Methods of Producing Haemogenic Endothelial Cells from Pluripotent Stem Cells

Field

This invention relates to the production of haemogenic endothelial cells (HECs), for example for use in generating T cells for immunotherapy.

Background

Immunotherapeutics are poised to transform the cancer treatment landscape with the promise of long-term survival (McDermott et al., Cancer Treat Rev. 2014 Oct; 40(9): 1056-64). There is a clear unmet medical need for new immunomodulatory drugs to expand patient population and range of tumor types. In addition, new agents are needed to enhance the magnitude and duration of anti-tumor responses. The development of these agents has been possible because of the in-depth understanding of the basic principles controlling T-cell immunity over the last two decades (Sharma and Allison, Cell. 2015 Apr 9; 161(2): 205-14). This typically requires tumor specific CD4+ and CD8+ T-cells recognising tumor-associated peptide antigens presented by MHC molecules. Different vaccination strategies and adoptive transfer of ex vivo expanded tumor infiltrated lymphocytes have in some cases demonstrated the ability of tumor specific T-cells to treat late-stage cancer (Rosenberg et al., Nat Med. 2004 Sep; 10(9): 909-15).

However, current adoptive T cell therapies are limited by a lack of suitable patient and tumor-specific T cells and there is a need for therapeutically sufficient and functional antigen-specific T cells for effective use in immunotherapy.

Summary

The present inventors have developed a process for the differentiation of induced pluripotent stem cells (iPSCs) into haemogenic endothelial cells that offers rapid and reproducible differentiation with high yields. This may be useful for example in the production of clinical grade blood cells, such as T cells, for use in immunotherapy.

In one aspect, the present disclosure provides a method of producing a population of haemogenic endothelial cells (HECs) comprising;

(a) differentiating a population of induced pluripotent stem cells (iPSCs) into mesoderm cells by culturing the iPSCs sequentially in first, second and third mesoderm induction media to induce differentiation into mesoderm cells; wherein

the first mesoderm induction medium stimulates SMAD1, SMAD5 and SMAD9 mediated signalling pathways;

the second mesoderm induction medium (i) stimulates SMAD1, SMAD5 and SMAD9 mediated signalling pathways and (ii) has fibroblast growth factor (FGF) activity; and

the third mesoderm induction medium (i) stimulates SMAD1, SMAD2, SMAD3, SMAD5 and SMAD9 mediated signalling pathways (ii) has fibroblast growth factor (FGF) activity and (iii) inhibits glycogen synthase kinase 3β; and;

(b) differentiating the mesoderm cells into HECs by culturing the mesoderm cells sequentially in first and second HE induction media to induce differentiation into HECs; wherein;

the first HE induction medium (i) stimulates VEGFR mediated signalling pathways and (ii) has fibroblast growth factor (FGF) activity; and

the second HE induction medium (i) stimulates cKIT receptor (CD117; KIT receptor tyrosine kinase) mediated signalling pathways, (ii) stimulates VEGFR mediated signalling pathways; and (iii) has fibroblast growth factor (FGF) activity.

A first example of the disclosure provides a method of producing a population of haemogenic endothelial cells (HECs) comprising;

(a) differentiating a population of induced pluripotent stem cells (iPSCs) into mesoderm cells by culturing the iPSCs sequentially in first, second and third mesoderm induction media to induce differentiation into mesoderm cells; and;

(b) differentiating the mesoderm cells into HECs by culturing the mesoderm cells sequentially in first and second haemogenic endothelium (HE) induction media to induce differentiation into HECs.

The first mesoderm induction medium stimulates SMAD1, SMAD5 and SMAD9 mediated signalling pathways. For example, the first mesoderm induction medium may comprise BMP4. The second mesoderm induction medium (i) stimulates SMAD1, SMAD5 and SMAD9 mediated signalling pathways and (ii) has fibroblast growth factor (FGF) activity. For example, the second mesoderm induction medium may comprise BMP4 and bFGF. The third mesoderm induction medium (i) stimulates SMAD1, SMAD2,

SMAD3, SMAD5 and SMAD9 mediated signalling pathways (ii) has fibroblast growth factor (FGF) activity and (iii) inhibits glycogen synthase kinase 3β. For example, the third mesoderm induction medium may comprise BMP4, bFGF, activin, and CHIR-99021.

The first HE induction medium (i) stimulates VEGFR mediated signalling pathways and (ii) has fibroblast growth factor (FGF) activity. For example, the first HE induction medium may comprise VEGF and bFGF. The second HE induction medium (i) stimulates cKIT receptor (CD117; KIT receptor tyrosine kinase) mediated signalling pathways, (ii) stimulates VEGFR mediated signalling pathways; and (iii) has fibroblast growth factor (FGF) activity. For example, the second HE induction medium may comprise SCF, VEGF and bFGF.

Steps (a) and (b) may be performed without purification or isolation of cells in the population.

A method of the first example may further comprise;

(c) differentiating the HECs into HPCs by culturing the population of HECs under suitable conditions to promote haematopoietic differentiation.

Steps (a), (b) and (c) above may be performed in defined culture media in the absence of feeder cells, stromal cells, serum or other undefined media supplements.

In some preferred examples, HPCs produced as described herein may be used in the production of T cells.

A method of the first example may further comprise;

(iv) differentiating the population of HPCs into T cell progenitor cells; and

(v) maturing the progenitor T cells to produce a population of DP CD4+CD8+ T cells.

A method of the first example may further comprise;

(vi) activating and expanding the DP CD4+CD8+ T cells to produce a population of SP CD8+ T cells or a population of SP CD4+ T cells.

In other examples, HPCs as described herein may be used in the production of NK cells. A method of the first example may further comprise;

(iv) differentiating the population of HPCs into NK cells.

In other examples, HPCs produced as described herein may be used in the production of B cells. A method of the first example may further comprise;

(iv) differentiating the population of HPCs into B cells.

These and other aspects and examples of the disclosure are described in more detail below.

Claims

1. A method of producing a population of haemogenic endothelial cells (HECs) comprising;

(a) differentiating a population of induced pluripotent stem cells (iPSCs) into mesoderm cells by culturing the iPSCs sequentially in first, second and third mesoderm induction media to induce differentiation into mesoderm cells; wherein

the first mesoderm induction medium stimulates SMAD1, SMAD5 and SMAD9 mediated signalling pathways;

the second mesoderm induction medium (i) stimulates SMAD1, SMAD5 and SMAD9 mediated signalling pathways and (ii) has fibroblast growth factor (FGF) activity; and

the third mesoderm induction medium (i) stimulates SMAD1, SMAD2, SMAD3, SMAD5 and SMAD9 mediated signalling pathways (ii) has fibroblast growth factor (FGF) activity and (iii) inhibits glycogen synthase kinase 3β; and;

(b) differentiating the mesoderm cells into HECs by culturing the mesoderm cells sequentially in first and second HE induction media to induce differentiation into HECs; wherein;

the first HE induction medium (i) stimulates VEGFR mediated signalling pathways and (ii) has fibroblast growth factor (FGF) activity; and

the second HE induction medium (i) stimulates cKIT receptor (CD117; KIT receptor tyrosine kinase) mediated signalling pathways, (ii) stimulates VEGFR mediated signalling pathways; and (iii) has fibroblast growth factor (FGF) activity.

2. A method according to claim 1 wherein the first mesoderm induction medium comprises BMP.

3. A method according to any one of the preceding claims wherein the second mesoderm induction medium comprises BMP and FGF.

4. A method according to any one of the preceding claims wherein the third mesoderm induction medium comprises activin, BMP, FGF, and a glycogen synthase kinase 3β inhibitor.

5. A method according to any one of the preceding claims wherein the first HE induction medium comprises FGF and VEGF.

6. A method according to any one of the preceding claims wherein the second HE induction medium comprises SCF, FGF and VEGF.

7. A method according to any one of the preceding claims further comprising

(c) differentiating the HECs into HPCs by culturing the population of HECs in a haematopoietic induction medium to induce differentiation into HPCs,

wherein the haematopoietic induction medium (i) stimulates cKIT receptor (CD117) or cKIT receptor (CD117) mediated signalling pathways and/or (ii) stimulates VEGFR or VEGFR mediated signalling pathways.

8. A method according to claim 7 wherein the haematopoietic induction medium comprises one or both of SCF and VEGF.

9. A method according to claim 8 wherein the haematopoietic induction medium comprises
(i) VEGF, SCF, Thrombopoietin (TPO), Flt3 ligand (Flt3L), IGF-1, IL-3 and IL-6; or
(ii) VEGF, SCF, Thrombopoietin (TPO), Flt3 ligand (Flt3L), IGF-1, IL-3, IL-6, and IL-7.

10. A method according to claim 8 or 9 wherein the haematopoietic induction medium is devoid of one or more of BMP, FGF, SHH, EPO, angiotensin II and losartan.

11. A method according to any one of claims 7-10 further comprising;
(d) differentiating the population of HPCs into progenitor T cells by a method comprising culturing the population of HPCs in a lymphoid expansion medium to produce the progenitor T cells.

12. A method according to claim 11 further comprising maturing the progenitor T cells to produce a population of double positive CD8+ CD4+ T cells by a method comprising culturing the population of progenitor T cells in a T cell maturation medium to produce the double positive CD8+ CD4+ T cells.

13. A method according to claim 12 comprising activating and expanding the double positive CD8+ CD4+ T cells to produce a population of T cells having a CD8+ single positive phenotype or a CD4+ single positive phenotype.

14. A method according to claim 13 wherein the T cells specifically bind to cancer cells expressing a tumour antigen.

15. A method according to any one of the preceding claims wherein the iPSCs are derived from T cells obtained from a donor individual.

16. A method according to claim 15 wherein the T cells obtained from the donor individual are specific for the target antigen, optionally wherein the T cells obtained from the donor individual are tumour-infiltrating lymphocytes (TILs).

17. A method according to any one of claims 1 to 16 wherein the method further comprises introducing heterologous nucleic acid encoding an antigen receptor into the iPSCs, HECs, HPCs or progenitor T cells.

18. A method according to claim 17 wherein the antigen receptor is a TCR, optionally an affinity enhanced TCR or a non-MHC restricted TCR.

19. A method according to claim 18 wherein the TCR binds specifically to an MHC displaying a peptide fragment of a tumour antigen expressed by the cancer cells or binds specifically to a tumour antigen or peptide fragment thereof expressed by cancer cells independently of MHC presentation.

20. A method according to claim 17 wherein the antigen receptor is a chimeric antigen receptor (CAR) or NKCR, optionally wherein the CAR or NKCR binds specifically to a target antigen expressed by cells.