SUMMARY

[0008] The present disclosure provides a methods of treating a vascular disease comprising administering to a subject a composition comprising hemogenic endothelial cells (HEs) obtained by *in vitro* differentiation of pluripotent stem cells.

[0008a] In one aspect, the present disclosure provides the use of a composition in the manufacture of a medicament for treating a vascular disease in a subject suffering from, or suspected of suffering from, a vascular disease, wherein the composition comprises hemogenic endothelial cells (HEs) obtained by in vitro differentiation of pluripotent stem cells, wherein the pluripotent stem cells comprise embryonic stem cells and/or induced pluripotent stem cells; and wherein the HE cells are positive for at least one microRNA (miRNA) selected from the group consisting of miRNA-214, miRNA-199A-3p, and miRNA-335.

[0008b] In another aspect, the present disclosure provides use of a composition in the manufacture of a medicament for increasing blood flow in a subject suffering from, or suspected of suffering from, a vascular disease, wherein the composition comprises HEs obtained by *in vitro* differentiation of pluripotent stem cells, wherein the pluripotent stem cells comprise embryonic stem cells and/or induced pluripotent stem cells; and wherein the HE cells are positive for at least one microRNA (miRNA) selected from the group consisting of miRNA-214, miRNA-199A-3p, and miRNA-335.

[0008c] In another aspect, the present disclosure provides use of a composition in the manufacture of a medicament for reducing blood pressure in a subject suffering from, or suspected of suffering from, a vascular disease, wherein the composition comprises HEs obtained by *in vitro* differentiation of pluripotent stem cells, wherein the pluripotent stem cells comprise embryonic stem cells and/or induced pluripotent stem cells; and wherein the HE cells are positive for at least one microRNA (miRNA) selected from the group consisting of miRNA-214, miRNA-199A-3p, and miRNA-335.

[0008d] In another aspect, the present disclosure provides a composition comprising HEs obtained by *in vitro* differentiation of pluripotent stem cells, wherein the pluripotent stem cells comprise embryonic stem cells and/or induced pluripotent stem cells; and wherein the HEs positive for at least one microRNA (miRNA) selected from the group consisting of miRNA-214, miRNA-199A-3p, and miRNA-335.

[0008e] In another aspect, the present disclosure provides a pharmaceutical composition comprising the HEs as disclosed herein.

[0009] In an example, the vascular disease is selected from the group consisting of coronary artery diseases (e.g., arteriosclerosis, atherosclerosis, and other diseases or injuries of the arteries, arterioles and capillaries or related complaint), myocardial infarction, (e.g. acute myocardial infarction), organizing myocardial infarct, ischemic heart disease, arrhythmia, left ventricular dilatation, emboli, heart failure, congestive heart failure, subendocardial fibrosis, left or right ventricular hypertrophy, myocarditis, chronic coronary ischemia, dilated cardiomyopathy, restenosis, arrhythmia, angina, hypertension (e.g. pulmonary hypertension, glomerular hypertension, portal hypertension), myocardial hypertrophy, peripheral arterial disease including critical limb ischemia, cerebrovascular disease, renal artery stenosis, aortic aneurysm, pulmonary heart disease, cardiac dysrhythmias, inflammatory heart disease, congenital heart disease, rheumatic heart disease, diabetic vascular diseases, and endothelial lung injury diseases (e.g., acute lung injury (ALI), acute respiratory distress syndrome (ARDS)). In a specific example, the vascular disease is pulmonary hypertension. In another example, the vascular disease is pulmonary arterial hypertension.

[0010] In an example of any of the methods disclosed herein, the mean pulmonary (artery) pressure is reduced in the subject.

[0011] The present disclosure also provides a method of increasing blood flow in pulmonary arteries comprising administering to a subject a composition comprising HEs obtained by *in vitro* differentiation of pluripotent stem cells. In an example, the subject has pulmonary hypertension. In a specific example, the subject has pulmonary arterial hypertension.

[0012] The present disclosure further provides a method of reducing blood pressure in a subject comprising administering to the subject a composition comprising HEs obtained by *in vitro* differentiation of pluripotent stem cells. In an example, the subject has pulmonary hypertension. In a specific example, the subject has pulmonary arterial hypertension. In another example, the blood pressure is diastolic pressure. In yet another example, the blood pressure is systolic pressure. In a further example, the blood pressure is mean pulmonary (artery) pressure. Moreover, the blood pressure may be reduced by at least 20% in the subject by any of the methods of the present disclosure.

CLAIMS

What is claimed is:

1. Use of a composition in the manufacture of a medicament for treating a vascular disease in a subject suffering from, or suspected of suffering from, a vascular disease, wherein the composition comprises hemogenic endothelial cells (HEs) obtained by *in vitro* differentiation of pluripotent stem cells, wherein the pluripotent stem cells comprise embryonic stem cells and/or induced pluripotent stem cells; and wherein the HE cells are positive for at least one microRNA (miRNA) selected from the group consisting of miRNA-214, miRNA-199A-3p, and miRNA-335.

2. The use of claim 1, wherein the vascular disease is selected from the group consisting of coronary artery diseases (e.g., arteriosclerosis, atherosclerosis, and other diseases or injuries of the arteries, arterioles and capillaries or related complaint), myocardial infarction, (e.g. acute myocardial infarction), organizing myocardial infarct, ischemic heart disease, arrhythmia, left ventricular dilatation, emboli, heart failure, congestive heart failure, subendocardial fibrosis, left or right ventricular hypertrophy, myocarditis, chronic coronary ischemia, dilated cardiomyopathy, restenosis, arrhythmia, angina, hypertension (eg. pulmonary hypertension, glomerular hypertension, portal hypertension), myocardial hypertrophy, peripheral arterial disease including critical limb ischemia, cerebrovascular disease, renal artery stenosis, aortic aneurysm, pulmonary heart disease, cardiac dysrhythmias, inflammatory heart disease, congenital heart disease, rheumatic heart disease, diabetic vascular diseases, endothelial lung injury diseases (e.g., acute lung injury (ALI), and acute respiratory distress syndrome (ARDS)).

3. The use of claim 1, wherein the vascular disease is pulmonary hypertension or pulmonary arterial hypertension.

4. The use of any one of claims 1-3, wherein the mean pulmonary (artery) pressure is reduced in the subject.

5. Use of a composition in the manufacture of a medicament for increasing blood flow in a subject suffering from, or suspected of suffering from, a vascular disease, wherein the

composition comprises HEs obtained by *in vitro* differentiation of pluripotent stem cells, wherein the pluripotent stem cells comprise embryonic stem cells and/or induced pluripotent stem cells; and wherein the HE cells are positive for at least one microRNA (miRNA) selected from the group consisting of miRNA-214, miRNA-199A-3p, and miRNA-335.

6. The use of claim 5, wherein the subject has pulmonary hypertension or pulmonary arterial hypertension.

7. Use of a composition in the manufacture of a medicament for reducing blood pressure in a subject suffering from, or suspected of suffering from, a vascular disease, wherein the composition comprises HEs obtained by *in vitro* differentiation of pluripotent stem cells, wherein the pluripotent stem cells comprise embryonic stem cells and/or induced pluripotent stem cells; and wherein the HE cells are positive for at least one microRNA (miRNA) selected from the group consisting of miRNA-214, miRNA-199A-3p, and miRNA-335.

8. The use of claim 7, wherein the subject has pulmonary hypertension or pulmonary arterial hypertension.

9. The use of claim 7, wherein the blood pressure is diastolic pressure, systolic pressure, and/or mean pulmonary (artery) pressure.

10. The use of any one of claims 7-9, wherein the blood pressure is reduced by at least 20% in the subject.

11. The use of any one of claims 1-10, wherein

(a) the HEs are further positive for at least one miRNA selected from the group consisting of miRNA-126, miRNA-24, miRNA-196-b, hsa-miR-11399, hsa-miR-196b-3p, hsa-miR-5690, and hsa-miR-7151-3p;

(b) the HEs are positive for miRNA-214, miRNA-199a-3p, and miRNA-335, hsa-miR-11399, hsa-miR-196b-3p, hsa-miR-5690, and hsa-miR-7151-3p;

(c) the HEs are positive for miRNA-126, miRNA-24, miRNA-196-b, miRNA-214, miRNA-199a-3p, and miRNA-335;

(d) the HEs are negative for at least one miRNA selected from the group consisting of miRNA-367, miRNA-302a, miRNA-302b, miRNA-302c, miRNA-223, and miRNA-142-3p;

(e) the HEs are negative for miRNA-223, and miRNA-142-3p;

(f) the HEs are negative for miRNA-367, miRNA-302a, miRNA-302b, miRNA-302c, miRNA-223, and miRNA-142-3p;

(g) the HEs further express at least one cell surface marker selected from the group consisting of CD24, CD73, CD31/PECAM1, CD309/KDR, CD144, CD34, CXCR4, CD146, Tie2, CD140b, CD90, CD271, and CD105;

(h) the HEs further express CD146, CXCR4, CD309/KDR, CD90, and CD271;

(i)the HEs further express CD146;

(j) the HEs further express CD144 (VECAD);

(k) the HEs further express at least one cell marker selected from the group consisting of CD24, CD31, CD309/KDR (FLK-1), PLVAP, GJA4, EGFL7, and ESAM;

(l) the HEs further express at least one cell marker selected from the group consisting of SOX9, PDGFRA, and EGFRA;

(m) the HEs further express at least one cell marker selected from the group consisting of KDR/VEGFR2, NOTCH4, collagen I, and collagen IV;

(n) the HEs further express CD31/PECAM1, CD309/KDR, CD144, CD34, and CD105;

(o) the HEs exhibit limited or no detection of at least one cell surface marker selected from the group consisting of CD34, CXCR7, CD43 and CD45;

(p) the HEs exhibit limited or no detection of CXCR7, CD43, and CD45;

(q) the HEs exhibit limited or no detection of CD43 and CD45;

(r) the HEs are CD73(+), CD43(-), CD45(-), and CD146 (+);

(s) the HEs further express (i) CD144 (VECAD) and (ii) CD73 and (iii) CD31 and/or CD309/KDR (FLK-1);

(t) the HEs further express at least one gene listed in Table 22 and Table 23; and/or

(u) the HE further express CD24.

12. The use of any one of claims 1-11, wherein the HEs are obtained (i) by culturing the pluripotent stem cells under adherent conditions in a differentiation medium in the absence of methylcellulose; and/or (ii) by in vitro differentiation of pluripotent stem cells without embryoid body formation.

13. The use of any one of claims 1-12, wherein the subject is a human.

14. A composition comprising HEs obtained by *in vitro* differentiation of pluripotent stem cells, wherein the pluripotent stem cells comprise embryonic stem cells and/or induced pluripotent stem cells; and wherein the HEs are positive for at least one microRNA (miRNA) selected from the group consisting of miRNA-214, miRNA-199A-3p, and miRNA-335.

15. The composition of claim 14, wherein

(a) the HEs are further positive for at least one miRNA selected from the group consisting of miRNA-126, mi-RNA-24, miRNA-196-b, hsa-miR-11399, hsa-miR-196b-3p, hsa-miR-5690, and hsa-miR-7151-3p;

(b) the HEs are positive for miRNA-214, miRNA-199a-3p, and miRNA-335 , hsa-miR-11399, hsa-miR-196b-3p, hsa-miR-5690, and hsa-miR-7151-3p;

(c) the HEs are positive for (i) miRNA-126, mi-RNA-24, miRNA-196-b, miRNA-214, miRNA-199a-3p, and miRNA-335;

(d) the HEs are negative for at least one miRNA selected from the group consisting of miRNA-367, miRNA-302a, miRNA-302b, miRNA-302c, miRNA-223, and miRNA-142-3p;

(e) the HEs are negative for miRNA-223, and miRNA-142-3p; and/or

(f) the HEs are negative for miRNA-367, miRNA-302a, miRNA-302b, miRNA-302c, miRNA-223, and miRNA-142-3p.

17. The composition of claim 15, wherein

(a) the HEs further express at least one cell marker selected from the group consisting of CD24, CD73, CD31, CD309/KDR (FLK-1), PLVAP, GJA4, EGFL7, and ESAM;

(b) the HEs further express at least one cell marker selected from the group consisting of SOX9, PDGFRA, and EGFRA;

(c) the HEs further express at least one cell marker selected from the group consisting of KDR/VEGFR2, NOTCH4, collagen I, and collagen IV; and/or

(d) the composition substantially lacks CD73-negative and CD144 (VECAD)-negative HE cells; and/or

(e) the HEs further express (i) CD144 (VECAD) and (ii) CD73 and (iii) CD31 and/or CD309/KDR (FLK-1);

(f) the HEs further express at least one gene listed in Table 22 and Table 23; and/or

(g) the HE further express CD24.

18. A pharmaceutical composition comprising the HEs of any one of claims 14-17.

19. The use of any one of claims 1-12, wherein the HEs are obtained by *in vitro* differentiation of pluripotent stem cells in the absence of TGFβ inhibitors.