Direct differentiation of hiPSCs towards Erythroid cells under Hypoxia condition without EB formation and Co-culture system

Almuraikhi N, Fauzi I, Mantalaris A

Current protocols used in the differentiation of human induced pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs) toward erythropoiesis utilize two main approaches; Embryoid Body (EB) formation, which influences heterogeneity of the produced population, and/or co-culture with mouse stromal cells, where obstacles of purification of the cells rise which makes the xeno-free culture requirement difficult to achieve, in addition to the cytokines supplements. Moreover, these protocols reported low efficiency in number and functionality, especially with hiPSCs, and required long time in culture.In this study, we designed an experiment on the differentiation of hiPSCs toward erythroid cells under hypoxia condition bypassing the EB formation step and no co-culture system was required. Hypoxia condition is a key enhancer for erythropoiesis and the activation of transferrin receptor and iron uptake. Our protocol involve three steps: 1) Hematopoietic induction, which starts with low serum condition (3-5%) with the addition of BMP4, VEGF, bFGF, to trigger the mesodermal formation, in addition to standard hematopoietic cytokines cocktails. i.e. Epo, SCF, FLT3, TPO, IL3, and IL6; followed by 2) erythroid differentiation by supplementing the cells with reduced number of cytokines; EPO, SCF and IL-3; and the final differentiation step 3) maturation of erythroid progenitors by exposing cells to EPO only.Early 7 days of culture showed an expression of an early hematopoietic marker, CD34 (19%) followed by a high expression of CD45 (88%) by day 14, which is a pan leukocyte marker in parallel to less expression of an early erythroid marker, CD71 (20%). Over the culture period, an increase in the expression of a late erythroid marker, CD235a was monitored that reached (55%) by the end of our 4-weeks culture protocol.Further studies on functional and morphological analysis using CFU assay and Wright-Giemsa staining on day 7 and day 14 showed that the cell population on day 14 were able to form hematopoietic colonies with relatively high formation of erythroid progenitor colonies, i.e. BFU-Es as compared to day 7. Positive expression of 3,3'-diaminobenzidine (DAB) staining showed the presence of heme-containing proteins in 4-weeks cells, which later confirmed by detection of relatively high haemoglobin expression from immunostaining. Interestingly, staining with new methylene blue confirmed the reticulocytes morphology which indicates a partial maturation process has successfully achieved within 4-week of culture. Further studies on maturation and enucleation of those cells are required in order to achieve fully mature and functional RBCs phenotype. Herein, we presented a direct and straight forward differentiation protocol toward erythoid cells using hiPSCs in feeder-free system, bypassing EB-stage resulting on high efficiency of erythroid cells formation within 4 weeks of culture, which include partial maturation under hypoxia condition.