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## Staying hydrated is important also for erythroblasts

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*doi:10.3324/haematol.2019.233999*

In this edition of *Haematologica*, Caulier and colleagues provide new insights into the role of PIEZO1, a mechanosensitive ion channel, in regulating normal human erythropoiesis.[1] Defects in PIEZO1 have also been shown to lead to disordered erythropoiesis in hereditary xerocytosis, an inherited red cell disorder leading to red cell dehydration.[2,3] Using *in vitro* cellular models of human erythropoiesis, the authors documented that the chemical activation of PIEZO1 either in an erythroid cell line model or in normal human hematopoietic stem cells (HSC) repressed erythroid differentiation. Importantly, they further documented that there was delayed erythroid differentiation in HSC from patients with PIEZO1 mutations. These findings provide unexpected and novel insights into the role of ion channels in the regulation of human erythropoiesis.[1]

Anemia is a significant health problem that affects nearly two billion people around the world. The major causes of anemia are: (i) an increased rate of destruction of circulating red cells in disorders that include red cell membrane disorders, sickle cell disease, immune hemolytic anemia, nutritional anemias and malaria; (ii) acute blood loss or splenic sequestration; and (iii) decreased production of red cells in the bone marrow due to ineffective erythropoiesis,which includes thalassemias, inherited bone marrow failure syndromes, infiltrative processes such as myelodysplastic syndrome and acute myeloid leukemia and suppression of erythropoiesis due to infection and medications. While significant progress has been made over the years to improve our understanding of the contribution of increased red cell destruction to anemia, much less is known about the extent of the effect of ineffective erythropoiesis and its contribution to anemia in the various red cell disorders. This is particularly true in the case of inherited red blood cell membrane disorders. The lack of progress has been due in part to a lack of an adequate and easily implementable methodology to study the complex process of human erythroid differentiation.

The generation of enucleated circulating human red cells is a complex biological process that begins in the bone marrow with the commitment of pluripotent HSC to the erythroid lineage (Figure 1). Subsequent stages of maturation include erythroid progenitors, burst-forming unit–erythroid and colony-forming unit–erythroid (CFU-E), which can be identified by their development into representative clonal colonies of red cells *in vitro*. The CFU-E then undergoes terminal differentiation, progressing through four to five morphological stages, each having characteristic light microscopic and ultrastructural features. During terminal erythroid differentiation there is an increasing amount of hemoglobin synthesis accompanied by nuclear chromatin condensation and in the final stage of differentiation there is nuclear extrusion to generate an anucleate reticulocyte which over 2 to 3 days matures, first in the marrow and then in the circulation, into the discoid erythrocyte.

Significant progress has been made during the last decade in developing culture systems to study the differentiation of human CD34 cells intto enucleate reticulocytes and using various cell surface markers to monitor the progression through all stages of erythroid differentiation.[4-7] These developments are enabling detailed characterization of normal and disordered human erythropoiesis.[8-13] Importantly, as a result of this progress it is now possible to obtain insights into at what stage of the complex process of erythroid differentiation various genes contribute to ineffective erythropoiesis.

Using these *in vitro* cellular models of human erythropoiesis, the study by Caulier and colleagues documented that the chemical activation of PIEZO1 either in an ery-

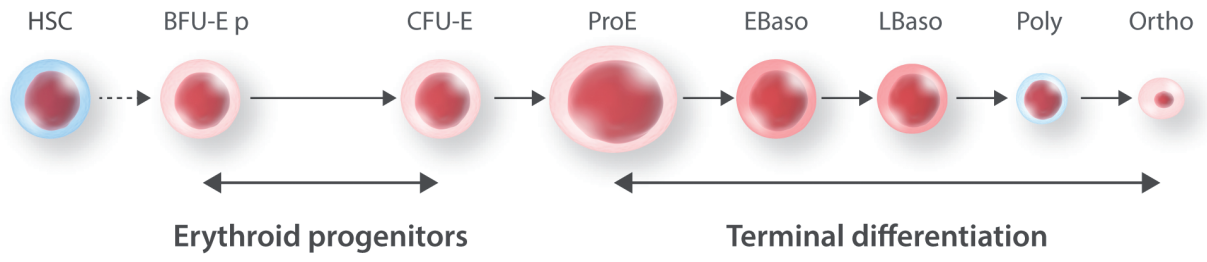


**Figure 1. Schematic representation of the various developmental stages of hematopoietic stem cells during erythroid differentiation.** The multipotent hematopoietic stem cell first commits to the erythroid lineage to generate erythroid progenitors, which are recognized by their ability to form erythroid colonies in a semisolid methylcellulose culture system in response to interleukin-3, stem cell factor and erythropoietin. They cannot be distinguished based on their morphology. It is estimated that hematopoietic stem cells undergo approximately eight to ten cell divisions prior to the generation of the first morphologically recognizable erythroid cell in the bone marrow, the proerythroblast. During terminal erythroid differentiation, the proerythroblast undergoes five mitoses to generate an orthochromatic erythroblast. This ordered progression during normal erythropoiesis may be disturbed at any of the different developmental stages in various pathological states, leading to ineffective erythropoiesis. HSC: hematopoietic stem cell; BFU-E: burst-forming unit–erythroid; CFU-E: colony-forming unit–erythroid; ProE: proerythroblast; EBaso: early basophilic erythroblast; LBaso: late basophilic erythroblast; Poly: polychromatic erythroblast; Ortho: orthochromatic erythroblast.

throid cell line model or in primary normal HSC repressed erythroid differentiation. Importantly, the authors also showed that there was delayed erythroid differentiation of HSC from patients carrying PIEZO1 mutations. Delayed erythroid differentiation due to PIEZO1 activation was shown to be dependent on calcium entry and transcriptional control through the phosphorylation of transcription factors NFAT, STAT5 and ERK1/2.

These findings provide unexpected and novel insights into the role of ion channels in regulating human erythropoiesis. Although the reported findings represent an important step in our understanding of the role of PIEZO1 in regulating human erythropoiesis and ineffective erythropoiesis in hereditary xerocytosis, a number of questions remain unanswered. The variability in the delayed erythoid differentiation among different patients has not been defined. Furthermore, while ineffective erythropoiesis has been documented to be a feature of terminally differentiating erythroblasts, it is less clear at what specific stage of terminal erythroid differentiation apoptosis dominates. There is also no information about whether ineffective erythropoiesis is a feature of erythroid progenitors. It is anticipated that these important issues will be pursued in future studies.

In spite of some of these unanswered questions, the studies by Caulier and colleagues are significant in that they provide new and previously unsuspected insights into the role of ion channels in regulating human erythropoiesis. These valuable insights expand our current understanding of the role not only of growth factors and cytokines but also of ion channels in human erythroid differentiation. It is likely that the experimental strategies used in the study will be useful in furthering our understanding of the regulation of human erythropoiesis in general and the contribution of ineffective erythropoiesis to anemia in various human red cell disorders.

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