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

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 one aspect, the present disclosure refers to 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 at least one of CD31 and CD144; and c. selecting a fraction that is CD31^{+}CD43^{-}, CD144^{+}CD43^{-}, or CD31^{+}CD43^{-} and CD144^{+}CD43^{-} wherein inducing hematopoietic differentiation is conducted serum free, 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 the sorting and cell fraction selection is performed at a time selected from about day 6 to about day 11 of differentiation.

In another aspect, the present disclosure refers to a population of hematopoietic progenitors obtained using the method as disclosed herein.

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 example, 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; 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 $CD144^{+}CD43^{-}$.

In a further example, there is provided a population of hematopoietic progenitors obtained using the method described herein.

In a further example, there is provided a method of enriching a population of stem cells for hematopoietic progenitors, comprising inhibiting activin/nodal signaling during hematopoietic differentiation. In an example, the inhibiting of activin/nodal signaling

comprises culturing the population with an activin/nodal inhibitor, preferably SB-431542.

In a further example, there is provided a use of an activin/nodal inhibitor for enriching a population of stem cells, undergoing hematopoietic differentiation, for hematopoietic progenitors. In an example, the activin/nodal inhibitor is SB-431542.

BRIEF DESCRIPTION OF FIGURES

These and other features of the preferred embodiments of the invention will become more apparent in the following detailed description in which reference is made to the appended drawings wherein:

Figure 1 shows hematopoietic induction of hESCs with Activin A and BMP4. (A) Differentiation scheme used for hematopoietic induction of human pluripotent stem cells. EBs were generated during the first 24 hours of culture in the presence of BMP-4, subsequently cultured in the presence of BMP-4 and bFGF for the next 24 hours and then in the presence of BMP4, bFGF and Activin A for the following 48 hours (days 2-4). For some experiments SB-431542 (SB) was added in place of Activin A. At day 4, BMP4 and Activin A (or SB) were removed and replaced with VEGF, IL-6, IL-11, IGF-1, SCF, EPO, TPO, Flt-3, IL-3 and DKK1 as indicated. (B) Frequency of hematopoietic progenitors detected in EBs over time. Ery: erythroid colonies; Ery/Myeloid: colonies consisting erythrocytes and low numbers of myeloid cells; and Myeloid: colonies consisting of either macrophages or mast cells. (C) Effect of Activin A stimulation or inhibition on hematopoietic progenitor development in day 13 EBs. (D) Flow cytometric analyses of CD34, CD43, CD41 and CD45 expression on the indicated days in EBs treated with 0.3 ng/ml Activin A plus the cytokines shown in (A).

Figure 2 shows primitive hematopoietic potential of day 9 CD34/CD43 populations. (A) Flow cytometric analyses showing the CD34 and CD43 fractions isolated from day 9 EBs; P1: 10%±3%, P2: 1.5%±0.7%, P3: 12.5%±-4%, P4: 21%±8% and P5: 35%±9% (±SD, n=5) (B) Erythroid and myeloid progenitor potential of the CD34/CD43 fractions (C) Flow cytometric analyses showing expression of CD41a, CD235a and CD45 on the fractions indicated in (A). (D) Hematopoietic potential of day 6 $CD34^{+}CD43^{-}$ and $CD34^{+}CD43^{+}$ populations. Day 6 sort (upper panels): Flow cytometric analyses

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 at least one of CD31 and CD144; and

    c. selecting a fraction that is $CD31^+CD43^-$, $CD144^+CD43^-$, or $CD31^+CD43^-$ and $CD144^+CD43^-$

wherein

inducing hematopoietic differentiation is conducted serum free,

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

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 claim 1 or 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 11, 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 S8-431542.

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 population is sorted based on expression of CD43 and CD31 and the selected fraction is $CD31^{+}CD43^{-}$.

10. The method of any one of claims 1-8, wherein the population is sorted based on expression of CD43 and CD144 and the selected fraction is $CD144^{+}CD43^{-}$.

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

12. A population of hematopoietic progenitors obtained using the method of any one of claims 1-11.