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Erythropoiesis

Production of erythroid cells from human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPS)

Production de cellules érythroïdes à partir de cellules souches humaines pluripotentes (hESC) et de cellules souches humaines pluripotentes induites (hiPS)

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Abstract

Erythroid progenitors can be generated *ex vivo* from human embryonic stem cells (hESC) or human induced pluripotent stem cells (hiPS). Development of laboratory scale culture conditions capable of generating mature functional erythrocytes from human embryonic stem cells or human induced pluripotent stem cells would open the possibility for manufacture of therapeutic quantities of red cells and thereby new clinical transfusion products. Current attempts to produce erythrocytes from human embryonic stem cells reveal the need for greater understanding of the process whereby primitive erythropoiesis switches to definitive fetal and adult erythropoiesis and the factors driving erythrocyte maturation. Studies with human embryonic stem cells have already yielded encouraging results but functional mature biconcave erythrocytes have yet to be generated from these cells.

Keywords: Erythrocytes; hESC; hiPS; Erythropoiesis

Résumé

Les progéniteurs érythroïdes peuvent être générés ex vivo à partir de cellules souches humaines embryonnaires (hESC) ou de cellules souches humaines pluripotentes induites (hiPS). Le développement de conditions de culture à l'échelle du laboratoire pouvant produire des globules rouges matures fonctionnels à partir de ces cellules souches (hESC ou hiPS) pourrait ouvrir la voie à une production à grande échelle de quantités nécessaires à un usage thérapeutique et, de ce fait, de nouveaux produits transfusionnels. De tels essais de production de globules rouges à partir de cellules souches nécessitent une meilleure compréhension, d'une part, des processus par lesquels l'érythropoïèse primitive évolue vers une érythropoïèse définitive fœtale et adulte, et, d'autre part, des facteurs nécessaires à la maturation érythrocytaire. Les études sur les cellules souches humaines embryonnaires ont déjà donné des résultats encourageants mais des globules rouges mature fonctionnels présentant une forme discoïde biconcave n'ont pas encore pu être obtenus à partir de ces cellules.

Mots clés : Érythrocytes ; hESC ; hiPS ; Érythropoïèse

1. Introduction

The laboratory scale generation of erythroid cells from human haematopoietic progenitors in peripheral blood and umbilical cord blood is routinely undertaken in many laboratories and considerable progress has been made in scaling up this process to produce therapeutic quantities of mature human erythrocytes [1,2]. Recently, there has been great interest in the possibility of generating erythroid cells from human embryonic stem cells (hESC) and induced pluripotent stem cells (hiPS [3,4]). There are two main reasons for this interest. Firstly, hES and iPS cells provide the possibility of maintaining pluripotent stem cells in culture and the potential for generating large quantities of haematopoietic progenitor cells *in vitro*. Secondly, since mature red cells lack nuclei and

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represent a well-characterized existing therapeutic product, they provide an attractive first generation stem cell product free of concerns of tumorgenicity, which accompany nucleated stem cell products.

However, the production of therapeutic quantities of red cells in culture requires the generation of large numbers of mature functional erythrocytes (2×10^{12} in a conventional therapeutic unit). Success in this endeavour will require greater understanding of erythropoiesis, particularly the processes effecting transition from primitive erythropoiesis in the yolk sac to definitive erythropoiesis in the adult bone marrow and the development of large-scale culture facilities and procedures allowing manufacture under GMP at economically acceptable cost.

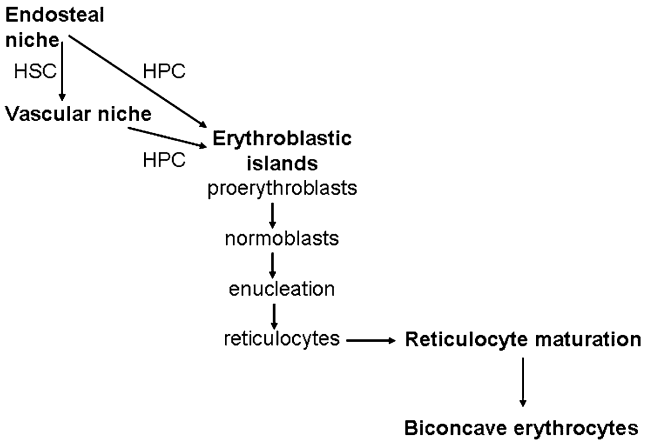

Fig. 1. Steps in the production of mature erythrocytes in bone marrow.

2. Erythropoiesis in bone marrow

Haematopoietic stem cells in bone marrow have the dual capability of self-renewal and differentiation into committed blood cell progenitors. These properties are thought to depend upon specific microenvironments which house stem cells in marrow, the co-called stem-cell niches. The number of different niches is presently unclear. Wilson and Trumpp distinguish endosteal and vascular bone marrow niches [5]. Cells involved in bone formation, most notably osteoblasts, support stem cells in endosteal niches through interactions between Notch receptors on the stem cells and Jagged 1 on osteoblasts [6]. Vascular bone marrow niches are located in the centre of the bone marrow and involve stem cell interaction with bone marrow sinusoidal endothelial cells (stromal cells). Wilson and Trumpp suggest dormant haematopoietic stem cells are primarily located in endosteal niches and that there is migration of stem cells from endosteal niches to vascular niches which contain self-renewing stem cells located close to sinusoids allowing rapid response to maintain homeostasis by effecting differentiation of haematopoietic stem cells to haematopoietic precursor cells (HPC). Following differentiation of HPC along the erythroid lineage, maturation from proerythroblast to immature reticulocytes takes place in association with bone marrow macrophages in erythroblastic islands [7]. Interaction with macrophages facilitates a three-fold proliferation of proerythroblasts and further maturation to normoblasts culminating in enucleation. Following enucleation, the immature reticulocyte undergoes extensive membrane remodelling to form a flexible, deformable and functional biconcave disc suitable for peripheral circulation. Successful replication of normal erythropoiesis *in vitro* would therefore contain the following stages: stem cell renewal, generation of HPC, conversion of HPC to proerythroblasts, maturation of erythroid cells, enucleation and reticulocyte remodeling to form functional biconcave discs (Fig. 1).

Mature erythrocytes have been produced in the laboratory using CD34+ cells isolated from umbilical cord blood [1,2]. Giarratana et al. used a three-stage system in which CD34+ cells were incubated in the presence of cytokines SCF, IL3 and EPO, human transferrin and a source of iron for 8 or more days before coculture on a stromal cell layer with EPO for 3 days, followed by coculture on stromal cells without cytokines for a further 10 days. Ninety to 100% of cells were enucleated by day 18. Fujimi et al. describe a four-stage system. In this system, CD34+ cells are cultured on a stromal monolayer for 14 days in the presence of SCF, TPO and Flt3 ligand, followed by culture to day 28 in the presence of SCF, IL3, EPO and human transferrin. Macrophages derived from a parallel culture of cord CD34+ cells were added on day 29 with EPO and human transferrin, the erythroid cells were isolated on day 34 by leukofiltration and enucleation/maturation effected by culture of the erythroid cells in the presence of mannitol and adenine from day 35–38. These authors calculate that 5–8 therapeutic units of mature red cells are attainable from one cord blood donation. These studies establish the feasibility of producing mature fetal erythrocytes from cord blood *in vitro* but clearly there is scope for increasing the efficiency of this process by more closely replicating the natural process particularly by mimicking the three dimensional nature of bone marrow niches. Blanco et al. report that cord blood mononuclear cells expand 300-fold in the absence of added growth factors in niche-like structures formed in synthetic scaffolds made of polyurethane coated with collagen [8]. The same group have developed a 3D hollow fibre bioreactor designed to mimic the microenvironment and sinusoidal structure of bone marrow [9]. Despite the clear potential for manufacture of clinical red cell products from cord blood progenitors, the demand for red cells for transfusion is such that a source of stem cells capable of indefinite expansion is desirable to generate cultured red cells for therapy in large quantities. It is for this reason that several groups are now focussed on the production of red cells from hESC and hiPS.

3. Generating erythroid cells from embryonic stem cells

Erythroid cells in the mammalian embryo are derived from mesodermal cells which are involved in the formation of yolk sac and placenta. Hemangioblast precursors derived from mesoderm generate primitive erythroid progenitors which mature and may enucleate in the blood stream and definitive erythroid progenitors which colonize fetal liver, expand in