PROGENITOR CELLS OF MESODERMAL LINEAGE

Field of the Invention

The invention relates to progenitor cells of mesodermal lineage and their use in therapy.

Background to the Invention

Mesenchymal stem cells (MSCs) are multipotent, adult stem cells. MSCs differentiate to form the different specialised cells found in the skeletal tissues. For example, they can differentiate into cartilage cells (chondrocytes), bone cells (osteoblasts) and fat cells (adipocytes).

MSCs are used in a variety of therapies, such as the treatment of Age-related Macular Degeneration (AMD) and myocardial infarct. Once administered to the patient, the MSCs typically migrate (or home) to the damaged tissue and exert their therapeutic effects through paracrine signaling and by promoting survival, repair and regeneration of the neighbouring cells in the damaged tissue.

Current therapies typically involve the infusion of a mixture of MSC subtypes some of which do not migrate efficiently to the tissue of interest. This necessitates the use of a high cell-dose which can lead to off-target side effects and volume-related side effects. MSCs are typically obtained from bone marrow and so it is difficult to obtain large amounts.

Summary

The inventors have surprisingly identified a new class of progenitor cells of mesodermal lineage (PMLs) having a specific marker expression pattern. Homogenous populations of the PMLs of the disclosure can be isolated from mononuclear cells (MCs), such as peripheral blood MCs. The PMLs are capable of efficiently migrating to and repairing damaged tissues.

The disclosure provides a progenitor cell of mesodermal lineage, wherein the cell (a) expresses detectable levels of CD29, CD44, CD73, CD90, CD105 and CD271 and (b) does not express detectable levels of CD14, CD34 and CD45. In one aspect, there is provided a progenitor cell of mesodermal lineage, wherein the cell (a) expresses detectable levels of CD29, CD44, CD73, CD90, CD105, CD271, CD120a (tumour-necrosis factor (TNF)-alpha Receptor 1), CD120b (TNF-alpha Receptor 2), CD50 (Intercellular Adhesion Molecule-3, ICAM-3), CD54 (ICAM-1), CD58 (Lymphocyte function-associated antigen-1, LFA-1), CD62E (E-selectin), CD62L (L-selectin), CD62P (P-selectin), CD106 (Vascular cell adhesion protein, VCAM-1), CD102 (ICAM-2), CD166 (Activated leukocyte cell adhesion molecule), CD104 (Beta 4 integrin), CD123 (Interleukin-3 Receptor), CD124 (Interleukin-4 Receptor), CD126 (Interleukin-6 Receptor), CD127 (Interleukin-7 Receptor) and fibroblast growth factor receptor (FGFR) and (b) does not express detectable levels of CD14, CD34 and CD45.

The disclosure also provides:

- a population comprising two or more progenitor cells of the disclosure;
- a pharmaceutical composition comprising (a) a progenitor cell of the disclosure or a population of the disclosure and (b) a pharmaceutically acceptable carrier or diluent;
- a method of producing a population the disclosure, comprising (a) culturing mononuclear cells (MCs) under conditions which induce the MCs to differentiate into progenitor cells of mesodermal

lineage and (b) harvesting and culturing those progenitor cells which have an expression pattern of the disclosure and thereby producing a population of the disclosure;

- a method of repairing a damaged tissue in a patient, comprising administering to the patient a population of the disclosure, wherein the population comprises a therapeutically effective number of cells, and thereby repairing the damaged tissue in the patient; and
- a population of the disclosure for use in repairing a damaged tissue in a patient.

In one aspect, there is provided a population comprising:

(a) two or more progenitor cells of mesodermal lineage of the disclosure; or

(b) at least about 5×10^5 cells of the disclosure.

In one aspect, there is provided a pharmaceutical composition comprising (a) a progenitor cell of the disclosure or a population of the disclosure and (b) a pharmaceutically acceptable carrier or diluent.

In one aspect, there is provided a method of producing a population of progenitor cells of mesodermal lineage of the disclosure, comprising (a) culturing mononuclear cells (MCs) with platelet lysate under oxygen conditions of less than 20% oxygen (O_2) and under conditions which allow the progenitor cells to adhere such that the MCs differentiate into progenitor cells of mesodermal lineage and (b) harvesting and culturing those progenitor cells which have an expression pattern as defined in the disclosure and thereby producing a population of the disclosure.

In one aspect, there is provided a population of the disclosure for use in a method of repairing a damaged tissue in a patient.

In one aspect, there is provided a population of the disclosure for use in a method of treating a cardiac injury or disease, age-related macular degeneration or a bone injury or disease in a patient in need thereof.

In one aspect, there is provided use of a population of the disclosure in the manufacture of a medicament for repairing a damaged tissue in a patient.

In one aspect, there is provided use of a population of the disclosure in the manufacture of a medicament for treating a cardiac injury or disease, age-related macular degeneration or a bone injury or disease in a patient in need thereof.

Brief Description of the Figures

Fig. 1 shows an RT-PCR gel confirming the presence of CD44 and the absence of CD34 in the PMLs of the invention.

Fig. 2 shows the results of FACS analysis on the PMLs of the invention. This confirms that the cells are positive for at least CD73 and CD90 and negative for CD14, CD34 and CD45.

Fig. 3 shows further results from FACS analysis, namely the histograms for CD90 (top) and CD73 (bottom).

Fig. 4 shows FACS histograms for lack of CD14, CD34 and CD45 in stained (Fig. 4a) and unstained (Fig. 4b) cells.

Fig. 5 shows that progenitor cells of mesodermal lineage migrate to the fracture site in a time- and CXCR4-dependent manner. Bioluminescence (BLI) was performed at days 1 (first row), 3 (second row), 7 (third row) and 14 (fourth row) after fracture/transplantation in mice with tibia fracture that received a transplant of either 106 PML-P-Act-Luc (PML) (left column), PML-fl-Act-Luc-CXCR4+ (CXCR4(+)) (middle column), or PML-P-Act-Luc-CXCR4- (CXCR4(-)) (right column).

Detailed Description of the Invention

It is to be understood that different applications of the disclosed products and methods may be tailored to the specific needs in the art. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

In addition, as used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a cell" includes "cells", reference to "a tissue" includes two or more such tissues, reference to "a patient" includes two or more such patients, and the like.

All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

CLAIMS

1. A progenitor cell of mesodermal lineage, wherein the cell (a) expresses detectable levels of CD29, CD44, CD73, CD90, CD105, CD271, CD120a (tumour-necrosis factor (TNF)-alpha Receptor 1), CD120b (TNF-alpha Receptor 2), CD50 (Intercellular Adhesion Molecule-3, ICAM-3), CD54 (ICAM-1), CD58 (Lymphocyte function-associated antigen-1, LFA-1), CD62E (E-selectin), CD62L (L-selectin), CD62P (P-selectin), CD106 (Vascular cell adhesion protein, VCAM-1), CD102 (ICAM-2), CD166 (Activated leukocyte cell adhesion molecule), CD104 (Beta 4 integrin), CD123 (Interleukin-3 Receptor), CD124 (Interleukin-4 Receptor), CD126 (Interleukin-6 Receptor), CD127 (Interleukin-7 Receptor) and fibroblast growth factor receptor (FGFR) and (b) does not express detectable levels of CD14, CD34 and CD45.

2. A progenitor cell according to claim 1, wherein:

(a) the cell is capable of migrating to a specific, damaged tissue in a patient;

(b) the cell expresses detectable levels of C-X-C chemokine receptor type 1 (CXCR1);

(c) the cell expresses detectable levels of C-X-C chemokine receptor type 2 (CXCR2); and/or

(d) the cell is capable of migrating to a specific, damaged tissue in a patient and the specific tissue is cardiac tissue, retinal tissue or bone tissue.

3. A progenitor cell according to claim 2, wherein the specific tissue is:

(a) heart tissue or bone tissue and the cell expresses detectable levels of C-X-C chemokine receptor type 4 (CXCR4);

(b) retinal tissue and the cell expresses detectable levels of CXCR4, vascular endothelial growth factor (VEGF), transforming growth factor beta 1 (TGF-beta 1), insulin-like growth factor-1 (IGF-1), fibroblast growth factor (FGF), tumour necrosis factor alpha (TNF-alpha), interferon gamma (IFN-gamma), interleukin-1 alpha (IL-1 alpha), CXCL12, CD109, CD119, nuclear factor kappa-light-chain-enhancer of activated B cells (NFkappa B), CD140a, CD140b, CD221, CD222, CD304, CD309 and CD325; or

(c) bone tissue and the cell expresses detectable levels of TGF-beta 3, bone morphogenetic protein-6 (BMP-6), SOX-9, Collagen-2, CD117 (c-kit), chemokine (C-C motif) ligand 12 (CCL12), CCL7, interleukin-8 (IL-8), platelet-derived growth factor-A (PDGF-A), PDGF-B, PDGF-C, PDGF-D, macrophage migration inhibitory factor (MIF), IGF-1, hepatocyte growth factor (HGF), PDGF-Rα,

PDGF-Rβ, CXCR4, C-C chemokine receptor type 1 (CCR1), IGF-1 receptor (IGF-1R), hepatocyte growth factor receptor (HGFR), CXCL12 and NFkappaB.

4. A progenitor cell according to any one of the preceding claims, wherein the cell:

(a) is capable of having anti-inflammatory effects in a damaged tissue in a patient;

(b) expresses detectable levels of one or more of (i) insulin-like growth factor-1 (IGF-1), (ii) IGF-1 receptor; (iii) C-C chemokine receptor type 1 (CCR1), (iv) stromal cell-derived factor-1 (SDF-1), (v) hypoxia-inducible factor-1 alpha (HIF-1 alpha), (vi) Akt1 and (vii) hepatocyte growth factor (HGF) and/or granulocyte colony-stimulating factor (G-CSF), or overexpresses one or more of (i) to (vii); and/or

(c) expresses detectable levels of one or more of (i) vascular endothelial growth factor (VEGF), (ii) transforming growth factor beta (TGF-beta), (iii) insulin-like growth factor-1 (IGF-1), (iv) fibroblast growth factor (FGF), (v) tumour necrosis factor alpha (TNF-alpha), (vi) interferon gamma (IFN-gamma) and (vii) interleukin-1 alpha (IL-1 alpha), or overexpresses one or more of (i) to (vii).

5. A progenitor cell according to any one of the preceding claims, wherein:

(a) the cell is autologous with respect to an individual into which it is to be administered; or

(b) the cell is allogeneic with respect to an individual into which it is to be administered.

6. A population comprising:

(a) two or more progenitor cells of mesodermal lineage as defined in any one of the preceding claims; or

(b) at least about 5×10^5 cells as defined in any one of the preceding claims.

7. A pharmaceutical composition comprising (a) a progenitor cell according to any one of claims 1 to 5 or a population according to claim 6 and (b) a pharmaceutically acceptable carrier or diluent.

8. A method of producing a population of progenitor cells of mesodermal lineage according to claim 6, comprising (a) culturing mononuclear cells (MCs) with platelet lysate under oxygen conditions of less than 20% oxygen (O_2) and under conditions which allow the progenitor cells to adhere such that the MCs differentiate into progenitor cells of mesodermal lineage and (b) harvesting and culturing those progenitor cells which have an expression pattern as defined in any one of claims 1, 2, 3 and 4 and thereby producing a population according to claim 6.

9. A method according to claim 8, wherein:

(a) the MCs are peripheral blood mononuclear cells (PBMCs); and/or

(b) the MCs are obtained from a patient or an allogeneic donor.

10. A population according to claim 6 for use in a method of repairing a damaged tissue in a patient.

11. A population according to claim 6 for use according to claim 10, wherein:

(a) the tissue is derived from the mesoderm;

(b) the tissue is cardiac tissue, retinal tissue or bone tissue;

(c) the method is (a) for repairing damaged cardiac tissue and the population comprises a therapeutically effective amount of cells as defined in claim 3, (b) for repairing damaged retinal tissue and the population comprises a therapeutically effective amount of cells as defined in claim 3 or (c) for repairing damaged bone tissue and the population comprises a therapeutically effective amount of cells as defined in claim 3; and/or

(d) the tissue is damaged by injury or disease.

12. A population according to claim 6 for use in a method of treating a cardiac injury or disease, age-related macular degeneration or a bone injury or disease in a patient in need thereof.

13. A population according to claim 6 for use according to claim 12, wherein:

(a) the cardiac injury or disease is selected from myocardial infarct, left ventricular hypertrophy, right ventricular hypertrophy, emboli, heart failure, congenital heart deficit, heart valve disease, arrhythmia and myocarditis; or

(b) the bone injury or disease is selected from fracture, Salter-Harris fracture, greenstick fracture, bone spur, craniosynostosis, Coffin-Lowry syndrome, fibrodysplasia ossificans progressive, fibrous dysplasia, Fong Disease (or Nail-patella syndrome), hypophosphatasia, Klippel-Feil syndrome, Metabolic Bone Disease, Nail-patella syndrome, osteoarthritis, osteitis deformans (or Paget's disease of bone), osteitis fibrosa cystica (or Osteitis fibrosa or Von Recklinghausen's disease of bone), osteitis pubis, condensing osteitis (or osteitis condensans), osteitis condensans ilii, osteochondritis dissecans, osteogenesis imperfecta, osteomalacia, osteomyelitis, osteopenia, osteopetrosis, osteoporosis, osteonecrosis, porotic hyperostosis, primary hyperparathyroidism, renal osteodystrophy, bone cancer, a

bone lesion associated with metastatic cancer, Gorham Stout disease, primary hyperparathyroidism, periodontal disease, and aseptic loosening of joint replacements.

14. A population according to claim 6 for use according to claim 12 or 13, wherein the method is (a) for treating a cardiac injury or disease and the population comprises a therapeutically effective amount of cells as defined in claim 3, (b) for treating age-related macular degeneration and the population comprises a therapeutically effective amount of cells as defined in claim 3 or (c) for treating a bone injury or disease and the population comprises a therapeutically effective amount of cells as defined in claim 3.

15. A population according to claim 6 for use according to any one of claims 10 to 14, wherein the population is produced using MCs obtained from the patient or an allogeneic donor.

16. Use of a population according to claim 6 in the manufacture of a medicament for repairing a damaged tissue in a patient.

17. The use according to claim 16, wherein:
(a) the tissue is derived from the mesoderm;
(b) the tissue is cardiac tissue, retinal tissue or bone tissue;
(c) the use is (a) for repairing damaged cardiac tissue and the population comprises a therapeutically effective amount of cells as defined in claim 3, (b) for repairing damaged retinal tissue and the population comprises a therapeutically effective amount of cells as defined in claim 3 or (c) for repairing damaged bone tissue and the population comprises a therapeutically effective amount of cells as defined in claim 3; and/or
(d) the tissue is damaged by injury or disease.

18. Use of a population according to claim 6 in the manufacture of a medicament for treating a cardiac injury or disease, age-related macular degeneration or a bone injury or disease in a patient in need thereof.

19. The use according to claim 18, wherein:

(a) the cardiac injury or disease is selected from myocardial infarct, left ventricular hypertrophy, right ventricular hypertrophy, emboli, heart failure, congenital heart deficit, heart valve disease, arrhythmia and myocarditis; or

(b) the bone injury or disease is selected from fracture, Salter-Harris fracture, greenstick fracture, bone spur, craniosynostosis, Coffin-Lowry syndrome, fibrodysplasia ossificans progressive, fibrous dysplasia, Fong Disease (or Nail-patella syndrome), hypophosphatasia, Klippel-Feil syndrome, Metabolic Bone Disease, Nail-patella syndrome, osteoarthritis, osteitis deformans (or Paget's disease of bone), osteitis fibrosa cystica (or Osteitis fibrosa or Von Recklinghausen's disease of bone), osteitis pubis, condensing osteitis (or osteitis condensans), osteitis condensans ilii, osteochondritis dissecans, osteogenesis imperfecta, osteomalacia, osteomyelitis, osteopenia, osteopetrosis, osteoporosis, osteonecrosis, porotic hyperostosis, primary hyperparathyroidism, renal osteodystrophy, bone cancer, a bone lesion associated with metastatic cancer, Gorham Stout disease, primary hyperparathyroidism, periodontal disease, and aseptic loosening of joint replacements.

20. The use according to claim 18 or 19, wherein the use is (a) for treating a cardiac injury or disease and the population comprises a therapeutically effective amount of cells as defined in claim 3, (b) for treating age-related macular degeneration and the population comprises a therapeutically effective amount of cells as defined in claim 3 or (c) for treating a bone injury or disease and the population comprises a therapeutically effective amount of cells as defined in claim 3.

21. The use according to any one of claims 16 to 20, wherein the population is produced using MCs obtained from the patient or an allogeneic donor.