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 ~~aspect~~example of the ~~invention~~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 ~~aspect~~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 ~~embodiment~~examples, HPCs produced as described herein may be used in the production of T cells.

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

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

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

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

These and other aspects and example~~embodiment~~s of the ~~invention~~ 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 steps (i) and (ii) are performed in the absence of stromal cells or serum.~~

~~3. A method according to any one of claims 1 to 2 wherein the iPSCs are differentiated into mesoderm cells by culturing the population of iPSCs under suitable conditions to promote mesodermal differentiation.~~

2~~4~~. A method according to claim 1 ~~any one of the preceding claims~~ wherein the first mesoderm induction medium comprises BMP.

~~5. A method according to claim 4 wherein the first mesoderm induction medium consists of a chemically defined nutrient medium supplemented with BMP.~~

~~6. A method according to any one of the preceding claims wherein the iPSCs are cultured in the first mesoderm induction medium for 1 day.~~

3~~7~~. A method according to any one of the preceding claims wherein the second mesoderm induction medium comprises BMP and FGF.

~~8. A method according to claim 7 wherein the second mesoderm induction medium consists of a chemically defined nutrient medium supplemented with BMP and FGF~~

~~9. A method according to any one of the preceding claims wherein the iPSCs are cultured in the second mesoderm induction medium for 1 day.~~

4~~10~~. 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.

~~11. A method according to claim 10 wherein the third mesoderm induction medium consists of a chemically defined nutrient medium supplemented with activin, BMP, FGF, and a GSK3 inhibitor.~~

~~12. A method according to any one of the preceding claims wherein the iPSCs are cultured in the third mesoderm induction medium for 2 days.~~

~~13. A method according to any one of the preceding claims wherein the iPSCs are cultured in the first second and third mesoderm induction medium for 4 days to produce the mesoderm cells.~~

~~14. A method according to any one of the preceding claims wherein the mesoderm cells express one or more mesoderm markers selected from Brachyury, Goosecoid, Mixl1, KDR, FoxA2, GATA6, and PDGFαR.~~

~~15. A method according to any one of the preceding claims wherein mesoderm cells in the population are not purified following culture in the first, second and third mesoderm induction media.~~

~~16. A method according to any one of the preceding claims wherein the mesoderm cells are differentiated into HECs by culturing the population of mesoderm cells under suitable conditions to promote haemogenic endothelial (HE) differentiation.~~

1~~7~~5. A method according to any one of the preceding claims wherein the first HE induction medium comprises FGF and VEGF.

~~18. A method according to claim 17 wherein the first HE induction medium consists of a chemically defined nutrient medium supplemented with FGF and VEGF.~~

1~~9~~ 6. A method according to any one of the preceding claims wherein the second HE induction medium comprises SCF, FGF and VEGF.

~~20. A method according to claim 19 wherein the second HE induction medium consists of a chemically defined nutrient medium supplemented with SCF, FGF and VEGF.~~

~~21. A method according to any one of the preceding claims wherein the FGF is FGF2 (bFGF).~~

~~22. A method according to any one of the preceding claims wherein the BMP is BMP4.~~

~~23. A method according to any one of the preceding claims wherein activin is activin A.~~

~~24. A method according to any one of the preceding claims wherein the GSK3 inhibitor is CHIR-99021.~~

~~25. A method according to any one of the preceding claims wherein the HECs display a CD34+ phenotype.~~

~~26. A method according to claim 25 wherein the HECs display a CD34+ CD73- CXCR4- phenotype.~~

~~27. A method according to any one of the preceding claims wherein HECs in the population are not purified following culture in the HE induction medium.~~

~~28. A method according to any one of the preceding claims wherein steps (i) and (ii) are performed without purification or isolation of cells in the population.~~

~~29~~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 ~~under suitable conditions to promote haematopoietic differentiation.~~

~~30. A method according to claim 29 wherein the HECs are cultured~~ in a haematopoietic induction medium to induce differentiation into HPCs~~.~~

~~31. A method according to claim 30,~~

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.

~~32~~8. A method according to claim ~~31~~7 wherein the haematopoietic induction medium comprises one or both of SCF and VEGF.

~~33. A method according to claim 32 wherein the haematopoietic induction medium comprises SCF and VEGF.~~

~~34. A method according to any one of claims 31 to 33 wherein the haematopoietic induction medium (iii) stimulates MPL (CD110) or MPL (CD110) mediated signalling pathways; (iv) stimulates *FLT3* or *FLT3* mediated signalling pathways (v) stimulates *IGF1R* or *IGF1R* mediated signalling pathways and (vi) displays interleukin (IL) activity.~~

~~35~~9. A method according to claim ~~34~~8 wherein the haematopoietic induction medium comprises (i) VEGF, SCF, Thrombopoietin (TPO), Flt3 ligand (Flt3L), IGF-1, IL-3 and IL-6; or ~~.~~

(ii)

~~36. A method according to claim 34 wherein the haematopoietic induction medium comprises~~ VEGF, SCF, Thrombopoietin (TPO), Flt3 ligand (Flt3L), IGF-1, IL-3, IL-6, and IL-7.

10~~37~~. A method according to ~~any one of claims 31 to 36~~claim 8 or 9 wherein the haematopoietic induction medium is devoid of one or more of BMP, FGF, SHH, EPO, angiotensin II and losartan.

~~38. A method according to any one of claims 31 to 33 wherein the haematopoietic induction medium consists of a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein the one or more differentiation factors consist of SCF and/or VEGF.~~

~~39. A method according to any one of claims 31 to 37 wherein the haematopoietic induction medium consists of a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein (i) the one or more differentiation factors consist of VEGF, SCF, Thrombopoietin (TPO), Flt3 ligand (Flt3L), IGF-1, IL-3 and IL-6; or (ii) the one or more differentiation factors consist of VEGF, SCF, Thrombopoietin (TPO), Flt3 ligand (Flt3L), IGF-1, IL-3, IL-6, and IL-7.~~

~~40. A method according to claim 39 wherein the differentiation factors in the haematopoietic induction medium are (i) VEGF, SCF, Thrombopoietin (TPO), Flt3 ligand (Flt3L), IGF-1, IL-3, IL-6, and IL-7; or (ii) VEGF, SCF, Thrombopoietin (TPO), Flt3 ligand (Flt3L), IGF-1, IL-3, and IL-6.~~

~~41. A method according to any one of claims 29 to 40 wherein the HPCs display a CD34+ phenotype.~~

~~42. A method according to claim 41 wherein the HPCs display a CD34+ CD45+ phenotype.~~

~~43. A method according to any one of claims 29 to 42 wherein the HPCs comprise thymopoietic HPCs (tHPCs).~~

~~44. A method according to claim 43 wherein the tHPCs display a CD34+ CD7+phenotype~~

~~45. A method according to any one of claims 29 to 44 wherein the HECs are cultured in the haematopoietic induction medium for 16-28 days to produce the HPCs.~~

~~46. A method according to any one of claims 29 to 45 comprising purifying the population of HPCs.~~

~~47~~11. A method according to any one of claims 7-10~~the preceding claims~~ further comprising;

(d) differentiating the population of HPCs into progenitor T cells~~.~~

~~48. A method according to claim 47 wherein the HPCs are differentiated~~ by a method comprising culturing the population of HPCs in a lymphoid expansion medium to produce the progenitor T cells.

~~49. A method according to claim 47 or claim 48 wherein the progenitor T cells have a CD5+ CD7+ phenotype.~~

~~50~~12. A method according to claim 11 ~~any one of claims 47 to 49~~ further comprising maturing the progenitor T cells to produce a population of double positive CD8+ CD4+ T cells~~.~~

~~51. A method according to claim 50 wherein the progenitor T cells are matured~~ 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.

~~52~~13. A method according to claim ~~50 or 51~~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.

~~53. A method according to any one of claims 50 to 52 wherein the T cells specifically bind to cells expressing a target antigen.~~

~~54. A method according to claim 53 wherein the target antigen is a tumour antigen.~~

14~~55~~. A method according to claim 13~~54~~ wherein the T cells specifically bind to cancer cells expressing ~~the~~ a tumour antigen.

~~51~~6~~5~~. A method according to any one of the preceding claims wherein the iPSCs are derived from T cells obtained from a donor individual.~~.~~

~~57~~16. A method according to claim 15~~6~~ wherein the T cells obtained from the donor individual are specific for the target antigen, ~~.~~

~~58. A method according to claim 56 or 57~~ optionally wherein the T cells obtained from the donor individual are tumour-infiltrating lymphocytes (TILs).

~~59~~17. A method according to any one of claims 1 to ~~58~~16 wherein the method further comprises introducing heterologous nucleic acid encoding an antigen receptor into the iPSCs, HECs, HPCs or progenitor T cells.

~~60. A method according to claim 59 wherein the heterologous nucleic acid encoding the antigen receptor is comprised in an expression vector.~~

~~61. A method according to claim 60 wherein the expression vector is a lentiviral vector or adeno-associated viral (AAV) vector.~~

~~62. A method according to claim 61 wherein the heterologous nucleic acid is incorporated into the genome of the iPSCs, HECs, haemogenic progenitor cells, or progenitor T cells using a gene editing system.~~

~~63. A method according to claim 62 wherein the gene editing system is CRISPR/Cas9 or AAV.~~

~~64~~18. A method according to ~~any one of claims 59 to 63~~claim 17 wherein the antigen receptor is a TCR, optionally ~~.~~

~~65. A method according to claim 64 wherein the TCR is TCR is~~ an affinity enhanced TCR or ~~.~~

~~66. A method according to claim 64 wherein the TCR is TCR is~~ a non-MHC restricted TCR.

~~67. A method according to one of claims 64 to 66 wherein the TCR binds specifically to an MHC displaying a peptide fragment of a target antigen expressed by cells or specifically binds to a target antigen or peptide thereof expressed by cells independently of MHC presentation.~~

~~68~~19. A method according to claim 1~~67~~8 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.

~~69~~20. A method according to ~~any one of claims 64 to 68~~claim 17 wherein the antigen receptor is a chimeric antigen receptor (CAR) or NKCR, ~~.~~

~~70. A method according to claim 69~~optionally wherein the CAR or NKCR binds specifically to a target antigen expressed by cells.

~~71. A method according to claim 70 wherein the CAR or NKCR binds specifically to an MHC displaying a peptide fragment of a tumour antigen expressed by cancer cells.~~