc2_OL2_Stop_Sal_RV: 5'- GTC GAC TTA TCA CAC GGC GAT CTT GCC GCC CTT C-3') flanking the T2A-luc2 PCR product with 5' BsrGI and 3'SalI restriction sites (in italics), respectively. T2A-luc2 was sequence verified, digested with BsrGI/SalI and cloned into pRRL.PPT.SF.hCXCR4co-P2A-eGFP.pre* via three fragment ligation and digestion with NheI/SalI and NheI/BsrGI resulting in pRRL.PPT.SF.hCXCR4co-P2A-eGFP-T2A-luc2.pre*.

The control vector pRRL.PPT.SF.eGFP-T2A-luc2.pre* was constructed by three fragment ligation after digestion of pRRL.PPT.SF.hCxCR4co-P2A-eGFP-T2A-luc2*.pre* with NotI/NcoI, BsrGI/NcoI and NotI/BsrGI, respectively.

Production of lentiviral supernatant

Plasmid DNA was isolated using a PureLink™ HiPure Plasmid Filter Purification Kit (Invitrogen, Karlsruhe, Germany). Lentivirus particles were produced by co-transfection of 293T cells with a VSV-G envelope plasmid, a Gag/Pol plasmid and the lentiviral vectors carrying either hCXCR4, eGFP and firefly luciferase (pRRL.PPT.SF.hCXCR4co-P2A-eGFP-T2A-luc2.pre*) or only eGFP and firefly luciferase (pRRL.PPT.SF.eGFP-T2A-luc2.pre*). 293T cells were seeded at a density of 65,000 cells/cm² and grown in complete DMEM medium (Gibco, Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (FBS, Gibco, Invitrogen) and 1% Anti/Anti (Gibco, Invitrogen) up to 70% confluency. Cells were then infected with the lentivirus vector mix prepared in DMEM without serum, containing 10 µg/mL Polyethylenimine (Sigma-Aldrich). Medium was replaced after 8 h with complete DMEM and again after another 12 h with complete DMEM. Viral supernatant was harvested 24, 48, and 72 h after transfection, centrifuged at 300 g for 5 min, filtered using 0.45 µm pore size filter (Corning Inc, Corning, NY), and frozen in aliquots at -80°C. Lentiviral titer was determined using a QuickTiter™ Lentivirus Quantitation Kit (Cell Biolabs, San Diego; CA).

Transduction of the MSCs

Primary murine MSCs were expanded in complete α-MEM medium (Thermo Fisher Scientific, Waltham, MA) with 10% FBS (Gibco, Invitrogen) and 1% Anti/Anti (Gibco, Invitrogen) up to passage 4. MSCs were transduced at 70% confluence, with 1:2 diluted virus vector supernatant (7.5×10^9 virus particles/mL), containing 7 µg/mL polybrene (Sigma-Aldrich) at 37°C for 24 h. Media was replaced after 24 h with complete α-MEM. After recovering 24 h in complete α-MEM medium, a second transduction was done for 24 h. Successful transduction was confirmed by FACS analysis 72 h after the second transduction.

Transduction of fat tissue

The transduction was performed according to the protocol by Betz et al.²² Fat tissue fragments were infected with 2×10^8 plaque forming units (pfu) of Ad-hSDF-1α-RFP (backbone: adenoviral—type 5 (dE1/E3); promoter CMV; Vector BioLabs, Philadelphia, PA). Control tissue grafts were transduced with 2×10^8 pfu of Ad-RFP (backbone:

adenoviral—type 5 (dE1/E3); promoter CMV; Vector BioLabs). Viral particles were appropriately diluted in phosphate buffered saline (Thermo Fisher Scientific) and a volume of 10 μ L was applied directly to the surface of the tissue grafts. Then, the tissue fragments were placed in an incubator (37°C, 5% CO₂) for 60 minutes without any extra medium. The virus solution and the humidified air in the incubator kept the tissue fragments moist during incubation. Then 250 μ L complete DMEM medium were added. After 24 h the virus-medium solution was replaced by complete DMEM medium.

Expression of transgenes

Lentiviral: Two groups were investigated: hCXCR4 positive (+) and hCXCR4 negative (–; control) MSCs. Nine cell culture dishes of 10 cm diameter were seeded with passage 4 MSCs from each group. Cells were grown up to 70% confluence and transduced as described above. FACS analysis was used to determine the expression of hCXCR4 and eGFP one, three and six weeks after transduction. At each time point, cells from three plates per group were measured separately.

The expression of eGFP was also evaluated by fluorescence microscopy (Observer.Z1) and the expression of firefly luciferase by bioluminescence imaging (IVIS Spectrum, Caliper Life Science, Hopkinton, MA).

Adenoviral: Nine fat tissue fragments per group were placed in a 48 well-plate and either activated with Ad-SDF-1 α -RFP (SDF-1 α +) or Ad-RFP (SDF-1 α –) as described above. The tissues were subsequently cultured in 500 μ L complete DMEM medium per well for 22 days. Medium was changed every second day and the supernatants were frozen at –80°C. At the end of the culture period, the concentration of the SDF-1 α protein in the supernatant was measured by ELISA (Quantikine, SDF-1 α Immunoassay, R&D Systems GmbH, Wiesbaden, Germany) according to the manufacturer's protocol.

Migration experiment

The migration experiment was done using transwells (24-well plates, pore size 8.0 μ m, 6.5 mm insert, Corning). Four groups ($n = 6$ wells for each) were investigated: (1) hCXCR4+ cells and SDF-1 α medium, (2) hCXCR4– cells and SDF-1 α medium, (3) hCXCR4+ cells and SDF-1 α – medium, and (4) hCXCR4– cells and SDF-1 α – medium.

For production of SDF-1 α and SDF-1 α – (control) medium, tissue grafts were transduced by adenovirus as described above. Medium was changed after 2 and 4 days. On day 7, medium was collected for the migration experiment. The concentration of the SDF-1 α protein in the medium was measured using an ELISA (Quantikine, SDF-1 α Immunoassay, R&D Systems GmbH, Wiesbaden, Germany). Pooled SDF-1 α medium was measured to have 41.0 ng/mL SDF-1 α protein whereas no SDF-1 α protein was detectable in control medium.

hCXCR4+ and hCXCR4– cells were cultured and transduced as described above. Sixty hours after the second transduction, cells were starved for 12 h in pure α -MEM with 0.2% bovine serum albumin (BSA, Sigma-Aldrich). The cells were then trypsinized, washed twice

in PBS, resuspended in pure α -MEM with 0.2% BSA, counted and adjusted to a concentration of 325,000 cells per mL.

According to group affiliation, 600 μ L of SDF-1 α + or SDF-1 α - medium were added to the lower compartment of each transwell. Two hundred microliters of pure α -MEM with 0.2% BSA and 65,000 CXCR4+ or CXCR4- MSCs, respectively were added to the upper compartment of the transwells. Cells were incubated at 37° and 5% CO₂.

After 36 h, medium from the lower compartment was removed and replaced by 500 μ L of 0.25% Trypsin/EDTA (Gibco, Invitrogen). After 10 min the migrated cells, now floating in the trypsin, were counted using a hemocytometer, separately for each well.

Statistics

Numerical data were statistically analyzed using Graphpad Prism 5.00 software (San Diego, CA). The results are presented as mean and standard error. Since data followed the Gaussian distribution, statistical significance was tested by one-way-ANOVA, followed by post hoc testing using Tukey's Multiple Comparison Test. The level of significance was set to $p < 0.05$.

Results

Characterization of MSCs

Flow cytometry—Using FACS analysis, cells of passage 4 were shown to be positive for the MSC-markers CD73 (88.1%), CD90.2 (50.0%), CD105 (20.0%), and Sca-1 (52.3%) and negative for the haematopoietic, endothelial, and lymphocyte markers CD11b, CD14, CD34, and CD45.

Differentiation of MSCs—To prove their differentiation potential, MSCs were cultured in osteogenic, adipogenic and chondrogenic medium. Osteogenic differentiation potential was shown by Van Kossa stain [Fig. 1(A,D)]. Cells cultured in osteogenic medium exhibited a high level of calcification, whereas controls had virtually no calcification. Adipogenic differentiation was obtained following culture in medium described above [Fig. 1(B,E), Oil-Red O staining]. Adipogenic differentiation was characterized by fat vacuoles and stained lipid droplets, whereas controls did not show these features. Cartilage proteoglycan matrix was positive for Toluidine blue staining [Fig. 1(C)]. MSCs also showed the typical plastic-adherent growth [Fig. 1(F)].

Expression of transgenes

SDF-1 α expression from transduced fat tissue—The expression of SDF-1 α during 22 days of cell culture is shown in Figure 2. The concentration of SDF-1 α increased in the first days after transduction, reached a peak at day 6, and then decreased to virtually no detectable SDF-1 α expression after 14 days. Because the adenoviral vector expressed SDF-1 α as well as RFP, successful transduction was also evident by fluorescence microscopy (Fig. 2).

hCXCR4 and eGFP expression from transduced MSCs—MSCs, lentiviral transduced to express hCXCR4, eGFP and firefly luciferase (hCXCR4+ MSCs) or only eGFP and firefly luciferase (hCXCR4– MSCs) were cultured up to 6 weeks. Table I shows the results from FACS analysis after 1, 3, and 6 weeks of cell culture. While the expression of hCXCR4 decreased in the hCXCR4+ MSCs group from $72.9 \pm 0.9\%$ one week after transduction to $40.0 \pm 1.1\%$ after 6 weeks, the eGFP expression was more stable and ranged between 88.8 ± 0.1 and 76.3 ± 1.0 for both groups. Successful transduction was also confirmed by fluorescence microscopy and bioluminescence imaging (Fig. 3). Untransfected MSCs were measured to have a natural murine CXCR4 expression of $2.8 \pm 0.6\%$ after 1 week, $2.9 \pm 0.4\%$ after 3 weeks and $2.4 \pm 0.2\%$ after 6 weeks.

Migration of MSCs toward SDF-1 α

The migration of lentiviral transduced, hCXCR4+ or hCXCR4– MSCs toward cell culture medium from SDF-1 α + or SDF-1 α –(control) expressing fat tissue grafts was investigated (Fig. 4). After 36 h of culture, 9025 ± 925 cells migrated through the membrane of the transwells in group 1 (CXCR4+/SDF-1 α +), 4817 ± 940 cells in group 2 (CXCR4–/SDF-1 α +), 2050 ± 766 cells in group 3 (CXCR4+/SDF-1 α –), and 2108 ± 426 cells in group 4 (CXCR4–/SDF-1 α –). Groups with SDF-1 α + medium had significantly higher migration rates than groups with SDF-1 α – medium ($p < 0.0001$). Furthermore, hCXCR4+ MSCs migrated significantly more often to medium containing SDF-1 α than hCXCR4– MSCs did ($p < 0.0001$).

Discussion

We showed that adenoviral transduced fat tissue grafts can express a sufficient amount of SDF-1 α to attract MSCs via the CXCR4/SDF-1 α axis. The MSCs transduced by an innovative lentiviral construct showed long expression of the CXCR4 surface protein over at least 6 weeks as well as a strong eGFP and firefly luciferase expression allowing detection of the labeled cells in further animal experiments by fluorescence microscopy and *in vivo* bioluminescence imaging.

We used adipose tissue to express SDF-1 α as it is easily available and accessible in humans and animals, and limits the risk to other important structures such as muscle, tendon or bone. The expression of SDF-1 α from fat tissue was slightly shorter in time than we expected, and lasted only 14 days. Although this is still longer than administration of a single dose of recombinant protein, other groups observed expression durations of 3–6 weeks using transduced muscle tissue.^{26,27} Adipose tissue seems to be less susceptible than muscle tissue to adenoviral transduction.²⁸ Furthermore, we used a relatively low amount of virus particles per tissue graft compared to other groups.^{26,28} Preliminary studies showed that no efficient transduction of the fat tissue grafts was accessible using the lentiviral system.

In our migration experiment, we had a relatively high migration rate for the control groups without SDF-1 α . First, this can be explained by the comparatively long migration time of 36 h, there is a known rate of spontaneous cell migration. Previous investigations showed that shorter migration periods of 12 or 24 h resulted in a lower number of migrated cells, which was less accurate for quantification. Migration periods longer than 36 h are not common

since the rate of spontaneous migration would become the main source of migrated cells. Furthermore the effect of the attracting protein disappears by increasing time. Second, we focused on the SDF-1 α /CXCR4 axis, but there are other pathways directing MSCs by growth factors and cytokines other than SDF-1 α .²⁹ The increased migration rate of hCXCR4– MSCs towards SDF-1+ medium can be explained by the natural expression of murine CXCR4 receptor. We measured an expression rate of about 2-3%. In the literature, natural CXCR4 receptor expression rates are described between 4 and 4.79% for rat and human MSCs.^{30,31} For our experiments, we used human genes for murine cells, which is acceptable because of the high cross-reactivity of human and murine CXCR4 and SDF-1 α .³² The experiment focusing on the temporal expression of hCXCR4 and eGFP from lentiviral transduced MSCs showed 1–2% positive hCXCR4 signal in the control group using FACS analysis. This can be explained by a partial cross reaction between natural murine CXCR4 receptor and hCXCR4-FACS-antibody.³²

Local SDF-1 α delivery has been shown to have positive effects on tissue regeneration. Using the adenoviral technique of activating tissue grafts, more MSCs can be attracted and could result in improved tissue healing. It was shown, that MSCs support regeneration of tendons and ligaments,³³ promote ingrowth of bone allografts, and help to regenerate the junction between bone and tendon.³⁴ It is therefore appealing to use adenoviral transduced fat tissue grafts not only to express SDF-1 α but also other factors to attract MSCs and increase vascularization. For minimally invasive applications, local direct injection of the adenoviral particles *in vivo* is one possible “minimally invasive” method to archive this goal. This means of delivery has been shown in animal studies using BMP-2.³⁵

Adenoviral gene therapy, primary developed to treat genetic disorders, is now of great interest for cancer therapy and already in clinical use.³⁶ It offers the ability to infect with high efficiency a variety of both quiescent and proliferating cell types.³⁷ Adenoviral vectors can be easily grown to a very high titer, allowing the researcher to transduce a large number of cells and/or a tissue target of large animals and humans.³⁷ An important safety feature includes the absence of vector genome integration, thereby reducing the likelihood for germ-line transmission and insertional mutagenesis.³⁷ As experience in the use of adenoviral gene therapy continues to increase, this method has potential applications for use in clinical regenerative medicine.

Conclusion

We showed that MSCs overexpressing the CXCR4 receptor by lentiviral transduction were highly attracted by medium from SDF-1 α expressing fat tissue *in vitro*. This method presents a promising way to attract MSCs *in vivo* by SDF-1 α activated tissue grafts, and use their positive effects towards healing of musculoskeletal and other diseases. The model presented, with adenoviral transduced tissue grafts expressing SDF-1 α combined with lentiviral labeled MSCs, is a model to investigate the favorable effects of SDF-1 α on tissue regeneration and systemic stem cell recruitment.

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Figure 1.

Differentiation potential of passage 4 primary murine MSCs: Osteogenic differentiation potential is shown by Van Kossa stain (**A** and **D**). Cells cultured 28 days in osteogenic medium exhibit a high grade of calcification (**A**), whereas controls have virtually no calcification (**D**). Adipogenic differentiation was obtained following culture (14 days) in medium as described. (**B** and **E**, Oil-Red O staining). Adipogenic differentiation is characterized by fat vacuoles and stained lipid droplets (**B**), whereas controls do not show these features (**E**). Cartilage proteoglycan matrix is positive for Toluidine blue staining (**C**). MSCs also show the typical plastic-adherent growth (**F**). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

Figure 2.

Expression of adenoviral transduced (Ad-SDF-1 α -RFP) fat tissue grafts *in vitro*. Fat tissue grafts of 3 mm diameter were transduced by 2×10^8 pfu adenovirus to express the genes for SDF-1 α and RFP. The graph on the left shows the time dependent concentration of SDF-1 α after adenoviral transduction. SDF-1 α concentrations were analyzed from the cell culture media by ELISA ($n = 9$ each time point). The image on the right shows RFP expression of transduced fat tissue 7 days after transduction. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

Figure 3. hCXCR4⁺ MSCs showing expression of green fluorescent protein and firefly luciferase after lentiviral transduction. On the left is a fluorescence microscopy image of lentiviral transduced hCXCR4⁺MSCs, 6 weeks after transduction. The right image shows a bioluminescence measurement of 300,000 MSCs 4 days after lentiviral transduction. Bioluminescence imaging was performed 10 min after adding 1.5 mg luciferin, exposure time 10 s. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

Figure 4.

Migration of transduced hCXCR4 positive (hCXCR4⁺) or negative (hCXCR4⁻) MSCs through transwells toward SDF-1 α positive (SDF-1 α ⁺) or negative (SDF-1 α ⁻) medium from transduced fat tissue grafts ($n = 6$ each group). A comparison of all groups showed statistically significant differences ($p < 0.0001$, one way ANOVA). Post hoc testing showed significant intergroup differences between the groups marked with an asterisk ($p < 0.0001$, Tukey's Multiple Comparison Test).

Table I
Expression of hCXCR4 and eGFP from MSCs After Lentiviral Transduction

Group	hCXCR4+ MSCs		hCXCR4-MSCs (Control)	
	hCXCR4 (%)	eGFP (%)	hCXCR4 (%)	eGFP (%)
Week 1	72.9 ± 0.9	88.7 ± 0.4	1.1 ± 0.1	80.1 ± 0.1
Week 3	44.5 ± 0.3	88.8 ± 0.1	1.5 ± 0.1	79.6 ± 0.7
Week 6	40.0 ± 1.1	82.8 ± 0.9	2.0 ± 0.2	76.3 ± 1.0

Passage 4 MSCs were lentiviral transduced and cultured for 1, 3, or 6 weeks, before expression of hCXCR4 and eGFP were measured by FACS analysis. $n = 3$ for each group and each time point.