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Development of *in vitro* erythroblast cultures for transfusion purposes

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Introduction: The demand for Red Blood Cell (RBC) transfusion is continuously increasing while the supply is not always sufficient. As there is no appropriate alternative to RBC transfusion, the *in vitro* manufacture of RBCs is a potential means to ensure an adequate and safe supply of blood products. Our previous studies developed an erythropoiesis model to culture human erythroblasts *in vitro* using media supplemented with Erythropoietin (Epo), Stem Cell Factor (SCF) and Dexamethasone (Dex) to allow survival, proliferation and self renewal capacity.

Aim: To enhance erythroblast growth capacity *in vitro* by constitutive activation of SCF and Epo signaling and investigating the cooperative mechanisms with Dex resulting in induced self renewal potential.

Methodology: Haematopoietic progenitors were isolated using Magnetic cell sorting. The CD34+ fraction was cultured in specific media and characterised by flow cytometry. The CD34- fraction was also kept in culture for erythroblast commitment and proliferation. The growth curves were calculated using the CASY cell counter and analyser. Cells were factor deprived for 4 hours and stimulated for 2 hours with Epo, SCF, Dex alone or selected combinations. Cells were harvested and the nuclear cell lysates isolated. The activation of the transcription factors NFkB, AP-1, CREB, NFAT and GR were investigated using Luminex technology. In addition, an electroporation protocol was optimised for expression studies. Currently Jak2 and cKit coding sequences are being amplified from cDNA samples.

Results: The erythroblast culture originating from the CD34- fraction was improved by a co-culture of a stromal cell layer. This co-culture showed enhanced proliferation and retained a mean erythroblast diameter of 9.5µm. In the absence of the stromal layer an erythroblast diameter of 8.5µm was obtained, with continuous cell purification. The transcription factor mulitplex profile of cultured erythroblasts showed a specific transcription activation upon the addition of Epo and Dex inducing CREB and NFAT activity. The CD34+ cell culture retained an immature cell morphology and the capacity to differentiate into erythroblasts upon culturing in a selective media (ESD).

Conclusion: The identification of the co-culture system merits further investigation. Studying the mechanism of self renewal of erythroblasts and expression of Jak2 and cKit will assess the possibility of factor-independent growth of the blasts. The outcome could provide key insights into mass production of erythrocytes in culture which would eventually allow additional research for production of transfusion-compatible erythrocyte units.

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