Factors that influence short-term homing of human bone marrow-derived mesenchymal stem cells in a xenogeneic animal model

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The online version of this article contains a supplementary appendix.

ABSTRACT

Background

Human mesenchymal stem cells are potential agents for tissue regeneration, enhancing hematopoietic stem cell transplantation and delivering genes of therapeutic interest. To implement any of these strategies successfully, we need a better understanding of factors that influence the tissue distribution of systemically administered mesenchymal stem cells.

Design and Methods

The present study was designed to investigate the short-term tissue homing of mesenchymal stem cells in immunodeficient mouse models, exploring the effects of animal age, duration of *ex vivo* expansion of mesenchymal stem cells, lentiviral transduction and CXCR4 over-expression. Dye-labeled mesenchymal stem cells ($1.5–2.0x10^6$/animal) were injected via the tail vein into unconditioned β2m/NOD/SCID animals. Animals were sacrificed 20-24 hours later and cell suspensions from tissues were examined by flow cytometry for the presence of PKH-positive cells.

Results

PKH-positive cells were readily detected in the bone marrow, spleen, liver and lungs at 20-24 hours after infusion. The homing of systemically infused mesenchymal stem cells to the bone marrow and spleen of unconditioned β2m/NOD/SCID animals was significantly (>2-fold, $p<0.001$) higher in younger (<10 weeks) animals, and was reduced with increasing passage number. Despite low surface CXCR4 expression, human mesenchymal stem cells migrated to SDF-1 *in vitro*, and this was enhanced by over-expression of CXCR4 using lentiviral transduction. Over-expression of CXCR4 by lentiviral transduction (>80%) did not alter the bone marrow homing of mesenchymal stem cells in unconditioned animals, but caused a significant ($p<0.05$) increase in homing to bone marrow and spleen of animals that had received prior irradiation.

Conclusions

Tissue homing of systemically administered mesenchymal stem cells is influenced by host factors such as age, is diminished by prolonged *in vitro* culture, and can be increased by enforced expression of CXCR4, at least in irradiated hosts.

Key words: mesenchymal cells, homing, chemokines.

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Introduction

Bone marrow-derived human mesenchymal stem cells (MSC) are an accessible population of progenitor cells that can differentiate into mesenchymal tissues including cartilage and bone[1] are easily expanded *ex vivo* and readily transduced by viral vectors.[2,3] Human MSC may prove useful for enhancing hematopoietic stem cell transplantation,[4] modulating graft-versus-host disease,[5] treating disorders of bone, cartilage[6] and muscle[7] and delivering therapeutic genes.[8] The successful employment of human MSC for these *in vivo* strategies relies upon the efficient localization and retention of cells within appropriate tissues. Site-directed administration of MSC can result in successful engraftment and integration of MSC under specific circumstances, most commonly in injured tissue.[9-11] It remains unresolved to what degree such effects are due to the local production of growth factors rather than direct participation of injected MSC in cellular regeneration.

Site-directed administration is practicable only for a limited number of applications, hence the localization of systemically administered MSC in appropriate tissues, for example bone marrow, is crucial to the success of many therapies. The initial localization of circulating cells to specific organs is referred to as *homing*. Homing of hematopoietic stem and progenitor cells to the bone marrow is a multistep process mediated by adhesion molecules and chemokines.[12-15] Far less is known about the *in vivo* homing of human MSC, partly because *in vivo* studies using systemic administration have employed different models and read-out systems. Evidence for tissue localization of systemically infused MSC is most convincing in models employing tissue injury.[16-18] Current evidence suggests that in the absence of tissue damage systemically administered MSC seed to the bone marrow only at low levels,[19-21] with large numbers of MSC lodging in the pulmonary vascular bed. Although MSC can engraft in non-pulmonary tissues, levels are at the limit of detection and of clinical use only in disorders such as osteogenesis imperfecta.[22]

The initial localization of exogenously administered MSC to target tissues or organs is critical, and clearly we need a better understanding of the mechanisms that regulate the migration and homing of MSC to the bone marrow and other tissues *in vivo*. The chemokine stromal derived factor-1 (SDF-1) has a major role in the homing and engraftment of hematopoietic stem and progenitor cells to the bone marrow,[15] and together with its receptor, CXCR4, functions in stem cell seeding of the bone marrow during embryonic development.[24] Enforced expression of CXCR4 on $CD34^+$ cells increased migration and engraftment in immunodeficient animals.[25] Levels of CXCR4 and SDF-1 are upregulated in stressed or injured tissues,[26,27] hence this receptor/ligand pair may mediate metastatic invasion,[28] or the migration of cells into damaged tissues.[29,30] Human MSC express low levels of CXCR4,[31,32] which may account for the low levels of seeding to the bone marrow following systemic infusion. Despite low levels of CXCR4, human MSC are reported to migrate to SDF-1 *in vitro*,[31,33,34] suggesting that, like hematopoietic stem and progenitor cells, human MSC may upregulate functional CXCR4 molecules to the cell surface. Other factors important in MSC homing and migration *in vivo* include vascular endothelial growth factor, fibroblast growth factor-2, interleukin-6, hepatocyte growth factor and monocyte chemotactic protein-1.[34-36] Irradiation or tissue damage may increase MSC migration by inducing such factors.[37,38]

The present study was designed to investigate the short-term homing of bone marrow-derived human MSC using unconditioned B6.CB17-Prkdc-SzJ SCID (NOD/SCID) and β2m/NOD/SCID animals. The aim was to evaluate factors, including animal age and CXCR4 expression, which influence the short-term biodistribution of human MSC in this unconditioned xenogeneic animal model.

Design and Methods

Human mesenchymal stem cells isolation and culture

Human MSC were obtained from the bone marrow of healthy donors undergoing bone marrow harvest after informed consent under a protocol approved by the local ethics committee. Human MSC were cultured from the adherent fraction of bone marrow mononuclear cells, and characterized for phenotype and differentiation function according to established protocols,[39,2] (*see the Online Supplementary Methods*).

Mice

B6.CB17-Prkdc-SzJ severe combined immunodeficiency (NOD/SCID) and β2-microglobulin non-obese diabetic/severe combined immunodeficiency (β2m NOD/SCID) mice were obtained from Jackson Laboratories and bred and maintained under defined flora conditions in individually ventilated sterile microisolater cages in accordance with the Animals Scientific Procedures Act 1986 approved by the Institutional Animal Care and Use and licensed by the Home Office. Mice underwent transplantation with MSC by lateral tail vein injection and were killed by CO_2 inhalation 20-24 hours after transplantation. For irradiation experiments mice were sublethally irradiated (325 cGy from a cesium 137 source).

Labeling human mesenchymal stem cells with PKH-26 and injection into animals

Cultured human MSC (passages 6-15) were labeled with PKH-26 according to manufacturer's (Sigma) instructions (*see the Online Supplementary Methods*). Labeled cells were then injected into the lateral tail vein of NOD/SCID or β2m NOD/SCID mice ($1.5–2.0\times10^6$ cells per animal).

Analysis of murine tissues for human mesenchymal stem cells homing

Animals were sacrificed 20-24 hours after transplantation. Mononuclear cells obtained from bone marrow and other tissues were analyzed for PKH-positive cells (*see the Online Supplementary Methods*).

Fluorescence in situ *hybridization assay*

FISH was performed using probes specific for the human X and Y chromosomes. Human MSC of male origin were transplanted into female mice. FISH analysis was performed on mononuclear cell suspensions from bone marrow and other organs using standard techniques. (*see the Online Supplementary Methods*).

Viral vector construction and transduction of human mesenchymal stem cells with CXCR4

Details of the bicistronic lentiviral vector containing cDNA for CXCR4 (Figure 1) are given in *the Online Supplementary Methods*. Human MSC at early passage (<3) were transduced using a multi-hit protocol as previously described[2] (*see the Online Supplementary Methods*).

Analysis of surface and intracellular CXCR4 expression

Human MSC were incubated with phycoerythrin-conjugated anti-human CXCR4 (BD PharMingen) for 30 minutes on ice, washed and analyzed by flow cytometry. For intracellular CXCR4 staining, surface CXCR4 molecules were blocked with non-conjugated antihuman CXCR4 monoclonal antibody (10 μg/mL) for 1 hour on ice. Cells were washed, fixed, permeabilized (BD Cytofix/Cytoperm, BD Biosciences), and subsequently incubated with anti-human CXCR4-phycoerythrin monoclonal antibody. After further washing, cells were analyzed on an EPICS Elite flow cytometer (Beckman-Coulter).

In vitro *migration assay*

Recombinant human SDF-1 (0-100 ng/mL, Peprotech) was placed in the lower chamber of fibronectin-coated Transwell filters (8.0 μm Pore Size, Corning Inc). CXCR4-transduced, or control hMSC were plated in the upper chamber and allowed to migrate over 4 hours at 37°C. Filters were removed, fixed using 100% ice cold methanol (-20° C for 10 minutes) and washed once in phosphate-buffered saline. Filters were stained with hematoxylin and eosin, washed and air dried. Cells on the upper surface of each filter were carefully removed using a damp cotton bud, filters were detached, placed on a glass slide and mounted using DPX mounting medium. Migrated cells were visualized by light microscopy, and counted by two independent observers (20 fields per slide, x100 power).

Statistical analysis

Differences between groups were analyzed using the Mann-Whitney U test; p values less than 0.05 were considered statistically significant (GraphPad Prism Version 4.0).

Results

In vivo *homing of human mesenchymal stem cells in unconditioned immunodeficient mice*

PKH-26 (PKH)-labeled human MSC (1.5–2.0×10^6 cells/animal; for optimization of cell doses, see *the Online Supplementary Methods*) were administered via the tail vein into unconditioned β2m/NOD/SCID animals. Animals were sacrificed 20-24 hours later, and single cell suspensions from bone marrow, spleen, lungs and liver were analyzed for PKH-positive cells by flow cytometry. The highest levels of homing were consistently found in the lungs, while there was considerable variation of PKH-positive cells in the bone marrow and spleen between animals (Figure 2A, Table 1). PKH-positive cells were also detected in the liver. In two experiments, homing was also analyzed at 48 hours, with similar results to those at 24 hours. There was some donor-to-donor variation; this did not, however, correlate with donor age or sex. These findings were supported by FISH analysis using human X and Y specific probes (*Online Supplementary Figure S1*). PKH-positive cells identified in murine tissues also stained positive with a human-specific anti-HLA class I monoclonal antibody (*data not shown*). We also used unconditioned NOD/SCID animals as recipients. There was a generally lower level of homing in these animals than in β2m/NOD/SCID recipients (Table 1).

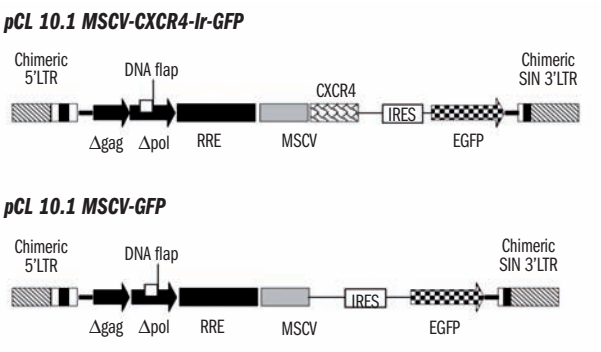

Figure 1. Schematic representation of the lentiviral vector constructs The pCL10.1 MSCV promoter is used to drive expression of either GFP cDNA in the control vector (bottom panel) or the CXCR4-IRES-GFP bisistronic cassette of the experimental vector (top panel).

Extended culture of cells is usually required to obtain sufficient numbers of human MSC for therapeutic applications. We studied the effect of passage number on homing behavior. The numbers of PKH-positive cells in the bone marrow of β2mNOD/SCID mice were lower in mice transplanted with advanced passage human MSC than in animals that had received cells from earlier passages (p<0.001, Figure 2B). A similar effect of passage number was seen in the spleen (p<0.01).

Efficiency of short-term homing alters with the animals' age

The numbers of PKH-positive cells detected in the bone marrow of younger (<10 weeks) animals were significantly higher (median 433/10^5; range, 294-521) than the numbers in older (>10 weeks) animals (median, 166/10^5; range, 120-306; p<0.001, Figure 2C). To control for any age-related changes in bone marrow cellularity we determined the numbers of PKH-positive cells per bone. This analysis confirmed that bone marrow homing was greater in younger (<10 weeks) animals (medi-

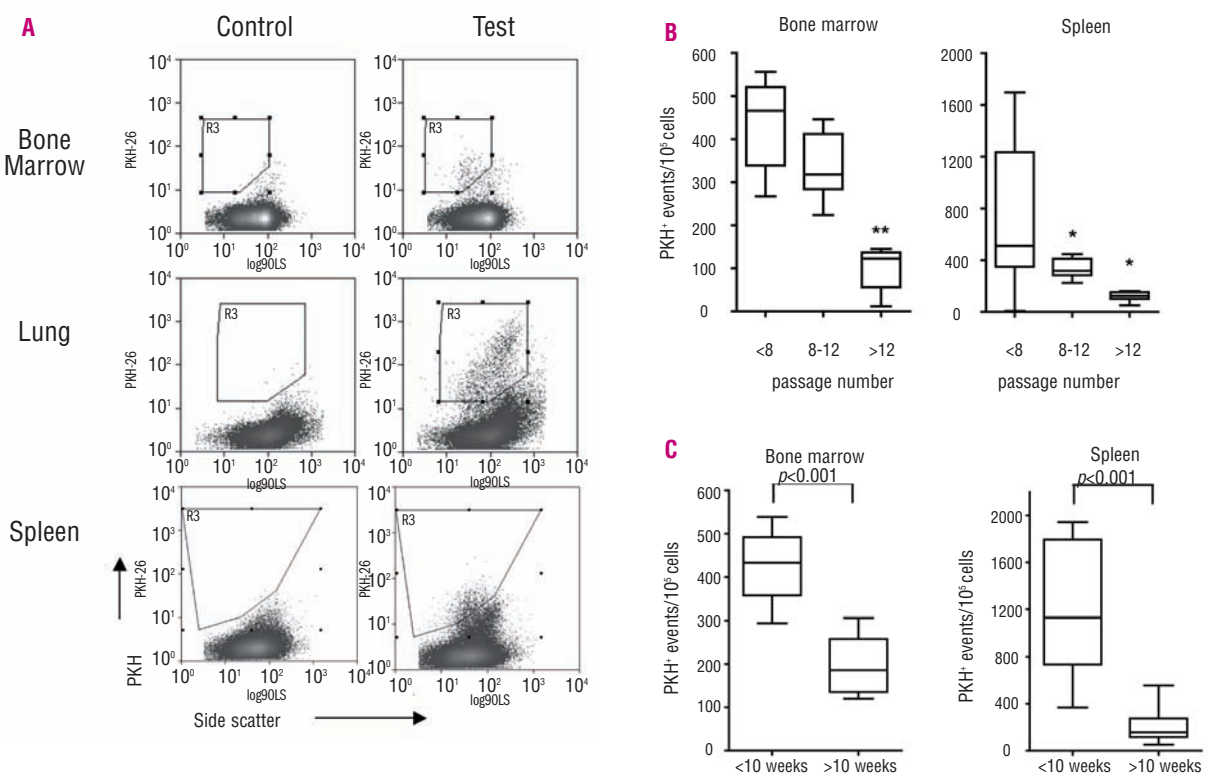

Figure 2. Short-term *in vivo* homing of human mesenchymal stem cells (MSC). (A) Flow cytometric identification of PKH-stained human MSC 20 hours after infusion. Cell suspensions from each organ were analyzed by flow cytometry for the presence of PKH-bright cells, using as negative control an animal infused with saline only. One representative animal. Control: animal infused with saline; test: animal infused with PKH-labeled human MSC. Gates indicate PKH-positive events. (B) Effect of passage number on homing to the bone marrow (left panel) and spleen (right panel). Data are given as number of PKH- positive events/10^5 cells analyzed, and are pooled from two different MSC donors, 5-7 animals per data point, *p<0.01, **p<0.001, compared with homing of MSC at passage 8 or less. (C) Effect of age on the homing efficiency of human MSC. Data presented as in (B), from 8-12 animals per data point, 4 experiments.

an, 8836/bone; range, 4600-15977, *vs* median, 2725/bone; range, 1750-7933 in older animals; p<0.001). This effect of the animals' age was also observed for human MSC homing to the spleen (p<0.001, Figure 2C), but not to lungs. (p=0.1457).

CXCR4 expression and homing of human mesenchymal stem cells

The consistent, albeit low, levels of homing to the bone marrow displayed by human MSC led us to investigate the possible contribution of CXCR4, as the bone marrow is reported to express high levels of SDF-1. We found that the surface expression of CXCR4 was low (2.3±1.8%, n=10) or absent (n=5) on all human MSC tested (Figure 3A). In contrast, human MSC harbored significant amounts of intracellular CXCR4 (63±12%, n=3, Figure 3A), as previously reported.[31] Next we investigated whether CXCR4 receptors, despite their low expression on infused human MSC, did contribute to bone marrow homing. To do this, we used a phycoerythrin-cyanin 5 (PE-Cy5)-conjugated anti-CXCR4 monoclonal antibody to label human MSC that had homed to the bone marrow. We confirmed that the PE-Cy5-conjugated monoclonal antibody was able to stain surface CXCR4 molecules on PKH-labeled human MSC (*Online Supplementary Figure S2*). Bone marrow cells recovered from animals 24 hours after infusion of unmanipulated human MSC were incubated with PE-Cy5-conjugated anti-CXCR4 monoclonal antibody or the isotype control, washed and analyzed by flow cytometry. We found that bone marrow-homed human MSC, identified as PKH-bright cells (gated in Panel A, Figure 3B) expressed significant levels of CXCR4 (28.8±8.1%, n=5), compared with levels on pre-

Table 1. Short-term homing of PKH-labeled human mesenchymal stem cells in two strains of unconditioned immunodeficient animals.

Organ	*β2m/NOD/SCID*[a] *Positive events/10^5 cells Median (range)*	*NOD/SCID*[b] *Positive events/10^5 cells Median (range)*
Bone marrow	376 (120-556)	296 (101-446)
Spleen	1115 (220-2433)	797 (286-1139)
Lung	4091 (1203-7900)	3164 (1396-7392)

PKH-labeled human MSC were infused via the tail vein (1.5 – 2.0×10^6/animal) and animals were sacrificed 20-24 hours later. Single cell suspensions isolated from organs were analyzed by flow cytometry for PKH-positive events.
[a]5 experiments, 22 animals; [b]3 experiments, 9 animals.

infusion human MSC (panel B, Figure 3B). This suggests that either human MSC upregulate intracellular CXCR4 *in vivo*, or there is preferential homing of a small population of human MSC expressing higher levels of CXCR4.

CXCR4 expression and in vitro *migration of human mesenchymal stem cells*

Human MSC were transduced with a bicistronic vector encoding CXCR4 and eGFP (MSC^{CXCR4}), or the control vector encoding eGFP (MSC^{GFP}). Significant levels of transgene expression were achieved (43-96% CXCR4, 50-93% eGFP positive, Figure 4A) and the expression was stable for up to 20 passages. CXCR4 expression on human MSC^{CXCR4} from five different donors was 41.8±9.8 (MCF) and 73.2±12.7 (% positive). Both human MSC^{GFP} and human MSC^{CXCR4} expressed intracellular CXCR4 (Figure 4A). *In vitro*, unmanipulated human MSC migrated to SDF-1, as previously reported.[31] Human MSC^{CXCR4} (>80% CXCR4-positive) displayed significantly higher levels of migration to SDF-1 (Figure 4B). No further increase in migration occurred at SDF-1 concentrations >100 ng/mL.

Enforced CXCR4 expression does not alter homing in unconditioned recipients

We found no significant differences in bone marrow homing between eGFP-transduced (median $422/10^5$ cells; range, 267-556), and unmanipulated human MSC (median $376/10^5$ cells; range, 247-539), suggesting that lentiviral transduction did not affect homing. The presence of eGFP-expressing human MSC in murine tissues was confirmed by direct visualization of fluorescent cells in bone marrow trephine rolls (*data not shown*).

We next tested the *in vivo* homing of gene-modified human MSC expressing high (>85%) levels of CXCR4 (MSC^{CXCR4}), using young animals (6-8 weeks), and unmanipulated or MSC^{GFP} from the same donor as controls. To our surprise, enforced expression of CXCR4 had no effect on homing of human MSC to the bone marrow, spleen, liver or lungs of unconditioned β2m/NOD/SCID animals (Figure 5A). When we repeated these experiments in NOD/SCID animals, we found a small increase in homing of MSC^{CXCR4} to the spleen (Figure 5B), but no effect on bone marrow homing.

CXCR4 over-expression enhances in vivo *homing in irradiated animals*

It is possible that an effect of CXCR4 over-expression on homing would only be revealed in the context of tissue injury. We, therefore, repeated our experiments in sub-lethally irradiated hosts. Irradiation leads to loss of bone marrow cellularity (of up to 80%), so that the percentage of PKH-positive cells detected by flow cytometry is artefactually increased. The absolute numbers of PKH-positive human MSC detected per bone, however, remained unchanged (*data not shown*). To investigate the homing of human human MSC^{CXCR4} in irradiated hosts, β2m/NOD/SCID animals were treated with 325 cGy 24 hours prior to infusion of human MSC^{CXCR4} or $hMSC^{GFP}$. Under these conditions, human MSC^{CXCR4} demonstrated significantly higher levels of homing to the bone marrow (median, $260/10^5$ cells; range, 219-283) compared with $hMSC^{GFP}$ (median, $177/10^5$ cells; range, 28-220; $p<0.05$, Figure 6A). Because irradiation reduces the total number of bone marrow cells, we also determined the number of PKH-positive cells/bone. These results confirmed the superior homing of CXCR4-expressing human MSC (Figure 6B). Similar effects of CXCR4 over-expression were also demonstrated for human MSC homing to the spleen (Figure 6A). The absolute number of human MSC homed to the spleen was also significantly higher for human MSC^{CXCR4} ($p<0.05$, Figure 6B).

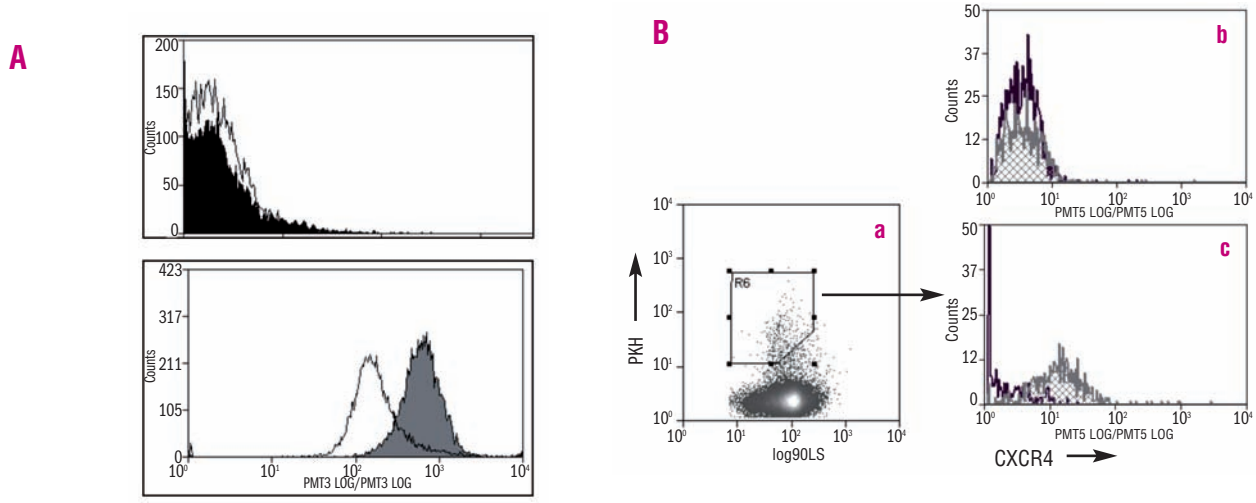

Figure 3. (A) Unmanipulated human mesenchymal stem cells (MSC) were stained for surface (top panel) or intracellular (bottom panel) CXCR4. Empty histograms indicate negative controls incubated with istoype-matched monoclonal antibody. (B) CXCR4 expression on MSC which had homed to bone marrow PKH^+ cells present in the bone marrow at 20 hours after infusion (shown gated in a) were analyzed for CXCR4 expression by staining with anti-CXCR4-PE-Cy5 monoclonal antibody (c). For comparison, the profile of CXCR4 expression on MSC prior to infusion into animals is shown (b). Empty histograms represent negative controls, gray hatched histograms positive samples stained with CXCR4 monoclonal antibody. One representative experiment.

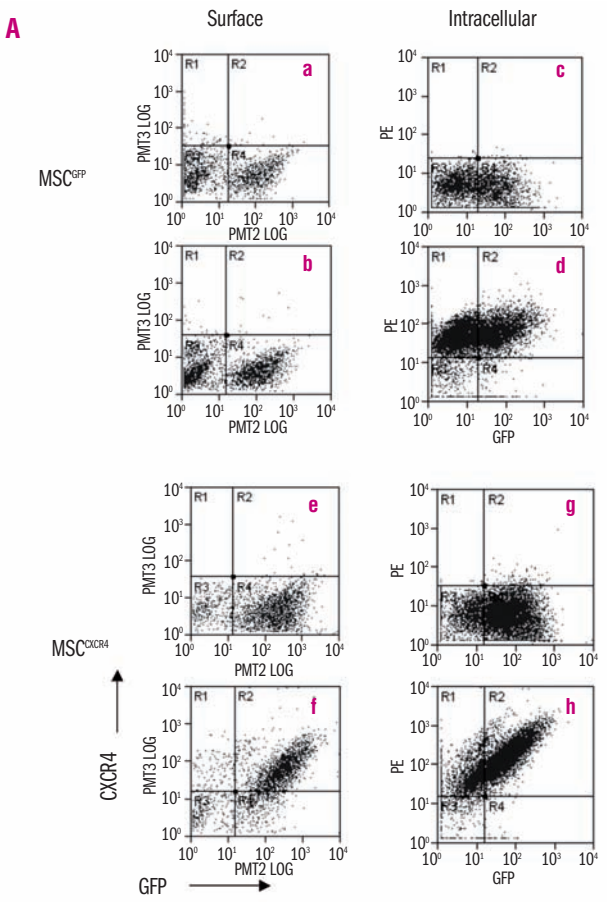

Figure 4. Enforced CXCR4 expression on human mesenchymal stem cells (MSC) enhances *in vitro* migration (A) CXCR4-transduced human MSC (MSC^{CXCR4}) or eGFP transduced (MSC^{GFP}) human MSC were stained for surface or intracellular CXCR4 as indicated. Panels a, c, e, and g represent negative controls stained with IgG-PE, while panels b, d, f and h are positive samples stained with anti-CXCR4-PE. GFP fluorescence is shown on the X-axis to indicate transduction efficiency. Note that in panel f only GFP-positive MSC are positive for surface CXCR4, while in panel h, all MSC are positive for intracellular CXCR4. (B) Enhanced migration of MSC^{CXCR4} (MSC/CXCR4) compared with that of MSC^{GFP} (control MSC). Migration to SDF-1 (0-100 ng/mL) over 4 hours. Mean±SEM of three experiments, with duplicate filters for each condition, and ten high power fields/filter counted by each of two observers. The right panel shows representative filters stained with hematoxylin and eosin (x100 magnification).

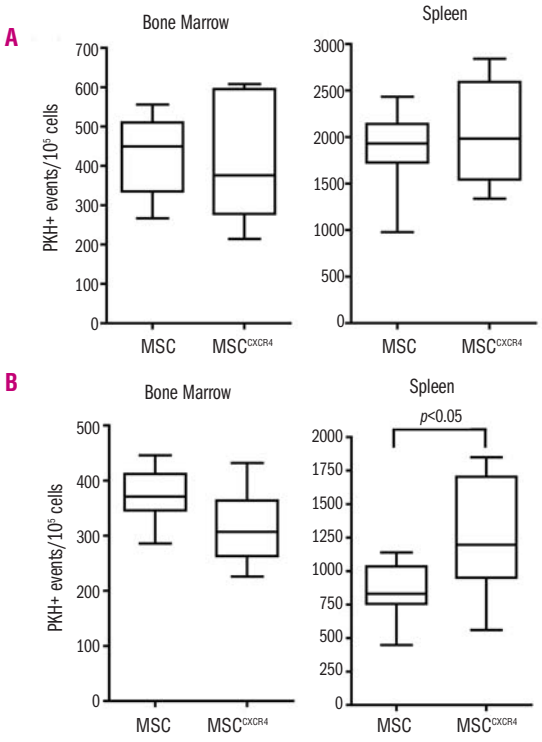

Figure 5. *In vivo* homing of human mesenchymal stem cells (MSC) expressing CXCR4 by lentiviral transduction (A). Comparison of *in vivo* homing to the bone marrow (left panel) and spleen (right panel) between human MSC^{CXCR4} and control e-GFP-transduced human MSC from the same donor (MSC) in unconditioned β2m/NOD/SCID animals. Ten to 15 animals per data point, three experiments. (B) *In vivo* homing of MSC^{CXCR4} and control MSC to the bone marrow (left panel) and spleen (right panel) in unconditioned NOD/SCID animals. Ten to 12 animals per data point, two experiments.

Discussion

In this study we used a sensitive dye-labeling method to quantify the tissue biodistribution of human MSC after systemic injection in a xenograft model, and showed that a proportion of transplanted cells was capable of localizing into the bone marrow, spleen and lungs of unconditioned animals. A key observation reported here is that the efficiency of homing is critically influenced by the age of the recipient animal, and also declines with increased duration of cell culture. The adverse effect of the recipient's age on short-term homing in this model remains to be confirmed in other models, and in human recipients. It may, however, have important implications for clinical transplantation in elderly recipients. A decline in homing may be related to the down-regulation of stromal cell-derived chemokines/cytokines during the aging process. The decline in homing efficiency with prolonged *ex vivo* culture of human bone marrow-derived MSC in this study using an unconditioned immunodeficient xenogeneic model is reminiscent of a similar previous report using murine MSC in a syngeneic model.[40] Many clinical applications require high doses of MSC, achievable only with considerable *ex vivo* expansion. A clearer under-

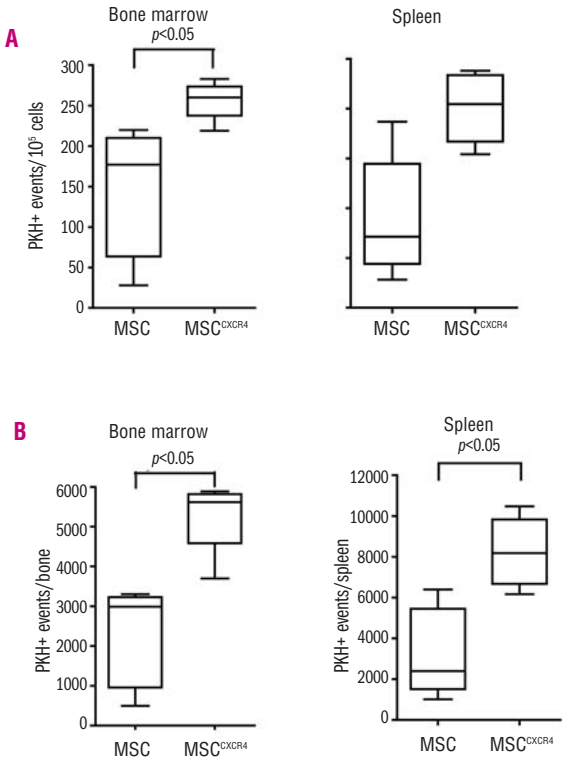

Figure 6. Effect of CXCR4 over-expression on *in vivo* homing of human mesenchymal stem cells (MSC) in irradiated recipients. Effect of enforced CXCR4 expression on homing of human MSC to the bone marrow and spleens of irradiated β2m/NOD/SCID animals. MSC^{CXCR4}, CXCR4-transduced human MSC, MSC, control e-GFP-transduced human MSC from the same donor. Results expressed as number of PKH-26 positive events/10^5 cells (A) or number of PKH-26-positive events/organ (B), five to seven animals per data point, two experiments.

standing of the effect of extended *in vitro* culture on adhesion and chemokine receptor expression, and on migratory capacity is needed.

Importantly, our observations confirm a potential role for the CXCR4 receptor in the short-term homing behavior of systemically administered human MSC. While unmanipulated human MSC express low levels of surface CXCR4, cells that had localized to the bone marrow at 20 hours post-transplantation showed significant levels of expression. We demonstrate for the first time that enforced surface expression of CXCR4 by lentiviral gene transfer was able to enhance *in vivo* short-term homing, but only in the context of an irradiated model. The reported surface expression of CXCR4 on human MSC varies from almost negligible to 20-40%.[33,34] Such variation may be related to differences in culture conditions. For example, CXCR4 is known to be upregulated when human MSC are exposed to cytokines, including insulin-like growth factor-1,[41] which is present in cell culture serum. Several studies now report that human MSC migrate *in vitro* in response to SDF-1,[31,34] perhaps mediated by upregulation of intracellular CXCR4 molecules. Similarly, the localization of human MSC to the bone marrow, despite low surface expression of CXCR4, may be explained by upregulation of intracellular CXCR4 molecules to the cell surface *in vivo*, in response to cytokines, sheer forces,[42] or exposure to hypoxia. Similarly, upregulation of CXCR4 by cytokines *in vitro* may enhance short-term localization of MSC to the bone marrow of irradiated NOD/SCID animals.[44]

In the present study we used a lentiviral system to stably over-express a functional CXCR4 receptor on human MSC and examined the effects on SDF-1-directed migration *in vitro* and on homing *in vivo*. Lentiviral vectors are well suited to these studies because their safety profile is better than that of onco-retroviral vectors.[45] Over-expression of CXCR4 on human MSC significantly promoted their migration *in vitro*, in confirmation of previous reports,[31,46] but did not influence short-term homing to the bone marrow following systemic administration in unconditioned mice. CXCR4 expression is dynamic and regulated by cytokines, adhesion molecules, ligand-binding and proteolytic enzymes.[15,28] Functional modulation by receptor internalization and trafficking, incorporation into lipid rafts, cleavage of both CXCR4 and SDF-1 by serine proteases and matrix metalloproteinases also occurs.[28,47] It is not clear if, and how, lentivirally expressed surface molecules are subject to these physiological regulatory mechanisms.

Irradiation, and consequent tissue injury, leads to increased secretion of cytokines and chemokines such as SDF-1, and, in some models, to increased homing/engraftment of MSC.[48,49] It is not surprising, therefore, that CXCR4–overexpressing human MSC demonstrate superior homing (compared with control human MSC) under these circumstances. What is perhaps surprising is that while more than 80% of infused cells express surface CXCR4 molecules, levels of homing remain below 1%. This may reflect the limited capacity of the system, or alternatively CXCR4-expressing human MSC may display increased localization to tissues other than the bone marrow and spleen. Lentivirally expressed receptors may not undergo physiological trafficking necessary for optimal function, as discussed above. Increased CXCR4 receptors on genetically modified human MSC may also affect the migratory response to other inflammatory or chemokinetic agents released following irradiation by receptor cross-talk.[50]

In conclusion we report that multiple factors influence the *in vivo* homing of human MSC in this xenogeneic model. *In vivo* homing to the bone marrow and spleen is greater in younger animals. Enforced surface expression of CXCR4 by lentiviral gene transfer leads to increased *in vitro* migration to SDF-1, and enhanced homing *in vivo* but this effect is only marked in irradiated hosts. These factors should be taken into account when using *in vivo* models for pre-clinical evaluation of MSC-based therapies.

Authorship and Disclosures

CK designed and performed the research, analyzed data and wrote the paper. NR performed the research. KY designed the research, analyzed data and wrote the paper. AN designed the research, AP performed the research. The authors reported no potential conflicts of interest.

References

1. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143-7.
2. Kyriakou CA, Yong KL, Benjamin R, Pizzey A, Dogan A, Singh N, et al. Human mesenchymal stem cells (hMSCs) expressing truncated soluble vascular endothelial growth factor receptor (tsFlk-1) following lentiviral-mediated gene transfer inhibit growth of Burkitt's lymphoma in a murine model. J Gene Med 2006;8:253-64.
3. Zhang XY, La Russa VF, Bao L, Kolls J, Schwarzenberger P, Reiser J. Lentiviral vectors for sustained transgene expression in human bone marrow-derived stromal cells. Mol Ther 2002;5:555-65.
4. in't Anker PS, Noort WA, Kruisselbrink AB, Scherjon SA, Beekhuizen W, Willemze R, et al. Nonexpanded primary lung and bone marrow-derived mesenchymal cells promote the engraftment of umbilical cord blood-derived CD34(+) cells in NOD/SCID mice. Exp Hematol 2003;31:881-9.
5. Le Blanc K, Rasmusson I, Sundberg B, Gotherstrom C, Hassan M, Uzunel M, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 2004; 363:1439-41.
6. Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, et al. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med 1999;5:309-13.
7. Dezawa M, Ishikawa H, Itokazu Y, Yoshihara T, Hoshino M, Takeda S, et al. Bone marrow stromal cells generate muscle cells and repair muscle degeneration. Science 2005; 309:314-7.
8. Studeny M, Marini FC, Champlin RE, Zompetta C, Fidler IJ, Andreeff M. Bone marrow-derived mesenchymal stem cells as vehicles for interferon-beta delivery into tumors. Cancer Res 2002;62:3603-8.
9. Zhao LR, Duan WM, Reyes M, Keene CD, Verfaillie CM, Low WC. Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats. Exp Neurol 2002;174:11-20.
10. Jin HK, Carter JE, Huntley GW, Schuchman EH. Intracerebral transplantation of mesenchymal stem cells into acid sphingomyelinase-deficient mice delays the onset of neurological abnormalities and extends their life span. J Clin Invest 2002;109:1183-91.
11. Turgeman G, Pittman DD, Muller R, Kurkalli BG, Zhou S, Pelled G, et al. Engineered human mesenchymal stem cells: a novel platform for skeletal cell mediated gene therapy. J Gene Med 2001;3:240-51.
12. Lapidot T, Dar A, Kollet O. How do stem cells find their way home? Blood 2005;106:1901-10.
13. Frenette PS, Subbarao S, Mazo IB, von Andrian UH, Wagner DD. Endothelial selectins and vascular cell adhesion molecule-1 promote hematopoietic progenitor homing to bone marrow. Proc Natl Acad Sci USA 1998;95:14423-8.
14. Papayannopoulou T, Priestley GV, Nakamoto B, Zafiropoulos V, Scott LM. Molecular pathways in bone marrow homing: dominant role of α(4)β(1) over β(2)-integrins and selectins. Blood 2001;98:2403-11.
15. Lapidot T, Kollet O. The essential roles of the chemokine SDF-1 and its receptor CXCR4 in human stem cell homing and repopulation of transplanted immune-deficient NOD/SCID and NOD/SCID/B2m (null) mice. Leukemia 2002;16:1992-2003.
16. Barbash IM, Chouraqui P, Baron J, Feinberg MS, Etzion S, Tessone A, et al. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. Circulation 2003;108: 863-8.
17. Ortiz LA, Gambelli F, McBride C, Gaupp D, Baddoo M, Kaminski N, et al. Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. Proc Natl Acad Sci USA 2003;100:8407-11.
18. Chapel A, Bertho JM, Bensidhoum M, Fouillard L, Young RG, Frick J, et al. Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multi-organ failure syndrome. J Gene Med 2003;5: 1028-38.
19. Devine SM, Cobbs C, Jennings M, Bartholomew A, Hoffman R. Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. Blood 2003;101:2999-3001.
20. Bentzon JF, Stenderup K, Hansen FD, Schroder HD, Abdallah BM, Jensen TG, et al. Tissue distribution and engraftment of human mesenchymal stem cells immortalized by human telomerase reverse transcriptase gene. Biochem Biophys Res Commun 2005;330:633-40.
21. Bensidhoum M, Chapel A, Francois S, Demarquay C, Mazurier C, Fouillard L, et al. Homing of in vitro expanded Stro-1- or Stro-1+ human mesenchymal stem cells into the NOD/SCID mouse and their role in supporting human CD34 cell engraftment. Blood 2004;103:3313-9.
22. Gao J, Dennis JE, Muzic RF, Lundberg M, Caplan AI. The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. Cells Tissues Organs 2001;169:12-20.
23. Horwitz EM, Gordon PL, Koo WK, Marx JC, Neel MD, McNall RY, et al. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: implications for cell therapy of bone. Proc Natl Acad Sci USA 2002;99:8932-7.
24. Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa S, Kitamura Y, et al. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature 1996;382:635-8.
25. Kahn J, Byk T, Jansson-Sjostrand L, Petit I, Shivtiel S, Nagler A, et al. Overexpression of CXCR4 on human CD34+ progenitors increases their proliferation, migration, and NOD/SCID repopulation. Blood 2004;103:2942-9.
26. Schioppa T, Uranchimeg B, Saccani A, Biswas SK, Doni A, Rapisarda A, et al. Regulation of the chemokine receptor CXCR4 by hypoxia. J Exp Med 2003;198:1391-402.
27. Ponomaryov T, Peled A, Petit I, Taichman RS, Habler L, Sandbank J, et al. Induction of the chemokine stromal-derived factor-1 following DNA damage improves human stem cell function. J Clin Invest 2000; 106:1331-9.
28. Kucia M, Reca R, Miekus K, Wanzeck J, Wojakowski W, Janowska-Wieczorek A, et al. Trafficking of normal stem cells and metastasis of cancer stem cells involve similar mechanisms: pivotal role of the SDF-1-CXCR4 axis. Stem Cells 2005;23:879-94.
29. Yamaguchi J, Kusano KF, Masuo O, Kawamoto A, Silver M, Murasawa S, et al. Stromal cell-derived factor-1 effects on ex vivo expanded endothelial progenitor cell recruitment for ischemic neovascularization. Circulation 2003;107:1322-8.
30. Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, Kiedrowski M, et al. Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet 2003;362: 697-703.
31. Wynn RF, Hart CA, Corradi-Perini C, O'Neill L, Evans CA, Wraith JE, et al. A small proportion of mesenchymal stem cells strongly expresses functionally active CXCR4 receptor capable of promoting migration to bone marrow. Blood 2004;104:2643-5.
32. Kortesidis A, Zannettino A, Isenmann S, Shi S, Lapidot T, Gronthos S. Stromal-derived factor-1 promotes the growth, survival, and development of human bone marrow stromal stem cells. Blood 2005;105:3793-801.
33. Sordi V, Malosio ML, Marchesi F, Mercalli A, Melzi R, Giordano T, et al. Bone marrow mesenchymal stem cells express a restricted set of functionally active chemokine receptors capable of promoting migration to pancreatic islets. Blood 2005;106: 419-27.
34. Son BR, Marquez-Curtis LA, Kucia M, Wysoczynski M, Turner AR, Ratajczak J, et al. Migration of bone marrow and cord blood mesenchy-

mal stem cells in vitro is regulated by stromal-derived factor-1-CXCR4 and hepatocyte growth factor-c-met axes and involves matrix metalloproteinases. Stem Cells 2006;24:1254-64.
35. Ball SG, Shuttleworth CA, Kielty CM. Mesenchymal stem cells and neovascularization: role of platelet-derived growth factor receptors. J Cell Mol Med 2007;11:1012-30.
36. Dwyer RM, Potter-Beirne SM, Harrington KA, Lowery AJ, Hennessy E, Murphy JM, et al. Monocyte chemotactic protein-1 secreted by primary breast tumors stimulates migration of mesenchymal stem cells. Clin Cancer Res 2007;13:5020-7.
37. Klopp AH, Spaeth EL, Dembinski JL, Woodward WA, Munshi A, Meyo RE, et al. Tumor irradiation increases the recruitment of circulating mesenchymal stem cells into the tumor microenvironment. Cancer Res 2007;67:11687-95.
38. Ji JF, He BP, Dheen ST, Tay SS. Interactions of chemokines and chemokine receptors mediate the migration of mesenchymal stem cells to the impaired site in the brain after hypoglossal nerve injury. Stem Cells 2004;22:415-27.
39. Majumdar MK, Thiede MA, Mosca JD, Moorman M, Gerson SL. Phenotypic and functional comparison of cultures of marrow-derived mesenchymal stem cells (MSCs) and stromal cells. Cell Physiol 1998;176:57-66.
40. Rombouts WJ, Ploemacher RE. Primary murine MSC show highly efficient homing to the bone marrow but lose homing ability following culture. Leukemia 2003;17:160-70.
41. Li Y, Yu X, Lin S, Li X, Zhang S, Song YH. Insulin-like growth factor 1 enhances the migratory capacity of mesenchymal stem cells. Biochem Biophys Res Commun 2007;356:780-4.
42. Ruster B, Gottig S, Ludwig RJ, Bistrian R, Muller S, Seifried E, et al. Mesenchymal stem cells display coordinated rolling and adhesion behavior on endothelial cells. Blood 2006;108:3938-44.
43. Hung SC, Pochampally RR, Hsu SC, Sanchez C, Chen SC, Spees J, et al. Short-term exposure of multipotent stromal cells to low oxygen increases their expression of CX3CR1 and CXCR4 and their engraftment in vivo. PLoS ONE 2007;2:e416.
44. Shi M, Li J, Liao L, Chen B, Li B, Chen L, et al. Regulation of CXCR4 expression in human mesenchymal stem cells by cytokine treatment: role in homing efficiency in NOD/SCID mice. Haematologica 2007;92:897-904.
45. Montini E, Cesana D, Schmidt M, Sanvito F, Ponzoni M, Bartholomae C, et al. Hematopoietic stem cell gene transfer in a tumor-prone mouse model uncovers low genotoxicity of lentiviral vector integration. Nat Biotechnol 2006;24:287-96.
46. Bhakta S, Hong P, Koc O. The surface adhesion molecule CXCR4 stimulates mesenchymal stem cell migration to stromal cell-derived factor-1 in vitro but does not decrease apoptosis under serum deprivation. Cardiovasc Revasc Med 2006;7:19-24.
47. Wysoczynski M, Reca R, Ratajczak J, Kucia M, Shirvaikar N, Honczarenko M, et al. Incorporation of CXCR4 into membrane lipid rafts primes homing-related responses of hematopoietic stem/progenitor cells to an SDF-1 gradient. Blood 2005;105:40-8.
48. Mouiseddine M, Francois S, Semont A, Sache A, Allenet B, Mathieu N, et al. Human mesenchymal stem cells home specifically to radiation-injured tissues in a non-obese diabetes/severe combined immunodeficiency mouse model. Br J Radiol 2007;80 Spec No 1:S49-S55.
49. Francois S, Bensidhoum M, Mouiseddine M, Mazurier C, Allenet B, Semont A, et al. Local irradiation not only induces homing of human mesenchymal stem cells at exposed sites but promotes their widespread engraftment to multiple organs: a study of their quantitative distribution after irradiation damage. Stem Cells 2006;24:1020-9.
50. Abbott JD, Huang Y, Liu D, Hickey R, Krause DS, Giordano FJ. Stromal cell-derived factor-1α plays a critical role in stem cell recruitment to the heart after myocardial infarction but is not sufficient to induce homing in the absence of injury. Circulation 2004;110:3300-5.