only promote HE development, but also on methods to identify these progenitors as they are specified, in an earlier study, we used T cell potential to map the onset of definitive hematopoiesis in mouse ESC differentiation cultures, and demonstrated that this program initiates from a Flk-1$^+$ *Sox17*$^+$ progenitor that emerged 48 hours following the onset of primitive hematopoiesis (Irion et al., 2010). Several studies have demonstrated that it is possible to generate T lymphocytes from hESCs (Galic et al., 2006; Timmermans et al., 2009).

SUMMARY OF THE INVENTION

There is described herein, use of T-cell potential to map the onset of the definitive hematopoietic program from hESCs and hiPSCs induced with specific morphogens in serum-free cultures.

In an example, a candidate definitive hematopoietic progenitor population was identified that emerges between days 6 and 9 of culture, expresses surface markers and genes indicative of HE/definitive hematopoietic progenitors, and displays T-cell potential following culture on OP9-DL4 stromal cells. In addition to T-cell progenitors, this population also gives rise to erythroid and myeloid progenitors following co-culture with stromal cells. The characteristics of this population suggest that it may represent the *in vitro* equivalent of the human P-Sp-derived definitive hematopoietic program and as such, progenitors of human HSCs.

In an aspect, there is provided a method of enriching a population of stem cells for hematopoietic progenitors, the method comprising: inducing hematopoietic differentiation in a population of human embryonic stem cells or human induced pluripotent stem cells wherein the inducing hematopoietic differentiation comprises culturing the population with BMP4, from about day 0 to about day 4 of differentiation, and with bFGF, from about day 1 up to about day 8 of differentiation; sorting the population based on expression of CD43 and ~~at least one of~~ CD34, ~~CD31 and CD144~~; and selecting a fraction that is ~~at least one of~~ CD34$^+$CD43$^-$, ~~CD31$^+$CD43$^-$ and CD144$^+$CD43~~, wherein inducing hematopoietic differentiation is conducted serum free wherein the inducing hematopoietic differentiation comprises inhibiting activin/nodal signaling within a period of time from about day 2 to about day 4 of differentiation; and wherein the sorting and cell fraction selection is performed at a time selected from about day 6 to about day 11 of differentiation.

Also disclosed is a population of hematopoietic progenitors obtained using the method described herein.

Also disclosed is a method of enriching a population of stem cells for hematopoietic progenitors, comprising inhibiting activin/nodal signaling during hematopoietic differentiation. In an embodiment, the inhibiting of activin/nodal signaling

CLAIMS

1. A method of enriching a population of stem cells for hematopoietic progenitors, the method comprising:

    a. inducing hematopoietic differentiation in a population of human embryonic stem cells or human induced pluripotent stem cells;

    b. sorting the population based on expression of CD43 and at least one of CD34, CD31 and CD144; and

    c. selecting a fraction that is at least one of $CD34^{+}CD43^{-}$, $CD31^{+}CD43^{-}$ and $CD144^{+}CD43^{-}$,

    wherein inducing hematopoietic differentiation is conducted serum free.

2. The method of claims 1, wherein inducing hematopoietic differentiation is conducted stroma free.

3. The method of any one of claims 1 or 2, wherein the inducing hematopoietic differentiation comprises culturing the population with BMP4, between days 0 and 4.

4. The method of any one of claims 1, 2 and 3, wherein the inducing hematopoietic differentiation comprises culturing the population with bFGF, between days 1 and 8.

5. The method of any one of claims 1-4, wherein the inducing hematopoietic differentiation comprises culturing the population with at least one of VEGF, DKK, IL-6 and IL-11, and/or combinations thereof, between days 3 and 13, or between days 4 and 9.

6. The method of any one of claims 1-5, wherein the inducing hematopoietic differentiation comprises culturing the population with at least one of SCF and EPO, between days 4 and 13, or between days 6 and 9.

7. The method of any one of claims 1-6, wherein the inducing hematopoietic differentiation comprises culturing the population with at least one of TPO, Flt-3 and IL-3, and/or combinations thereof, between days 6 and 13, or between days 8 and 9.

8. The method of any one of claims 1-7, wherein the inducing hematopoietic differentiation comprises culturing the population with up to 1 ng/ml Activin A, preferably about 0.3 ng/ml, and further preferably about 0 ng/ml.

9. The method of any one of claims 1-7, wherein the inducing hematopoietic differentiation comprises inhibiting activin/nodal signaling.

10. The method of claim 9, wherein inhibiting activin/nodal signaling comprises culturing the population with an activin/nodal inhibitor.

11. The method of claim 10, wherein the inhibitor is SB-431542.

12. The method of any one of claims 10-11, wherein the inhibitor is added to the culture of cells between days 1.5 and 5, or between days 2 and 4 of differentiation or about day 1.75 of differentiation.

13. The method of any one of claims 1-12, wherein the sorting is performed between about day 6 and about day 13, or between about day 7 and about day 10 of differentiation.

14. The method of any one of claims 1-13, wherein the population is sorted based on expression of CD43 and CD34 and the selected fraction is $CD34^{+}CD43^{-}$.

15. The method of any one of claims 1-13, wherein the population is sorted based on expression of CD43 and CD31 and the selected fraction is $CD31^{+}CD43^{-}$.

16. The method of any one of claims 1-13, wherein the population is sorted based on expression of CD43 and CD144 and the selected fraction is $CD144^{+}CD43^{-}$.

17. The method of any one of claims 1-16, wherein the fraction comprises at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% hematopoietic progenitors.

CLAIMS

1. A method of enriching a population of stem cells for hematopoietic progenitors, the method comprising:

    a. inducing hematopoietic differentiation in a population of human embryonic stem cells or human induced pluripotent stem cells, wherein the inducing hematopoietic differentiation comprises culturing the population with BMP4, from about day 0 to about day 4 of differentiation, and with bFGF, from about day 1 up to about day 8 of differentiation;

    b. sorting the population based on expression of CD43 and CD34; and

    c. selecting a fraction that is $CD34^{+}CD43^{-}$,

    wherein inducing hematopoietic differentiation is conducted serum free

    wherein the inducing hematopoietic differentiation comprises inhibiting activin/nodal signaling within a period of time from about day 2 to about day 4 of differentiation; and

    wherein the sorting and cell fraction selection is performed at a time selected from about day 6 to about day 11 of differentiation.

2. The method of claims 1, wherein inducing hematopoietic differentiation is conducted stroma free.

3. The method of any one of claims 1-2, wherein the inducing hematopoietic differentiation comprises culturing the population with at least one of VEGF, DKK, IL-6 and IL-11, and/or combinations thereof, from about day 3 to about day 11, or from about day 4 to about day 9 of differentiation.

4. The method of any one of claims 1-3, wherein the inducing hematopoietic differentiation comprises culturing the population with at least one of SCF and EPO, from about day 4 to about day 1, or from about day 6 to about day 9 of differentiation.

5. The method of any one of claims 1-4, wherein the inducing hematopoietic differentiation comprises culturing the population with at least one of TPO, Flt-3 and IL-3, and/or combinations thereof, from about day 6 to about day 11, or to about day 8 to about day 9 of differentiation.

6. The method of claim 1, wherein inhibiting activin/nodal signaling comprises culturing the population with an activin/nodal inhibitor.

7. The method of claim 6, wherein the inhibitor is SB-431542.

8. The method of any one of claims 1-7, wherein the sorting is performed at a time selected from about day 7 to about day 10 of differentiation.

9. The method of any one of claims 1-8, wherein the fraction comprises at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% hematopoietic progenitors.