Multipotent Mesenchymal Stem Cells Acquire a Lymphendothelial Phenotype and Enhance Lymphatic Regeneration In Vivo

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Background—The importance and therapeutic value of stem cells in lymphangiogenesis are poorly understood. We evaluated the potential of human and murine mesenchymal stem cells (MSCs) to acquire a lymphatic phenotype in vitro and to enhance lymphatic regeneration in vivo.

Methods and Results—We assessed the lymphendothelial differentiation of human and murine MSCs after induction with supernatant derived from human dermal microvascular endothelial cells, isolated lymphatic endothelial cells, and purified vascular endothelial growth factor (VEGF)-C in vitro. We used human or murine progenitor MSC lines and then characterized the lymphatic phenotype by morphology, migratory capacity, and the expression of lymphatic markers such as Prox-1, podoplanin, Lyve-1, VEGF receptor-2, and VEGF receptor-3. Using a murine lymphatic edema model, we assessed the potential of these cells to form a functional lymphatic vasculature in vivo after injection of syngeneic MSCs. Incubation with supernatant from lymphatic endothelial cells induced an endothelium-like morphology and the expression of lymphendothelial markers in both human and murine MSCs in vitro. MSCs showed migratory activity along a VEGF-C gradient, which was enhanced by VEGF-C conditioning. In vivo, the local application of MSCs resulted in a significant decrease in edema formation (~20.1%; \( P < 0.01 \) versus untreated tails) after 3 weekly cell injections and restored the drainage of intradermally injected methylene blue after 7 weekly injections.

Conclusions—MSCs were capable of expressing a lymphatic phenotype when exposed to lymph-inductive media and purified VEGF-C. Migratory activity toward VEGF-C in vitro suggests homing capability in vivo. Restoration of lymphatic drainage after injection of MSCs in a lymphedema model indicates that MSCs play a role in lymphatic regeneration. The potential clinical application of MSC in wound healing and reduction of lymphatic edema warrants further research. (Circulation. 2009;119:281-289.)

Key Words: lymphangiogenesis • Lyve-1 • mesenchymal stem cell • Prox-1 • VEGF

Lymphatic capillaries drain interstitial fluid and proteins back into the venous circulation. Moreover, the lymphatic system plays a central role in the immune response through trafficking of immune cells and represents an escape route for metastasizing tumor cells.1,2 The molecular basis of lymphangiogenesis is only partially understood, in part because of an overlap between markers of endothelial cells (ECs) and lymphatic endothelial cells (LECs), the lack of specific lymphatic molecular markers, and the unavailability of good experimental models. The most studied mediators of lymphangiogenesis are the members of the vascular endothelial growth factor (VEGF) family, which are crucial players in the regulation of lymphangiogenesis as well as hemangiogenesis.3 Treatment of lymph node–excised mice with adenovirus delivered VEGF-C– or VEGF-D–induced robust growth of the lymphatic capillaries, which gradually underwent intrinsic remodeling, differentiation, and maturation into functional collecting lymphatic vessels, including the formation of uniform endothelial cell-cell junctions and intraluminal valves.4 In addition, the blockade of VEGF receptor-3 (VEGFR-3) signaling with monoclonal antibodies was shown to result in decreased sprouting, vascular density, vessel branching, and EC proliferation in mouse angiogenesis models.5 A clinical correlate can be found in the well-described development of lymphoceles in transplant recipients receiving sirolimus.6,7

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To date, the role of stem cells in physiological and pathological lymphangiogenic processes remains unclear. The cell type that contributes to lymphatic regeneration may depend to a degree on the tissue environment, as demonstrated in human, gender-mismatched renal transplant patients, in whom recipient-derived lymphatic progenitor cells were found to contribute to lymphangiogenesis in the graft. However, the lymphatic vessels of normal tissues and those around posttransplant carcinomas did not incorporate donor-derived progenitors. Conflicting evidence regarding the source of lymphatic progenitors has been provided through reports that tumor-associated lymphatics are primarily formed from a preexisting lymphatic network through vessel sprouting. The potential role of mesenchymal stem cells (MSCs) in lymphangiogenesis has not been investigated to date. However, the source of the cells that contribute to lymphangiogenesis may mirror to a degree the description for haematopoietic endothelial precursors.

Methods

Cell Lines

Either the human MSC line V542 obtained from the peripheral blood of living donors or murine MSC lines obtained from the bone marrow of either Balb/c or C57B16 mice were used for in vitro experiments. For in vivo experiments, murine MSC line p53−/− MSC obtained from the bone marrow of p53−/− C57B16 mice (as published previously) was used.12 These stem cells were positive for CD73 (SH3) and CD105 (SH2) and negative for the myelogenic markers CD34, CD14, CD45, and MHC class II. V542 was also positive for CXCR1, CXCR2, CXCR3, CXCR2, CXCR7, CXCR5, and CCR5. p53−/− MSCs expressed CCR2, CXCR1, CXCR2, CXCR3, and CCR7.12-14 The aim of the present study was to characterize the potential of MSCs to acquire a lymphatic phenotype in vitro and to regenerate lymphatic vasculature after tissue injury in vivo and, in this way, to assess a potential role for these cells in clinical applications related to wound healing and the reduction of lymphatic edema.

Isolation of LECs

HMVECs (PromoCell, Heidelberg, Germany) were expanded in NU-4 (UNC) (NUNC Roskilde, Denmark) T-75 or T-175 flasks over 3 or 4 passages in endothelial growth medium MV (PromoCell) without supplements. They were incubated with trypsin for 5 minutes, and the reaction was stopped with the use of trypsin neutralization solution (PromoCell) cells and resuspended in HEPES balanced salt solution (PromoCell). The cells were incubated with a biotinylated VEGFR-3 antibody (R&D Systems, Minneapolis, Minn) for 1 hour, followed by incubation with streptavidin microbeads (Miltenyi Biotech, Bergish Gladbach, Germany). Labeled cells were isolated with a MidiMACS magnet and MS columns (Miltenyi Biotech). Ten microfilters of the original stock solution of each antibody (Becton Dickinson, Heidelberg, Germany) were used for 106 cells in 100 μL FACS buffer.

Migration Assay

VEGF-C stimulation was assessed with the use of 8-μm pore size polycarbonate filters with a modified Boyden chamber assay system. The assay contains 180 μL medium (RPMI 1640, 0.1% BSA, and 10 mM/L HEPES) with 300 ng of highly purified human VEGF-C (R&D Systems) in the lower chamber. Primary MSCs or MSCs pretreated with VEGF-C were labeled with 10 μg/mL calcein (Molecular Probes, Eugene, Ore) and resuspended at 105 cells/mL in assay medium, and 50 μL cell suspension was added to the upper

RNA Isolation and cDNA Synthesis

Total RNA of the harvested cells was extracted with the use of the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. RNA was purified with the DNA-free kit (Ambion Inc, Austin, Tex). Quality and concentration of the isolated RNA were verified by measuring the optical density, and the RNA was stored at −80°C until analysis. cDNA was synthesized from total RNA with the use of M-MLV reverse transcriptase (Promega, Madison, Wis).

Reverse Transcription Polymerase Chain Reaction

For the murine lymphatic marker podoplanin, the following forward primers were used: 5'-CGC TCT CTC TGC GGT TGT A-3'; as reverse primer: 5'-ATG TGG ACC GTC CCA GTG T-3'. The forward primer for VEGFR-3/FLT-4 was as follows: 5'-CTT TGA CCA TTG GAA CTC C-3'; the reverse primer: 5'-CCA ATC TCT TCG TCG TGC A-3'. The forward primer for Prox-1 had the following sequence: 5'-GCC AGA GAC CTT AAA ACA GG-3'; the reverse primer: 5'-GAC TCT GAA ATG GAT AAG CCA C-3' (all primers from Thermo Electron Inc, Ulm, Germany). PCR was performed with the use of recombinant Taq DNA polymerase (TaKaRa Bio Inc, Japan) and cycled 35 times at a temperature of 60°C in a Biometra Personal Cycler.

Flow Cytometry

Flow cytometry was performed as described previously. The immune phenotype was defined with the use of monoclonal antibody reagents directed against CD10, CD14, CD45, CD68, HLA-DR (Becton-Dickinson, Heidelberg, Germany), and CD105 (Ansell, Bayport, Minn) used according to the manufacturer’s recommendations. The following anti-human chemokine receptor antibody was used: CCR7 (1:1) (E. Kremmer, GSF, Munich, Germany). For detection, anti-mouse (PE, Dako, Copenhagen, Denmark), anti-rat (PE, Biomedica, Germany), or anti-goat (PE, DPC Biermann, Bad Nauheim, Germany) antibodies were used, respectively.

Antibodies against podoplanin, VEGFR-2, and VEGFR-3 were purchased from BD Pharmingen. Ten microfilters of the original stock solution of each antibody (Becton Dickinson, Heidelberg, Germany) was used for 106 cells in 100 μL FACS buffer.

Migration Assay

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chamber of the transwells. Plates were incubated at 37°C, and the increase of fluorescence in the lower chamber was measured over time (30 minutes to 3 hours) with the use of a TECAN GENios Plus enzyme-linked immunosorbent assay reader (TECAN, Grödig, Austria; emission, 535 nm; excitation, 485 nm) and XFluor 4 software (TECAN). The results represent at least 2 independent experiments performed in triplicate.

Animal Model

We modified the surgical preparation of the C57Bl/6 mouse tail as previously described by Boardman and Swartz. The animals were operated on under general anesthesia with the use of inhalation of isoflurane and adequate pain control. On tails of female C57Bl/6 mice, a circumferential 2-mm-wide piece of skin located 1 cm distal of the tail base was removed. Particular care was taken to ensure that the deeper draining lymphatics running alongside the major blood vessels were severed as well. For this purpose, 5 μL of methylene blue was injected intradermally at the distal end of the tail, and all blue-stained lymphatic vessels at the site of preparation were severed with the use of an operating microscope. We applied stem cells locally to the site of injury once a week using subcutaneous injections of 10⁷ syngenic murine mesenchymal p53⁻/− stem cells per animal. To compare lymphatic vessel regeneration on day 57 between mice that received stem cells locally and mice that did not receive stem cells, we injected 5 μL of methylene blue intradermally at the distal end of the tail and waited for 15 minutes to allow lymphatic drainage of the dye. At the end of the experiment, mice tails were surgically removed, fixed in formalin for 24 hours, and kept in EDTA for 5 days at 37°C. Lymphatic vessels were identified by immunohistochemistry with the use of rabbit primary antibodies against murine LYVE-1 and podoplanin (Acris Antibodies), a biotinylated goat secondary antibody to rabbit IgG (DakoCytomation, Glostrup, Denmark), and Texas Red Avidin D (Vector, Burlingame, Calif).

Statement of Responsibility

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Morphology

We observed a change in morphology after incubating the human MSC line for 4 weeks with supernatant from isolated

Figure 1. Endothelial differentiation of human V54/2 stem cells in vitro with the use of LEC supernatant. A, Representative image of V54/2 cells kept under standard growth conditions (magnification ×50). B, Representative image of V54/2 cells conditioned for 4 weeks with supernatant from LECs that had been isolated from human vascular ECs (magnification ×50). Marked changes in the morphology toward spindle shape with podocytic protrusions are suggestive of endothelial morphology.

Figure 2. Expression of the lymphatic transcription factor Prox-1 in the human MSC lines V54/1-2. RT-PCR analysis revealed a strong band for Prox-1 in the human MSC lines V54/1 (1) and V54/2 (2) derived from peripheral blood. Water was used as negative control (3).

Figure 3. Expression of podoplanin, Prox-, and VEGFR-3 in conditioned immortalized MSC Balb/c. A, Podoplanin. RT-PCR analysis revealed no expression of podoplanin in the unconditioned (1) and the EC-medium–incubated (4) groups. However, there was an upregulation of podoplanin in the human VEGF-C (C156S)–conditioned (2) and the HMVEC supernatant–conditioned (3) groups. Water was used as negative control (5). B, Prox-1. The only expression of Prox-1 mRNA was found in imMSCs conditioned with VEGF-C (C156S) (2). C, VEGFR-3. All 4 groups showed expression of VEGFR-3. Water was used as negative control.
Figure 4. Surface expression of podoplanin (a), VEGFR-2 (b), and VEGFR-3 (c) assessed by standard flow cytometry. For podoplanin, unconditioned cells for negative control (row 1) and HMVECs as positive control (row 2) are shown. V54/2 conditioned with supernatant from HMVEC (row 3), VEGF-C (C156S) (row 4), and supernatant from LEC (row 5) show progressively enhanced expression of podoplanin, suggesting the existence of unidentified inductors for a lymphatic phenotype released by LECs. For VEGFR-2, enhanced surface expression of VEGFR-2 in V54/2 conditioned with HMVEC supernatant or VEGF-C (C156S) exceeded 70%. For VEGFR-3, VEGFR-3 expression was ~2-fold higher in V54/2 conditioned with HMVEC supernatant or VEGF-C (C156S) compared with unconditioned cells.
LECs (Figure 1). Both cell lines grew adherently with a fibroblast-like morphology in cell culture, but only cells that were induced with LEC supernatant acquired a spindle shape with long podocytic protrusions suggestive of endothelial differentiation (Figure 1).

Reverse Transcriptase Polymerase Chain Reaction
Molecular evidence for lymphendothelial differentiation of the cells was provided by RT-PCR. The signal for the mRNA encoding Prox-1, the main known transcription factor for lymphangiogenesis, was detected in the human MSC line V54/2 (Figure 2).

Furthermore, PCR analysis was performed with the use of mRNA isolated from the murine Balb/c MSC line. The following groups were used during the experiment: unconditioned, conditioned with human VEGF-C (C156S), and conditioned with HMVEC supernatant. An upregulation of podoplanin was detected in cells conditioned with human VEGF-C (C156S) and HMVEC supernatant, respectively (Figure 3). Subsequent DNA sequencing of the band verified the specificity of the results.

Lymphangiogenic Phenotype
Flow cytometric analysis was then performed for VEGF-R2, VEGFR-3, and podoplanin, epitopes on LECs and on treated V54/2 (Figure 4). HMVECs were used as positive controls and isotype antibodies as negative controls. Upregulation of podoplanin was highest in the group that had been incubated with supernatant from isolated LECs (10.9%), whereas the upregulation of podoplanin in HMVEC supernatant–conditioned cells (3.9%) and VEGF-C–induced cells (5.9%) was more moderate (Figure 4a).

VEGF-R2 is expressed on blood endothelial cells as well as LECs. In the experiments performed here, an upregulation of VEGF-R2 was found in ~75% of the conditioned stem cell population versus 17.6% in the unconditioned cell line (Figure 4b). VEGFR-3, which is more specific for LECs, was found to be expressed in only 4.7% of the V54/2 cells but was upregulated after the induction of a lymphangiogenic phenotype in cells conditioned with the HMVEC supernatant group (9.8%) and the VEGF-C–induced group (7.5%) (Figure 4c).

Migration Assay
To test the functionality of VEGFR-3 and active homing of MSCs toward a VEGF-C gradient, a modified Boyden chamber assay was used to determine the response of cells to VEGF-C. An increased migration index of V54/2 toward purified VEGF-C was demonstrated with the use of a modified Boyden chamber (Figure 5). The migration index of the conditioned MSC population was almost twice that of the unconditioned MSCs. The human peripheral blood–derived MSC V54/2 cells were conditioned with VEGF-C over a period of 4 weeks.

MSCs Express Functional CCR7
The chemokine receptor CCR7 has been implicated in lymphangiogenesis. V54/2 and the murine stem cell lines

Figure 5. Migration assay with the use of a modified Boyden chamber. We compared the migration indices of native V54/2- and VEGF-C–induced V54/2 toward a VEGF-C gradient. Migration was assessed after 1 and 3 hours. The migration index was significantly higher in the VEGF-C–induced population, suggesting upregulation of migratory mechanisms in MSCs after their induction toward a lymphatic phenotype.

Figure 6. Human and mouse MSC lines express functional CCR7. A, Chemokine receptor mRNA expression in human and murine MSC lines. Triangles indicate values of single RT-PCR experiments determining chemokine receptor expression in individual Balb/c MSC preparations; circles, values of single RT-PCR experiments determining chemokine receptor expression in individual V54/2 MSC preparations; and line, median values of chemokine receptor expression. All values were normalized against rRNA. The y axis represents the ratio to rRNA. B, Surface expression of CCR7 was demonstrated on MSC by FACS analysis. Gates were set on the living cell population determined by propidium iodine staining. The histogram is a representative example of several experiments. C, Functionality was determined with the use of a modified Boyden chamber migration assay. The results show expression at the mRNA level, with the use of RT-PCR, of a low level of surface expression of CCR7 in the human cell lines. The MSC line V54/2 shows migration to the ligand CCL19. The migratory capacity of V54/2 toward CCL19 is compared with the migration of HT1080 toward FCS. The y axis represents the migration index. The migration index is calculated as described.
were analyzed for CCR7 expression at the mRNA level with the use of TaqMan RT-PCR, at the cell surface level with the use of CCR7-specific monoclonal antibodies, and finally at the functional level with the use of modified Boyden chamber assays. The human and murine cell lines result show expression at the mRNA level and a low but functional level of surface expression of CCR7 (Figure 6).12

In Vivo Lymphatic Regeneration

With an animal model of lymphatic edema, the ability of syngenic C57Bl/6 stem cells to reduce edema formation was evaluated. In this model, drainage of interstitial fluid was completely abolished through microsurgical severing of the lymphatic vasculature along the entire circumference of the tail. A significant lymphatic edema developed within a few days after the procedure (Figure 7). In stem cell–treated animals, a marked reduction of the edema was observed after day 27 compared with untreated animals (P<0.05). In the latter control group, the edema persisted over the entire study period, whereas in the treatment group, the edema was significantly reduced. Moreover, intradermal injections of methylene blue on day 56 after the primary operation resulted in drainage of the dye across the site of incision only in mice treated with stem cells (Figure 7A). The mouse tails were harvested and analyzed for formation of neolymphangiogenesis. Consistently, in histological sections, regenerated Lyve-1– and podoplanin-positive lymphatic vessels, which are specific makers of lymphangiogenesis, were found at the site of surgical preparation (Figure 8) in the group that received syngenic MSCs.

Discussion

In the present study, we assessed the ability of well-characterized MSC lines to acquire a lymphatic phenotype in vitro and in vivo. The administration of MSCs in vivo may contribute to the reduction of lymphatic edema, a prevalent and clinically relevant problem. To date, the contribution of stem cells to lymphangiogenesis is not well understood, and the available reports describe conflicting evidence. He et al18 demonstrated that preexisting lymphatic endothelium but not bone marrow–derived endothelial progenitor cells are essential for tumor lymphangiogenesis and lymphatic metastasis. Recently, others have suggested a potential role for bone marrow–derived circulating progenitor ECs.19–22 The involvement of circulating endothelial progenitors in human lymphangiogenesis was demonstrated by Kerjaschki et al16 by examining the origin of ECs of newly formed lymphatic vessels in the kidneys of patients with gender-mismatched renal transplants. This group also suggested a central role for the chemokine receptor CCR7 in the recruitment of lymphatic endothelial precursors.17 Functional expression of CCR7 on the MSC lines used in these experiments could be demonstrated.

![Figure 7. Animal model. A, Intradermal injection of methylene blue on day 56 after the primary operation resulted in transportation of the dye across the incision site in mice receiving local application of stem cells. Mice receiving no stem cells showed no visible transportation of the dye across the incision site. B, Assessment of the circumference directly at the incision site (0 cm) as well as 1 cm distal of the incision site showed progressively reduced edema formation in mice treated with MSC. *Probability value on day 56.](image-url)
Isolation of LECs from HMVEC preparations allowed the use of their supernatant as a mean of inducing stem cell differentiation. The experiments were based on the hypothesis that LECs release paracrine factors for lymphatic differentiation, which may also have effects on stem cell differentiation. The LEC supernatant changed the fibroblast-like morphology of the adherent growing stem cell line V54/2 toward an endothelial-like morphology. Moreover, flow cytometry analysis revealed enhanced surface expression of podoplanin, VEGFR-2, and VEGFR-3 in cells conditioned with supernatant from HMVECs and LECs as well as with purified VEGF-C (C156S). Most importantly, there was a marked upregulation of podoplanin and of the only known transcription factor of lymphangiogenesis, Prox-1, on the mRNA level after the stem cell line was conditioned with VEGF-C (C156S) and HMVEC supernatant, respectively. Previous studies have demonstrated that isolated blood ECs, for example, produce VEGF-C in a 4-fold higher concentration than LECs. In addition, some of our data show that treatment of the stem cells with VEGFR-3–specific VEGF-C (C156S) was less potent in inducing a lymphatic phenotype than supernatant derived from LECs. This suggests the necessity for MSCs to be exposed to VEGFR-2, VEGFR-3, and possibly other factors to differentiate toward a lymphatic endothelial phenotype. These findings are consistent with the findings of other authors assessing the differentiability of mouse embryonic stem cell–derived endothelial progenitor cells under VEGFR-3 signaling. However, an additional explanation for the differences in differentiation potential at hand may be found in the recently discovered importance of several known growth factors for lymphangiogenesis.

Ligands to VEGFR-3 appear to be crucial for the embryonic development of lymphatic vasculature. This has been demonstrated in transgenic mice expressing a soluble form of the VEGFR-3 (and thereby neutralizing its ligands) in select tissues. In addition, it has recently been shown that genetic targeting of VEGFR-3 or blocking of VEGFR-3 signaling with monoclonal antibodies resulted in decreased sprouting, vascular density, vessel branching, and EC proliferation in mouse angiogenesis models. Further studies that seek to characterize unidentified factors will be of crucial importance for the understanding of regenerative as well as tumor-associated lymphangiogenesis.

To investigate whether an active migratory mechanism of MSCs toward a VEGF-C gradient may exist, a modified Boyden chamber assay was used. V54/2 cells were compared with VEGF-C–induced stem cells. The increased migration index of the treated V54/2 cells suggests that VEGF-C triggers stem cells to acquire a lymphatic phenotype that subsequently switches on active migration mechanisms in these stem cells. Furthermore, VEGF-C, which is upregulated during lymphatic regeneration and secreted by lymphogenic metastasizing tumors such as melanoma and breast cancer, may recruit circulating stem cells to the site of lymphangiogenesis by the mechanism of VEGF-C secretion.

Despite the suggestion that a blockade of VEGFR-3 ligands or downregulation of VEGF-C by means of a soluble antibody, endostatins, or small interfering RNA leads to reduced metastasization of VEGF-C–producing cancers and impaired adult lymphatic regeneration, the mechanism and the impact of these therapies on VEGF-3–endothelial progenitor cells have yet to be revealed. In hemangiogenesis,
the dependence of growing tumors on VEGF-responsive bone marrow–derived hematopoietic and endothelial precursor cells has been demonstrated.36 Reciprocity in lymphangiogenesis seems likely, especially given the results presented here.

Finally, a significant reduction of the lymphatic edema was found in mice that received local administration of stem cells in the in vivo lymphatic edema model. Evidence was also provided for the restoration of lymphatic fluid drainage as transportation of intradermally injected methylene blue across the injury site of mice that received stem cells was observed, whereas untreated animals showed no evidence for lymphatic regeneration in this model. Together with the histological evidence for regenerated lymphatic vessels, these data clearly show that stem cells are capable of inducing regeneration of functionally intact lymphatic vessels. However, it remains unclear whether trauma to the lymphatic vasculature as performed in this animal model represents a sufficient inductor of stem cell mobilization and homing to the injury site as demonstrated for hemangiogenesis.37 We have previously shown that rapamycin is a potent inhibitor of downstream signaling of the VEGFR-3/C pathway.7 The widespread clinical use of rapamycin has led to an increasing incidence of generalized and localized lymphatic edema formation.38 Whether MSCs have a role in treating lymphatic edema in the future will depend on further research, which will enhance the understanding of the role of stem cells in lymphatic biology.

In conclusion, the experiments presented here in concert with evidence generated by others strongly suggest that peripheral blood–derived stem cells are involved in lymphatic regeneration. The results suggest that these relatively easily accessible peripheral blood–derived stem cells may be of therapeutic value in patients with chronic or acute lymphatic edema.39 It will also be important in the future to assess the role of stem cells in tumor-associated lymphangiogenesis, as will the characterization of the LEC-secreted factors that induce stem cells toward lymphatic differentiation. The fact that many tumors produce large amounts of VEGF-C accompanied by a high incidence of lymphatic metastases40,41 underscores the necessity of further investigating the role of stem cells in the setting of tumor lymphangiogenesis.

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Disclosures

None.

References

In the present study, we assessed the ability of well-characterized mesenchymal stem cell (MSC) lines to acquire a lymphatic phenotype in vitro and in vivo. To date, the contribution of stem cells to lymphangiogenesis is not well understood, and the available reports describe conflicting evidence. However, we were able to demonstrate that MSCs are capable of expressing a lymphatic phenotype when exposed to lymph-inductive media and purified vascular endothelial growth factor-C. Migratory activity toward vascular endothelial growth factor-C in vitro suggests homing capability in living systems. To date, the contribution of stem cells to lymphangiogenesis is not well understood, and the available reports describe conflicting evidence. However, we were able to demonstrate that MSCs are capable of expressing a lymphatic phenotype when exposed to lymph-inductive media and purified vascular endothelial growth factor-C. Migratory activity toward vascular endothelial growth factor-C in vitro suggests homing capability in lymphatic vessels. The observed involvement of MSCs in restoration of lymphatic drainage leads to further questions about the role of MSCs in tumor-associated lymphangiogenesis and lymphatic endothelial cell–secreted factors that drive MSCs toward lymphatic differentiation.
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