A Novel System to enhance the Production of Erythroid Cells from Human Induced Pluripotent Stem Cells using HIF-1 α

Almuraikhi N, Panoskaltsis N, Mantalaris A

Blood shortage is one of the major global concerns as there is a significant imbalance in donations comparing to transfusion demands, which encouraged researchers to develop appropriate substitutes for donated blood using pluripotent stem cells. There has been a rising excitement lately that human induced pluripotent stem cells (iPS cells) could provide patient-specific cells for cellular therapy in addition to their differentiation capability into any cell type, which can be exploited in erythroid cell production. Erythropoiesis is the process of making erythrocytes that can be enhanced by hypoxia-inducible factors 1-alpha (HIF-1α), a transcription factor known to facilitates cellular adaptation to hypoxia by over-expressing specific genes and stimulating many metabolic processes, including erythropoiesis, and angiogenesis.

In this study, we have established a novel protocol to generate erythroid cells from human iPS cells using HIF-1α as a key enhancer. Beside the use of the standard cytokine cocktail used for erythroid induction; Epo, SCF, FLT3, TPO, IL3, and IL6, other growth factors were used; BMP4, VEGF, FGF, in addition to 5% serum. Supplementing the cells in 2D culture system with our novel optimized concentrations of the said cocktail under hypoxic condition showed the highest yield erythroid markers, mainly CD235a.

Further maturation of those cells is required in order to achieve fully mature and functional RBCs phenotype. These results must be supported by the detection of Rh type and ABO grouping to ensure the presence of RBCs antigens. Eventually, after optimizing an enucleation protocol, globin detection and O2 equilibrium curve must be also made to ensure functionality of the hemoglobin (incomplete work).

Thus, considering all the above, the ultimate aim of this study is the efficient production of mature and functional RBCs *in vitro* from patient-specific iPS cells using HIF-1**α** to facilitate erythroid progenitor maturation and proliferation.