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Enhanced Expression of N-Formyl Peptide Receptor in Mesenchymal Stem Cells Facilitates Homing to Inflammatory Lungs

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Abstract

Marrow-derived mesenchymal stem cells (MSCs) exhibit certain intrinsic tropism to inflammatory tissues. However, effective stem cell therapy often requires high levels of engraftment, which may not be sufficed by the MSC natural homing process. Here we investigated if lentiviral vector-enhanced expression of N-formyl peptide receptor (FPR) in MSCs could increase their sensitivity to N-formylated peptides (N-FP) and facilitates MSC homing to inflammatory lungs. HIV-1-based lentiviral vectors, expressing the FPR-EGFP fusion protein (HIV-FPR-EGFP) or co-expression of the FPR and EGFP proteins (HIV-FPR-IRES2-EGFP), were engineered. Expression of FPR in 293T cells, a cell line without the endogenous receptor, rendered the cells responsive to N-FP by intracellular calcium mobilization. Human MSCs, transduced with the FPR-IRES2-EGFP vector, showed a greater sensitivity to and an enhanced chemotaxis towards a low level of N-FP. The FPR-engineered hMSCs, expressing a luciferase reporter, were infused systemically into nu/nu mice which were pre-intubated intratracheally with or without a sublethal dose of Pseudomonas aeruginosa. In both groups, the MSCs were largely located in the lungs initially and cleared rapidly within days as shown by in vivo whole body bioluminescence imaging. However, MSC retention in the bacterium-challenged lungs a week after infusion was ~2-fold higher than the non-challenged controls. Biochemical measurement of luciferase enzymatic activity demonstrated low but definite homing of the MSCs in the bacteriumchallenged lungs. Engraftment of MSCs to the lungs was immunohistochemically confirmed. These data provide a proof of principle that engineering MSCs with FPR can enhance the stem cell homing to inflammatory tissues for potential repair.

Keywords: N-formyl peptide receptor; Mesenchymal stem cells; MSC homing and engraftment

Introduction

Multipotent mesenchymal stem cells (MSCs) derived from bone marrow have demonstrated great promise in regenerative medicine. A wealth of literature has documented the potency of MSCs to differentiate into many types of cells beyond their germinal boundary [1-3]. Moreover, MSCs can also secrete paracrine soluble factors to modulate local cells to promote damage recovery [4-7]. In order to achieve these effects, MSCs are required to be physically present in target tissues or organs [8,9]. MSCs have shown some tropism for sites of damage tissues [10,11]. However, experimental data only show low levels of engraftment [12], which limits greater clinical benefits for stem cell therapies. Therefore, engineering MSCs to increase their homing and engraftment to target tissues is critical for potential application of the cells for therapy to achieve maximal clinical benefits.

Even though the mechanisms underlying MSC migration and homing have not been fully elucidated, many receptors involved in migration of leukocytes and other cell types have been identified on MSCs [13]. Thus, it is believed that many of the same receptors are also involved in governing MSC migration. *In vitro* comparisons of chemotactic activities demonstrate a panel of growth factors and chemokines as MSC chemoattractants [14]. MSCs genetically modified to overexpress insulin-like growth factor (IGF)-1 or CXCR4 accelerate their mobilization for myocardial repair [15-17]. We and others have previously reported that human marrow-derived MSCs functionally express N-formyl peptide receptor (FPR) [18,19]. This G proteincoupled receptor binds to N-formylated peptides (N-FP) released from intruding bacteria or dead cells [20,21], allowing leukocytes to approach and infiltrate inflammatory tissues [20-24]. Fibroblast cells also use this same mechanism to migrate into inflammatory sites to repopulate wounds, deposit extracellular matrices for tissue growth, and secrete growth factors and immune modulating cytokines and chemokines [25]. Furthermore, overexpression of FPR in cells of non-leukocyte origin confers these cells with responsiveness to N-FP and results in directional chemotaxis [26-30]. The data from these publications altogether suggest the possibility of engineering MSCs with FPR to enhance their directional migration and homing. The present report demonstrates that lentiviral vector-mediated overexpression of FPR in human MSCs enhances their sensitivity to N-FP stimulation and increases MSC homing to inflammatory lungs.

Materials and Methods

Cell lines and reagents

Human MSCs were isolated from healthy volunteer donors by the NCRR/NIH-sponsored Tulane center for distribution of MSCs prepared with a standardized protocol [11]. These cells were cultured in α -MEM (Invitrogen-Gibco, Carlsbad, CA), containing 20% lot-specific fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA), 100 units/ml penicillin, 100 µg/ml streptomycin and 2 mM L-glutamine (Invitrogen-

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Gibco). Bovine serum albumin (BSA), N-FMLP (N-formyl-Met-Leu-Phe), N-formyl hexapeptide (N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys), human placental collagen IV and common chemicals were purchased from Sigma (St. Louis, MO).

Lentiviral vector construction and production

Total RNAs from human MSCs were extracted using the RNeasy extraction kit (Qiagen, Valencia, CA) and reverse-transcribed with the ImProm-IITM reverse transcription system (Promega, Madison, WI). The FPR cDNA was amplified using the sense primer 5'-CAGGAGCAGACAAGATGGAGACAA-3' and the antisense primer 5'-TCACTTTGCC TGTAACTCCACCTC-3'. The PCR product was cloned into the ZeroBlunt II-Topo vector plasmid (Invitrogen) and sequenced for verification. The FPR cDNA was further subcloned into pIRES2-EGFP or pEGFP-N1 (Clontech, Mountain View, CA), resulting in the following two cassettes: CMV-FPR- IRES2-EGFP or CMV-FPR-EGFP (fusion protein). Then, both cassettes were, respectively, used to replace the CMV-EGFP cassette in the parental lentiviral transgene plasmid (pNL-CMV-EGFP-WPRE-dU3) [31]. The corresponding HIVbased viral vectors (HIV-CMV-EGFP, HIV-FPR-EGFP, and HIV-FPR-IRES2-EGFP) were produced by triple-plasmid calcium-phosphate transfection method [32]. Viral particles in the culture medium were collected three times at 48, 60 and 72 hours after HEK293T cells were transfected with the envelope plasmid pLTR-G, the packaging plasmid pCD/NL- BH* $\Delta\Delta\Delta$, and one of the transgene plasmids [31,32]. Vector concentration was achieved by ultracentrifugation on a 20% sucrose cushion. Viral titers were obtained by serial dilution and transduction of HT1080 or HOS cells followed by real-time PCR for quantitation of vector genome.

Vector transduction of MSCs

MSCs were grown in 150 mm culture dishes (Thermo Fisher Scientific, Rochester, NY) at a low density (5000 cells per 150 mm dish). The cells were transduced with either HIV-CMV-EGFP or HIV-CMV-FPR-IRES2-EGFP virus at an MOI of 20 in the presence of polybrene (8 μ g/ml). Sixty hours after viral transduction, the cells were harvested by trypsin dissociation for use in subsequent experiments. In the case of *in vivo* application, MSCs were co-transduced with two lentiviral vectors: HIV-CMV-FPR-IRES2-EGFP and HIV-CMV-Luciferase-IRESs-DsRed at an MOI of 20 for each.

Calcium mobilization assay

Vector-transduced HEK293 cells were dissociated from cultures using 0.25% trypsin/EDTA and resuspended in PBS with 1% bovine serum albumin and 1.25 mM CaCl₂. The cells were incubated with 2 μ M Indo-1 AM (Invitrogen-Molecular Probes, Carlsbad, CA) for 30 minutes at 37°C. After washes to remove excess Indo-1 AM, the cells were resuspended in PBS, followed by fluorescent measurement by spectrofluorometry using excitation wavelength of 338 nm. The ratios of fluorescence emitted at 400 nm and 475 nm from the excitation wavelength were obtained, which reflects the changes of free calcium levels within the cells. After a base line was established, the cells were stimulated with 100 nM N-formyl hexapeptide.

MSC chemotaxis assay

Trypsin-dissociated MSCs were resuspended in PBS solution with 1% BSA. These cells were fluorescently labeled with 15 μ M CellTracker Green CMFDA (Invitrogen-Molecular Probes) for 30-45 minutes and then applied to the apical side of the Fluoroblok 24-well transwells (BD, Franklin Lake, NJ). The cells were allowed to migrate towards the

N-formyl hexapeptide (5 nM) applied to the basal side of the transwells for 2, 4 and 8 hours. Data were collected by measuring the fluorescence intensities from the basal side using the microplate fluorescence reader. Because the filter membrane of the transwells was specially designed to block any fluorescence from the apical side, the basal fluorescence reading represents cell migration to the basal side [18]. To convert fluorescence readings to cell numbers, a standard curve was preestablished for each experiment. The actual numbers of cells migrated to the basal side in response to the chemoattractant were obtained after subtracting the baseline cell migration of the control group receiving no N-formyl hexapeptide stimulation.

MSC differentiation assays

To assess pluripotency of the vector-transduced MSCs, standard differentiation assay schemes were followed [33]. Human MSCs, transduced with 50 MOIs of HIV-CMV-FPR-IRES2-EGFP vector, were plated at an initial density of 50 cell/cm² on a 60 mm dish. For osteogenesis, MSCs were cultured with a complete culture medium with 20 mM glycerol phosphate, 50 ng/ml thyroxine, 1 nM dexamethasone, and 0.5 μ M ascorbate-2-phosphates (Sigma-Aldrich). After 3 weeks, the cells were fixed with 10% formalin for 20 minutes at room temperature and stained with Alizarin Red (Sigma-Aldrich). For adipogenesis, MSCs were cultured with a complete culture medium with 5 μ g/ml insulin, 50 μ M indomethacin, 1 x 10⁻⁶ M dexamethasone, and 0.5 μ M 3-isobutyl-1- methylxanthine (Sigma-Aldrich). After 3 weeks, the cells were fixed with 10% formalin and stained with 0.5% Oil Red O (Sigma-Aldrich).

Bioluminescence imaging of MSCs in mice and luciferase assay of mouse lungs

The animal protocol for this experiment was approved by the LSUHSC institutional IACUC committee. Athymic (nu/nu) male mice received a midline cervical incision to expose the tracheas, followed by an intratracheal intubation of 50 µl of PBS solution with Pseudomonas aeruginosa (PAO1, 5 x 10⁵ cfu) or control PBS. After a 1-day recovery, MSCs (1 x 106), co-transduced with the HIV-CMV-FPR-IRES2-EGFP and HIV-CMV-Luc-IRES2-DsRed vectors at an MOI of 20 for each, were infused into the mice by tail vein injection. For in vivo imaging, the mice were anesthetized by isoflurane inhalation and injected with 150 mg/kg of luciferin (Xenogen, Hopkinton, MA). Bioluminescent signals were detected at 5 minutes after luciferin injection at an integration time of 1 second to 2 minutes using an in vivo imaging system with a cooled charge-coupled device camera (IVIS100; Xenogen, Hopkinton, MA). Similarly, the animals were measured for bioluminescence emission at the different time points (1, 4, and 7 days). To assess the luciferase enzyme activity in the lungs biochemically, the mouse lungs were harvested at 1, 4, 7 days post MSC infusion and homogenized into 1 ml of the luciferase assay lysis solution (Promega, Madison, WI). The homogenates were assayed immediately after addition of luciferin (Promega) in a luminometer (Turner, Sunnyvale, CA) and the detected luciferase activities were normalized against the protein concentrations determined by BCA analyses (Pierce, Rockford, IL).

Immunohistochemistry staining

Mouse lungs, 8 days post intratracheal intubation of PBS or PAO1, were dissected out and fixed in 2% paraformaldehyde/PBS solution by intra-tracheal inflation and *en bloc* immersion overnight at 4°C. Then, the fixative was removed by exchange with PBS repeatedly. Paraffin embedment and tissue section were performed using the routine protocol. The tissue slices were stained immunohistochemically with

the chicken polyclonal antibody against GFP as the first antibody (Novus Biologicals, Littleton, CO) and the donkey anti-chicken IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) as the second antibody. The biotin/streptavidin-conjugated HRP-DAB system was used for color development. The slides were weakly counterstained with hematoxylin.

Results

Lentiviral vector construction and functional expression of FPR

In order to genetically modify MSCs with FPR, we constructed the following three self-inactivating lentiviral vectors: 1) HIV-CMV-EGFP, 2) HIV-CMV-FPR-EGFP, and 3) HIV-CMV-FPR-IRES2-EGFP (Figure 1A). The high-titer VSV-G-pseudotyped vectors were produced by triple-plasmid co-transfection. To test if each of the vectors correctly expressed the expected transgene, we transduced 293T cells, a cell line without endogenous FPR expression. As displayed, EGFP was expressed in all three transduced cells (Figures 1B-1D). Noticeably, the EGFP subcellular localization patterns appeared different. The FPR-EGFP fusion protein was present largely on cytoplasmic membrane (Figure 1C) due to the transmembrane nature of the FPR receptor, while EGFP from the HIV-CMV-EGFP or HIV-CMV-FPR-IRES2-EGFP vector gave rise to the expected cytosolic distribution pattern. Because FPR is a G-protein coupled membrane-bound receptor, its functional expression can be validated by intracellular calcium response to N-FP

stimulation. The three transduced cell lines (HEK293-EGFP, HEK293-FPR-EGFP, and HEK293-FPR-IRES2-EGFP) were labeled with 2 μ M of Indo1-AM, a ratioable calcium probe, and subjected to intracellular calcium mobilization assays by spectrofluorometry. After a baseline was established, 100 nM of N-FMLP was used to stimulate the cells. The results demonstrated that cells receiving the HIV-CMV-FPR-IRES2-EGFP vector had a higher sensitivity to N-FP than those transduced with the HIV-CMV-FPR-EGFP vector (Figure 1E). In contrast, the cells receiving HIV-CMV-EGFP, a control vector, did not respond to N-FP stimulation at all. Given the strongest response to N-FP from the cells transduced with the HIV-CMV-FPR-IRES2-EGFP vector, we chose this vector to engineer MSCs hereafter.

Lentivector-enhanced FPR expression in MSCs induces greater cell sensitivity to N-FP

Human marrow-derived MSCs were transduced with 20 MOIs of HIV-CMV-FPR-IRES2-EGFP or HIV-CMV-EGFP. The micrographs (Figures 2A-2D) and flow cytometry histograms (Figures 2F-2H) demonstrated that the MSCs were efficiently transduced. The HIV-CMV-EGFP transduction gave rise to 95% EGPF-positive cells and the HIV-CMV-FPR-IRES2-EGFP transduction resulted in 53% EGFP-positive cells. Given that equal MOIs of vectors were applied, the EGFP expression difference may have been caused by the IRES sequence. The transduced MSCs were trypsin-dissociated, fluorescently labeled with 15 μ M Cell Tracker Green CMFDA and applied to the apical side of the Fluoroblok transwell inserts for chemotaxis assays. To the basal medium,







also examined as shown (F).

N-FP (0 or 5 nM) was applied. After 2, 4 and 8 hours of incubation, the fluorescent intensities were measured with a fluorescence plate reader from the basal side. To correlate the fluorescence readings to cell numbers, a standard curve was pre-established for each experiment. The actual numbers of cells that migrated to the basal side in response to N-FP was obtained after subtracting the base level random migration of the control cells receiving 0 mM N-FP treatment. As shown in Figure 2E, MSCs with FPR expression showed an increased sensitivity to N-FP by undergoing chemotaxis towards the low nano-molar concentration of N-FP. This level of sensitivity was not seen in the MSCs transduced with HIV-CMV-EGFP, negating any possibility of a lentiviral transduction affecting the sensitivity of hMSCs to N-FP. We also assayed the cells for intracellular calcium response to N-FP by spectrofluorometry. The N-FP (5 nM) stimulation led to a rapid and pronounced elevation of the cytosolic levels of free Ca²⁺. In contrast, the MSCs transduced with the EGFP control vector had no response to the lower level N-FP stimulation (data not shown).

These results indicate that an enhanced expression of FPR in MSCs causes the cells to have a greater sensitivity to and a greater capacity of migration towards a low concentration of N-FP. This finding suggests that the FPR-mediated chemotaxis mechanism can be potentially exploited to enhance MSC homing and engraftment.

MSCs with lentivector-enhancing FPR expression retain their pluripotency

Retention of stem cell pluripotency after ex vivo engineering is

important for potential therapeutic applications. To confirm if our vector-enhanced FPR expression affects such a property, we assayed the MSCs for their ability to differentiate into osteocytes and adipocytes. MSCs, transduced with 20 MOIs of HIV-CMV-FPR-IRES2-EGFP, expressed EGFP and FPR in all the cells (data not shown). By following the published protocol [33], the transduced cells were cultured in differentiation media that induces either osteogenesis or adipogenesis. After 3 weeks of continuous culture, differentiation was validated by Alizarin red staining for osteogenesis and oil red O staining for adipogenesis. As shown in Figure 3, MSCs modified by vector-mediated expression of FPR showed a rich extracellular matrix and calcium phosphate deposition, indicative of osteogenesis (Figures 3A and 3B). Furthermore, the cells also demonstrated an abundant intracellular accumulation of lipid droplets with variable sizes, suggesting adipogeneis (Figures 3C and 3D). Hence, the stem cells engineered with FPR via a lentiviral vector retain their authentic pluripotency.

MSCs with lentivector-enhanced expression of FPR display an increased homing to bacterial challenged lungs

In order to longitudinally observe homing of the MSCs with enhanced FPR expression *in vivo*, hMSCs were co-transduced with lentiviral vectors expressing FPR and firefly luciferase, as described in Materials and Methods. The experimental scheme is displayed (Figure 4A). Nude mice were challenged with a sublethal dose of *Pseudomonas aeruginosa* (PAO1, 5 x 10⁵ CFU) or PBS via intra-tracheal intubation. After recovery for one day, the mice were infused with 1 x 10⁶ of the vector-transduced MSCs via tail vein injection. One, 4 and 7 days



after MSC administration, the same animals from each treatment were anesthetized and injected with 150 mg/kg of luciferin for *in vivo* bioluminescent imaging. As shown (Figure 4B), one day after MSC administration, the stem cells were predominantly located in the lungs. At Days 4 and 7 most of the MSCs disappeared from the lungs regardless of treatments. However, the PAO1-challanged lungs appeared to have a slower rate of clearance than those of the controls (Figures 4B and 4C). By Day 7, bioluminescence fell below the detection threshold of this method for both groups (data not shown).

To confirm the observation from the in vivo imaging, we quantitatively measured the luciferase enzymatic activities of the lungs from the animals similarly treated as those for the whole body imaging. One, 4, and 7 days post MSC infusion, the lungs were harvested, homogenized, and quantified for luciferase activities by bioluminescence assays. Consistent with the imaging data, MSCs, infused through tail veins, were largely lodged into the lungs. Analyses of the Day-1 homogenates showed very high bioluminescence. No significant difference was detected between the control and PAO1challanged lungs. At Day 4 after MSC administration, there was a dramatic reduction in the level of bioluminescence within the lungs of both groups. At Day 7, the control group showed little bioluminescence. Interestingly, the PAO1-treated group maintained a comparable bioluminescence as Day 4 and is significantly higher than that of the day-7 control animals by Student's t-test (p<0.05, n= at least 3). Thus, the MSCs with FPR overexpression had an enhanced retention and homing to inflamed lungs.

Engraftment of FPR-engineered MSCs to distal lungs and airways

The next question we asked was whether the i.v. infused MSCs extravasated blood vessels and engrafted into lung epithelia. Immunohistochemical staining with the first antibody specific to

EGFP was performed on tissue sections of paraffin-embedded lungs. The biotinylated secondary antibody, coupled with streptavidinhorseradish peroxidase, oxidizes 3,3'-diaminobenzidine (DAB) to produce a brown staining for EGFP-positive cells. The nu/nu mice, 8 days after receiving intra-tracheal intubation of PBS followed by i.v. infusion of the FPR-engineered MSCs the next day, resulted in no detectable MSC engraftment in the lungs (Figures 5A and 5B). However, the parallel group of mice intubated with sub-lethal PAO1 (5 x 10⁵ CFU) intratracheally gave rise to MSC engraftment in the airways and alveoli (Figures 5C-5F). Interestingly, clonal expansion of MSCs was clearly seen (Figures 5D and 5E). The positive cells in the examined samples of distal lungs were counted and the percent of positive cells was estimated to be ~0.5%. In contrast, MSC airway engraftment was much lower (~0.03%). Because the samples were collected at Day 8 after intra-tracheal intubation when lung inflammation had been resolved, few inflammatory cells were observed in the lungs. Noticeably, the EGFP-positive cells integrated into the alveolar and airway epithelial structures, suggesting that the FPR-engineered MSCs have the potential of not only homing, but also engrafting to lung epithelia under the condition of bacterial challenge.

Discussion

Even though MSCs possess the great capacity of tissue regeneration and repair, a significant barrier to effective therapy is the inability of MSCs to target tissues of interest with high efficiency of engraftment [34]. Engineering MSCs to enhance their ability to home to particular tissues represent a novel strategy to overcome the low engraftment hurdle facing the stem cell field. In order for MSCs to migrate, the cells must have the ability to sense chemoattractant gradients prior to engaging in directional movement. Marrow-derived MSCs express FPR and migrate towards an N-FP gradient [18,19]. Taking advantage of such an existing chemotactic signaling pathway in MSCs, we demonstrate in



Figure 4: Lentivector-enhanced expression of FPR in human MSCs facilitates their homing to inflamed lungs. *Panel A*: Experimental scheme: at Day -1, athymic (nu/nu) mice were intubated intra- tracheally with 50 µl of *Pseudomonas aeruginosa* suspension solution (5 x 10⁵ CFU) or the same volume of PBS for control. At Day 0, human MSCs (1 x 10⁶), co-transduced with 20 MOI of HIV- CMV-FPR-IRES2-EGFP and HIV-CMV-Luciferase-IRES2-DSRed vectors, were infused into the mice by tail vein injection. At the specified time points (Day +1, +4 and +7), the animals were either subjected to *in vivo* bioluminescent imaging or sacrificed for measurement of firefly luciferase enzymatic activity in the lung. *Panels B & C*: Whole-body bioluminescent imaging of MSC localization. Two animals from each group were anesthetized by isoflurane inhalation and injected with 150 mg/kg of luciferin. Bioluminescent signals were detected at 5 minutes after luciferin injection at an integration time of 1 second to 2 minutes using the Xenogen *in vivo* imaging system. The intensity of bioluminescent emission was pseudo-colored and displayed (**B**). Photon emission rates in the thorax area were obtained and demonstrated (**C**). *Panel D*: Biochemical measurement of firefly luciferase enzymatic activity in the lungs. The animals were sacrificed at Day +1, +4 and +7 post MSC-infusion. The lungs were harvested and homogenized. Luminescence was measured immediately after addition of luciferin in a luminometer and normalized against the protein concentrations determined by BCA analyses. The data were expressed as Relative Luminescent Units (RLU) per milligram protein (mg) per minute. By day 7, significant retention of the MSCs in the lungs challenged with *Pseudomonas aeruginosa* was detected.

this report that lentivector-enhanced expression of FPR enhances MSC homing and engraftment to inflammatory targets such as bacterium-infected lungs. Because N-FP and its receptor FPR constitute a unique ligand-receptor pair for chemotaxis towards inflammation, the strategy of engineering MSCs with FPR for therapy may have broad clinical applications in potential intervention of inflammation-related disorders.

Lung diseases including asthma, emphysema, pulmonary fibrosis, and cystic fibrosis remain major causes of morbidity and mortality globally. Although important advances in supportive treatments have been made, there has been no cure for each of these diseases. MSCs are emerging as a promising cell-based therapy for a wide range of disorders. Clinical trials are evaluating the therapeutic effects of MSCs in patients with multiple sclerosis, graft-versus-host disease, Crohn disease and severe chronic myocardial ischemia [35]. Compelling preclinical data



Figure 5: Morphological examination of MSC homing and engratment in the lungs. Paratin sections of the lungs, 8 days post intubation with either PBS or PAO1, were immunohistochemically staining for EGFP expression. *Panels A & B*: Lung sections from the control animals received PBS intra-tracheal intubation and MSC intra-vascular infusion. *Panels C-F*: Lung sections from the experimental animals received PAO1 intra-tracheal intubation and MSC intra-vascular infusion. The EGFP-positive cells were stained brown (Red arrows). MSCs extravasated out of the blood vessel (C). MSCs engrafted into lung airway epithelia and underwent clonal expansion (D & E). MSCs engrafted into lung alveolar epithelia (F).

also demonstrate the promise of using MSCs for inflammatory lung diseases [36-40]. However, MSCs engraft undamaged lungs poorly [41-43], even though intravenously infused MSCs are largely lodged into the lungs initially. To increase MSC homing and engraftment, many reagents including bleomycin [44,45], naphthalene [41,43], LPS [46], irradiation [42] and polidocanol [47], were used to damage the lungs to create inflammation milieus. Using E. coli endotoxin or E. coli live bacteria to induce acute injury in isolated human lungs, Lee and colleagues found therapeutic effects of human mesenchymal stem cells on alveolar fluid clearance, inflammation reduction and microbicidal activity [39]. In this report, we used live Pseudomonas to infect mouse lungs to evaluate FPR-engineered MSC homing and engraftment. This model is of clinical relevance because bacterium-induced lung inflammation and injury has a high clinical occurrence. MSCs could have therapeutic effects on lung diseases through two possible ways: 1) direct structural constitution via transdifferentiation into lung cells, and 2) indirect functional modulation via secretion of paracrine factors such as growth factors, cytokines and chemokines. Regardless of their working modes, MSCs have to be present in the target tissues. Our data, showing that FPR-overexpression enhances MSC homing and engraftment into inflammatory lungs, suggest the possibility of achieving N-FP-guided stem cell targeting.

Stem cell therapy for different lung diseases may need different modalities [48,49]. For some lung diseases such as acute lung injury and acute respiratory distress syndrome, transient presence of MSCs in the lungs may be sufficient for injury repair via paracrine mechanisms [38,39,50,51]. However, for some other lung diseases such as cystic fibrosis and COPD, long-term engraftment may be required. As shown in Figure 5, the engrafted MSCs appeared to assume epithelial morphology and underwent clonal expansion in some cases. With regard to therapy, differentiation of the engrafted MSCs into pulmonary epithelia is critical for certain diseases, such as cystic fibrosis, which can correct and replace the diseased lung epithelia. Our previous publication has shown that CFTR gene-corrected CF-MSCs can differentiate into airway epithelial cells and contribute to chloride transport in airway epithelia *in vitro* in an air-liquid interface system [33]. The data from this report demonstrate that the MSCs engineered with FPR can engraft into distal lung and airway epithelia *in vivo*, indicating the potential for targeting of gene-engineered MSCs to CF lungs.

In addition to their stem/progenitor properties, MSCs have shown broad immune modulation abilities. They can be either proinflammatory or anti-inflammatory depending on their local environmental cues which induce them into different phenotypes [5,6,52,53]. Our data in Figure 3 demonstrate that the MSCs with FPR overexpression retain their pluropotency. It awaits further characterization as to whether the engineered stem cells retain their natural ability to sense and respond to their surrounding conditions for function.

In conclusion, this research provides the evidence suggesting that marrow-derived MSCs can be engineered to overexpress FPR for specific tissue targeting. Such a manipulation does not affect pluripotency of the MSCs. Importantly, overexpression of FPR enhances the MSC ability to home and engraft to inflammatory tissues such as bacteriuminfected lungs. Thus, it is possible to exploit the existing FPR signaling mechanism and chemotactic machinery within MSCs to achieve a guided targeting of the stem cells to inflammatory sites for therapy.

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References

- Prockop DJ (1997) Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 276: 71-74.
- Pittenger MF1, Mackay AM, Beck SC, Jaiswal RK, Douglas R, et al. (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284: 143-147.
- Mundra V, Gerling IC, Mahato RI (2013) Mesenchymal stem cell-based therapy. Mol Pharm 10: 77-89.
- Uccelli A, Moretta L, Pistoia V (2008) Mesenchymal stem cells in health and disease. Nat Rev Immunol 8: 726-736.
- Le Blanc K, Mougiakakos D (2012) Multipotent mesenchymal stromal cells and the innate immune system. Nat Rev Immunol 12: 383-396.
- Prockop DJ, Oh JY (2012) Mesenchymal stem/stromal cells (MSCs): role as guardians of inflammation. Mol Ther 20: 14-20.
- Maxson S, Lopez EA, Yoo D, Danilkovitch-Miagkova A, Leroux MA (2012) Concise review: role of mesenchymal stem cells in wound repair. Stem Cells Transl Med 1: 142-149.
- Chavakis E, Urbich C, Dimmeler S (2008) Homing and engraftment of progenitor cells: a prerequisite for cell therapy. J Mol Cell Cardiol 45: 514-522.
- 9. Khaldoyanidi S (2008) Directing stem cell homing. Cell Stem Cell 2: 198-200.
- Ren G, Chen X, Dong F, Li W, Ren X, et al. (2012) Concise review: mesenchymal stem cells and translational medicine: emerging issues. Stem Cells Transl Med 1: 51-58.
- 11. Prockop DJ (2009) Repair of tissues by adult stem/progenitor cells (MSCs): controversies, myths, and changing paradigms. Mol Ther 17: 939-946.
- Lau AN, Goodwin M, Kim CF, Weiss DJ (2012) Stem cells and regenerative medicine in lung biology and diseases. Mol Ther 20: 1116-1130.
- Spaeth E, Klopp A, Dembinski J, Andreeff M, Marini F (2008) Inflammation and tumor microenvironments: defining the migratory itinerary of mesenchymal stem cells. Gene Ther 15: 730-738.
- 14. Ponte AL, Marais E, Gallay N, Langonné A, Delorme B, et al. (2007) The in vitro migration capacity of human bone marrow mesenchymal stem cells: comparison of chemokine and growth factor chemotactic activities. Stem Cells 25: 1737-1745.

- Cheng Z, Ou L, Zhou X, Li F, Jia X, et al. (2008) Targeted migration of mesenchymal stem cells modified with CXCR4 gene to infarcted myocardium improves cardiac performance. Mol Ther 16: 571-579.
- Haider HKh, Jiang S, Idris NM, Ashraf M (2008) IGF-1-overexpressing mesenchymal stem cells accelerate bone marrow stem cell mobilization via paracrine activation of SDF-1alpha/CXCR4 signaling to promote myocardial repair. Circ Res 103: 1300-1308.
- Zhang J, Gong JF, Zhang W, Zhu WM, Li JS (2008) Effects of transplanted bone marrow mesenchymal stem cells on the irradiated intestine of mice. J Biomed Sci 15: 585-594.
- Viswanathan A, Painter RG, Lanson NA Jr, Wang G (2007) Functional expression of N-formyl peptide receptors in human bone marrow-derived mesenchymal stem cells. Stem Cells 25: 1263-1269.
- Kim MK, Min do S, Park YJ, Kim JH, Ryu SH, et al. (2007) Expression and functional role of formyl peptide receptor in human bone marrow-derived mesenchymal stem cells. FEBS Lett 581: 1917-1922.
- Murphy PM (1994) The molecular biology of leukocyte chemoattractant receptors. Annu Rev Immunol 12: 593-633.
- Seifert R, Wenzel-Seifert K (2003) The human formyl peptide receptor as model system for constitutively active G-protein-coupled receptors. Life Sci 73: 2263-2280.
- Ye RD, Boulay F (1997) Structure and function of leukocyte chemoattractant receptors. Adv Pharmacol 39: 221-289.
- 23. Le Y, Gong W, Li B, Dunlop NM, Shen W, et al. (1999) Utilization of two seventransmembrane, G protein-coupled receptors, formyl peptide receptor-like 1 and formyl peptide receptor, by the synthetic hexapeptide WKYMVm for human phagocyte activation. J Immunol 163: 6777-84.
- Miller AF, Falke JJ (2004) Chemotaxis receptors and signaling. Adv Protein Chem 68: 393-444.
- Le Y, Murphy PM, Wang JM (2002) Formyl-peptide receptors revisited. Trends Immunol 23: 541-548.
- Coats WD Jr, Navarro J (1990) Functional reconstitution of fMet-Leu-Phe receptor in Xenopus laevis oocytes. J Biol Chem 265: 5964-5966.
- 27. Kew RR, Peng T, DiMartino SJ, Madhavan D, Weinman SJ, et al. (1997) Undifferentiated U937 cells transfected with chemoattractant receptors: a model system to investigate chemotactic mechanisms and receptor structure/ function relationships. J Leukoc Biol 61: 329-37.
- Miettinen HM, Gripentrog JM, Jesaitis AJ (1998) Chemotaxis of chinese hamster ovary cells expressing the human neutrophil formyl peptide receptor: role of signal transduction molecules and alpha5beta1 integrin. J Cell Sci 111: 1921-8.
- Didsbury JR, Uhing RJ, Tomhave E, Gerard C, Gerard N, et al. (1992) Functional high efficiency expression of cloned leukocyte chemoattractant receptor cDNAs. FEBS Lett 297: 275-279.
- 30. Verghese MW, Fox K, McPhail LC, Snyderman R (1985) Chemoattractantelicited alterations of cAMP levels in human polymorphonuclear leukocytes require a Ca2+-dependent mechanism which is independent of transmembrane activation of adenylate cyclase. J Biol Chem 260: 6769-75.
- Ricks DM, Kutner R, Zhang XY, Welsh DA, Reiser J (2008) Optimized lentiviral transduction of mouse bone marrow-derived mesenchymal stem cells. Stem Cells Dev 17: 441-450.
- Kutner RH, Zhang XY, Reiser J (2009) Production, concentration and titration of pseudotyped HIV-1-based lentiviral vectors. Nat Protoc 4: 495-505.
- 33. Wang G, Bunnell BA, Painter RG, Quiniones BC, Tom S, et al. (2005) Adult stem cells from bone marrow stroma differentiate into airway epithelial cells: potential therapy for cystic fibrosis. Proc Natl Acad Sci U S A 102: 186-91.
- Karp JM, Leng Teo GS (2009) Mesenchymal stem cell homing: the devil is in the details. Cell Stem Cell 4: 206-216.
- 35. Minguell JJ, Allers C, Lasala GP (2013) Mesenchymal stem cells and the treatment of conditions and diseases: the less glittering side of a conspicuous stem cell for basic research. Stem Cells Dev 22: 193-203.
- Mei SH, McCarter SD, Deng Y, Parker CH, Liles WC, et al. (2007) Prevention of LPS-induced acute lung injury in mice by mesenchymal stem cells overexpressing angiopoietin 1. PLoS Med 4: e269.

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- Matthay MA, Goolaerts A, Howard JP, Lee JW (2010) Mesenchymal stem cells for acute lung injury: preclinical evidence. Crit Care Med 38: S569-573.
- Matthay MA, Thompson BT, Read EJ, McKenna DH Jr, Liu KD, et al. (2010) Therapeutic potential of mesenchymal stem cells for severe acute lung injury. Chest 138: 965-972.
- 39. Lee JW, Krasnodembskaya A, McKenna DH, Song Y, Abbott J, et al. (2013) Therapeutic effects of human mesenchymal stem cells in ex vivo human lungs injured with live bacteria. Am J Respir Crit Care Med 187: 751-760.
- Loi R, Beckett T, Goncz KK, Suratt BT, Weiss DJ (2006) Limited restoration of cystic fibrosis lung epithelium in vivo with adult bone marrow-derived cells. Am J Respir Crit Care Med 173: 171-179.
- Herzog EL, Van Arnam J, Hu B, Krause DS (2006) Threshold of lung injury required for the appearance of marrow-derived lung epithelia. Stem Cells 24: 1986-1992.
- 42. Serikov VB, Popov B, Mikhailov VM, Gupta N, Matthay MA (2007) Evidence of temporary airway epithelial repopulation and rare clonal formation by BMderived cells following naphthalene injury in mice. Anat Rec (Hoboken) 290: 1033-1045.
- 43. Ortiz LA, Dutreil M, Fattman C, Pandey AC, Torres G, et al. (2007) Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. Proc Natl Acad Sci U S A 104: 11002-11007.
- 44. Rojas M, Xu J, Woods CR, Mora AL, Spears W, et al. (2005) Bone marrowderived mesenchymal stem cells in repair of the injured lung. Am J Respir Cell Mol Biol 33: 145-152.

- 45. Gupta N, Su X, Popov B, Lee JW, Serikov V, et al. (2007) Intrapulmonary delivery of bone marrow-derived mesenchymal stem cells improves survival and attenuates endotoxin-induced acute lung injury in mice. J Immunol 179: 1855-1863.
- 46. Leblond AL, Naud P, Forest V, Gourden C, Sagan C, et al. (2009) Developing cell therapy techniques for respiratory disease: Intratracheal delivery of genetically engineered stem cells in a murine model of airway injury. Hum Gene Ther.
- 47. Lee JW, Fang X, Gupta N, Serikov V, Matthay MA (2009) Allogeneic human mesenchymal stem cells for treatment of E. coli endotoxin-induced acute lung injury in the ex vivo perfused human lung. Proc Natl Acad Sci U S A 106: 16357-16362.
- Weiss DJ (2014) Concise review: current status of stem cells and regenerative medicine in lung biology and diseases. Stem Cells 32: 16-25.
- Chimenti L, Luque T, Bonsignore MR, Ramírez J, Navajas D, et al. (2012) Pretreatment with mesenchymal stem cells reduces ventilator-induced lung injury. Eur Respir J 40: 939-948.
- Curley GF, Hayes M, Ansari B, Shaw G, Ryan A, et al. (2012) Mesenchymal stem cells enhance recovery and repair following ventilator-induced lung injury in the rat. Thorax 67: 496-501.
- Bernardo ME, Fibbe WE (2013) Mesenchymal stromal cells: sensors and switchers of inflammation. Cell Stem Cell 13: 392-402.
- 52. Keating A (2012) Mesenchymal stromal cells: new directions. Cell Stem Cell 10: 709-716.