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Erythropoiesis in vertebrates: from ontogeny to clinical relevance

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1. ABSTRACT

Erythropoiesis is a complex process that starts in the course of embryo formation and it is maintained throughout the life of an organism. During the fetal development, erythropoiesis arises from different body sites and erythroblast maturation occurs in the fetal liver. After birth, erythropoiesis and erythroblast maturation take place exclusively in the bone marrow, generating a lifetime reservoir of red blood cells (RBCs), which are responsible for transporting oxygen through the bloodstream to tissues and organs. Several transcription factors and cytokines, such as GATA-1, GATA-2, FOG-1 and erythropoietin (EPO), constitute an elaborated molecular network that regulates erythropoiesis as they are involved in the differentiation and maturation of RBCs. The profound understanding of erythropoiesis is fundamental to avoid, treat or even soften the effects of erythropoietic clinical disorders and may be useful to improve patients' well-being.

2. INTRODUCTION

Erythropoiesis is a process by which hematopoietic stem cells (HSCs) proliferate and differentiate to produce mature red blood cells (RBCs). Because erythroid lineages are extensively studied at cellular and molecular levels, the knowledge on their phenotypic markers, regulating transcriptions factors and related erythroid diseases is well consolidated. Nevertheless, deeply understanding this complex process, especially in humans, may shed light on basic cellular biology and on the pathophysiology of various diseases including bone marrow failure, degenerative diseases and cancers (1).

3. ERYTHROPOIESIS

3.1. Fetal erythropoiesis

Erythropoiesis in the intrauterine stage is essential to support embryo survival, growth and development to fetus (2,3). During the intrauterine stage of development, erythropoiesis comprises three transient hematopoietic waves (2,3). The first erythropoietic wave takes place entirely in the yolk sac blood islands, generating all primitive erythrocytes-myeloid progenitors (3). At this stage, primitive erythroid cells emerge and remain predominantly in the bloodstream until a second wave arises, which produces definitive erythroid cells (4). In the course of the second erythropoietic wave, the maturation of erythroid precursors occurs in the bloodstream and in the liver, decreasing the number of precursors and increasing the presence of definitive erythroid cells in the blood (5,6). This erythropoietic wave in mice may correspond to the first hepatic colonization of erythroid progenitors in humans (7). During the third wave, HSCs emerge from the main arteries between the embryonic days 7.5-11.5 (E7.5-E11.5), residing in the aorta-gonad-mesonephros of the embryo, yolk sac and placenta, migrate to the fetal liver (E14.5 murine/day 23-27 humans), proliferate and accumulate in the bone marrow (E18.5 murine/10-11 weeks humans) (8,9). Concomitantly, burst forming unit-erythroid (BFU-E) arises from HSCs in the fetal liver originating mature erythroblasts (10,11). Towards the end of gestation, erythropoiesis moves from the liver to the bone marrow (4).

Mammal intrauterine erythropoiesis consists of primitive and definitive phases, both taking place in different sites of the embryo and fetus. Whereas primitive erythropoiesis occurs in the early formation

of the embryo, close to E7.5 in mice and E17 in humans, definitive erythropoiesis begins during late fetal development, around E14 in mice and 7-8 weeks in humans (3,4). Primitive and definitive erythropoiesis involve cell trafficking through erythroid progenitors, erythroblast precursors, and RBCs compartments (3).

Erythroid progenitors originate from hemangioblasts (E7.5) and comprise two morphologically and developmentally distinct and temporally overlapping populations of erythroid lineages - primitive and definitive erythroid cells - which were firstly described more than a century ago (12-16). Primitive progenitors are nucleated cells that differentiate from mesodermal cells present in the yolk sac of the mammalian embryo during its early postimplantation development soon after the onset of gastrulation (E6.5) (3,4,17). They express adhesion molecules on their surface, so they are able to form colonies of maturing erythroid cells *in vitro* and may be found associated with endothelial cells *in vivo* (2,18). Definitive yolk sac erythropoiesis in mice and humans produces great amount of erythroid progenitors (E9.5) (3,7,19,20). The limited knowledge regarding primitive erythropoiesis in humans is mainly due to ethical issues concerning the isolation of embryonic stem cells and physical inaccessibility to early embryos. Nevertheless, it is suggested that this process happens in human embryos similarly to what occurs in mice (21). In human embryos, nucleated primitive erythroid cells appear in the fetal circulation during the first trimester (3). Primitive erythroblasts are the only circulating erythroid cells from 3 to 6 weeks of gestation. For instance, BFU-E are detected within 4-5 weeks gestation (22), after the second wave of colonization of the fetal liver by HSCs (4). Among many biological functions of erythrocyte progenitors during embryo growth and development, oxygen delivery, scavenge reactive oxygen species and vascular remodeling into mature blood vessels can be highlighted (23,24).

As the embryo develops, its vascular network, yolk sac and placenta links to each other, connecting the circulatory system and initiating the heart beating. The large size of erythroblasts precursors may create shear forces that correlate with the embryo vascular remodeling (7,23). Prior to the second wave of erythropoiesis, erythroid progenitors produced in the yolk sac loss adhesion molecules and secrete metalloproteases, facilitating their entry into the bloodstream (25). Active circulation starts at E9 (26).

In primitive erythropoiesis, progenitor cells differentiate into erythroblast precursors in the bloodstream (E9.5-E17.5) (24,27), although signals that induce this phenomenon remain unclear (26,28). Throughout definitive erythropoiesis, progenitor cells migrate to the fetal liver (E9.5) and accumulate into blood islands, which contain macrophages surrounded by differentiating progenitors and newly formed precursor cells at various stages of maturation (E9.5-E12.5) (29). The intimate contact of macrophages with erythroblasts allows phagocytosis and destruction of the expelled nuclei from erythroid precursors by macrophages. Besides that, macrophages promote erythroblast proliferation under stress conditions, as it happens in hypoxia (30-32). In this way, macrophage activity in the blood islands is essential for erythroblast maturation and proliferation (33). Therefore, the fetal liver provides a suitable microenvironment for the expansion and differentiation of definitive erythroid cells (25,33). This organ also acts as a niche for the expansion and differentiation of definitive HSCs, generating definitive erythroid cells that differentiate through a hierarchy of progenitors (5,6).

Erythroblast precursors (proerythroblasts, basophilic, polychromatophilic and orthochromatic erythroblasts) are nucleated cells found and matured either in the bloodstream (primitive erythropoiesis) or liver (definitive erythropoiesis). These cells differ from each other according to morphological changes that occur during their development. Losses of nucleoli (E9.5-10.5), nuclei condensation (E10.5), cytoskeleton remodeling accompanied by a decrease in cell size (E10.5-E11.5), enucleation of orthochromatic erythroblasts (E12.5), intracellular hemoglobin (Hb) accumulation and organelle clearance are reflected by changes in cytoplasmic staining of these cells as they mature (24,34-37). Similarly to what happens to mice, human erythroblasts enucleate *in vivo* as they interact with macrophages within the placenta (38). Enucleation results in formation of pyrenocytes and reticulocytes and may favor primitive RBCs to acquire a more hydrodynamic shape that is useful for their transit through the bloodstream. At least, reticulocytes will mature into RBCs (37,39-42).

During RBCs maturation, their progenitor loses surface area and cytoplasmic volume (E14.5-17.5) (42). Circulating reticulocytes and mature RBCs composes the third erythropoietic compartment. The production and release of reticulocytes into the bloodstream is responsible for the maintenance of RBC levels in the blood during homeostasis (43). Definitive erythropoiesis begins in the yolk sac of mice and human embryos, originating the first circulating definitive RBCs that emerge from the liver (E11.5-12.5). Later in the fetal development, this initial transient phase is replaced by a life-lasting erythropoietic system that uses HSCs as a cell source for erythropoiesis (44). Erythroid-myeloid progenitors definitive erythropoiesis acts as a developmental transition between primitive and HSC-derived erythropoiesis (5). In mammals, definitive RBCs circulate as smaller and anucleated cells through fetal and postnatal life.

Transcriptional regulators play an important role in erythropoiesis as they drive lineage-specific cellular development and maturation. Primitive and definitive erythroid cells have particular requirements for transcriptional and soluble factors, expressing endless gene signatures according to their stage of maturation (24,25,45). Therefore, gene expression studies are extremely important as they enrich the knowledge on the biology of primitive and definitive erythropoiesis. Many authors have investigated erythroid cells at transcriptional, posttranscriptional, and epigenetic levels (44,46-51). A transcriptional roadmap for primitive erythropoiesis can be found online at Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE24127). Besides that, gene expression profile in morphologically comparable states of primitive and definitive erythroid precursors is also available online (http://www.cbil.upenn.edu/ErythronDB).

3.2. Adult erythropoiesis

Erythropoiesis is tightly regulated by cytokines, such as erythropoietin (EPO), transcriptions factors, such as GATA-1, GATA-2, FOG-1, and micronutrients, such as iron and vitamin B12 (52,53). During early stages of erythropoiesis, HSCs give rise to common myeloid progenitors, megakaryocyte-erythrocyte progenitors, colony forming unit-erythroid (CFU-E) cells and BFU-E (54). During late stages (also referred as *terminal erythroid differentiation*), morphologically recognizable proerythroblasts undergo mitosis to produce basophilic, polychromatic, and orthochromatic erythroblasts. Orthochromatic erythroblasts expel their nuclei to generate reticulocytes. Finally, reticulocytes mature in the bone marrow, loosing their intracellular organelles, such as mitochondria (55,56) and ribosomes, and undergo extensive membrane remodeling, generating RBCs that enter the bloodstream (29,54).

BFU-E and CFU-E cells have been traditionally defined by colony assays (57, 58). BFU-E is characterized by $CD45^{+}GPA^{-}IL\text{-}3R^{-}CD34^{+}CD36^{-}CD71^{low}$ phenotype and CFU-E is characterized by $CD45^{+}GPA^{-}IL\text{-}3R^{-}CD34^{-}CD36^{+}CD71^{high}$ phenotype. Whereas the BFU-E colony-forming ability of $CD45^{+}GPA^{-}IL\text{-}3R^{-}CD34^{+}CD36^{-}CD71^{low}$ cells requires stem cells factor (SCF) and EPO, the CFU-E colony-forming ability of $CD45^{+}GPA^{-}IL\text{-}3R^{-}CD34^{-}CD36^{+}CD71^{high}$ cells requires only EPO, demonstrating specific requirements related to the development of RBCs. Techniques that ensure the isolation of highly pure human BFU-E and CFU-E progenitors are extremely useful to study disordered erythropoiesis in inherited and acquired hematological diseases (54).

BFU-E-derived colonies require 14 days in human systems to form mature colonies that typically contain more than a thousand erythroid cells. In contrast, CFU-E progenitors require only 7 days to form mature colonies consisted of only 16–32 cells. Thus, CFU-E are only 4–5 cell divisions upstream of mature RBCs (3).

Lately, researchers have been focused on elucidating how erythroid differentiation is regulated. Recent discoveries have clarified that lineage-specific transcription factor networks are essential for proper differentiation of erythroid cells, being GATA-1 and GATA-2 important transcription factors involved in the regulation of erythroid gene expression (52).

GATA transcription factor family is composed of six members in mammals, showing a highly conserved expression pattern in vertebrates. GATA-1, GATA-2 and GATA-3 are classified into the hematopoietic GATA subfamily based on their expression profiles and domain structures (59,60). Several advanced experimental approaches have further revealed the importance of GATA-1 in adult hematopoiesis, especially for erythropoiesis. Friend of GATA (FOG-1), as the name implies, is a very close interacting partner of GATA-1 (51). It is a large nine zinc finger-containing protein, which does not bind directly to DNA. Four of the FOG-1 zinc fingers contribute to its interactions with GATA-1 (61). FOG-1 is highly expressed in erythroid cells and megakaryocytes, mirroring expression of GATA-1 in these lineages. The phenotype of the FOG-1 gene knockout is very similar to that of the GATA-1 knockout as it results in mid embryonic lethality due to severe anemia (62).

FOG-1 is an essential coregulator of GATA-1 during hematopoiesis, mediating both transcriptional activation and repression (62,63). The importance of FOG-1 is underscored by the finding that FOG-1–deficient mice die from anemia with defects resembling loss of GATA-1 (62-64).

In contrast, GATA-2 is necessary for maintaining storage pools of hematopoietic stem and progenitor cells. Both, GATA-1 and GATA-2 expression is dynamically changed during erythroid differentiation and have particularly functions, which remain to be clarified (52). Further *in vitro* and *in vivo* studies focusing on transcription factors may provide valuable understanding regarding molecular mechanisms that regulate erythropoiesis.

3.3. Hemoglobin, role of erythropoietin in erythropoiesis and the iron metabolism

Hb is the main oxygen and carbon dioxide carrier present in erythroid cells and is responsible for oxygen delivery to body tissues (65).

During gestation, a sequential activation of genes responsible for the production of globin chains occurs, leading to the formation of different types of embryonic and fetal Hb that differ from each other according to their constitutive chains (66). These newly synthesized chains are capable of binding to heme and iron, forming the Hb molecule.

Embryonic Hb is found exclusively during the first 10 to 12 weeks of gestation and is synthesized by primitive erythroblasts in hematopoietic centers of the mesenteric onfalo-channel (67). Two ζ (zeta) and two ε (epsilon) chains, two α (alpha) and two ε chains, and two ζ chains and two γ (gamma) chains respectively form Gower 1 Hb, Gower 2 Hb and Portland Hb (68). Embryonic Hb has a high affinity to oxygen, hampering the release of oxygen at tissue level (69). During the third trimester of pregnancy, the production of ε chains decreases as β (beta) chains gradually replace them, resulting in the production of fetal Hb (HbF) (70). This phenomenon facilitates the release of oxygen to tissues in a more efficient manner than embryonic Hb (71,72).

In healthy newborns, two thirds of Hb are type A (Adult Hb) and one third belongs to the HbF (73). Adult Hb is a tetramer composed of both alpha and beta like polypeptide subunits ($\alpha_2\beta_2$), called HbA. A normal variant of HbA (HbA2) consisting of two alpha and two delta chains ($\alpha_2\delta_2$) can be found at low levels in normal human blood. The composition of these subunits varies over the course of ontogeny, leading to assembly of Hb molecules with different physiologic properties. In humans, two developmental switches take place for the production of the β-like subunits of the hemoglobin molecule. This switch is known as the primitive to definitive Hb switch at the β-globin locus (45). Kruppel-like factor 1 (KLF1) and B-cell CLL/lymphoma 11A (BCL11A) are two transcription factors with key roles in the developmental regulation of Hb expression. In particular, KLF1 activates β-globin and BCL11A, which in turn represses γ-globin expression (74).

The constant blood Hb concentration is maintained by EPO. Synergistically to EPO signaling effects, Stem cell Factor (SCF) signaling pathway also plays an essential role in erythroid cell development. SCF receptor is a member of the type III subfamily of receptor tyrosine kinase, which also includes the receptor for macrophage colony-stimulating factor (M-CSF) and platelet-derived growth factor (PDGF) (75). Similarly, interleukin-3 (IL-3) can also interact with EPO to stimulate more immature erythroid progenitors (76). EPO is a 30.4 kD acidic glycoprotein with 165 amino acids that acts as a hormone, cytokine and growth factor (77,78). It affects not only erythroid cells, but also other blood cell lines, such as myeloid cells, lymphocytes and megakaryocytes. Thus, EPO is able to enhance phagocytes function of polymorph nuclear cells and reduce the activation of macrophages, modulating the inflammatory process (79).

EPO is primarily expressed by hepatocytes during the fetal stage (78). It is an important cytokine that is required for the growth, survival and differentiation of red blood progenitor cells, exerting its physiological role by binding to its specific cell surface receptor (EpoR), which is predominantly found on erythroid precursor cells (80,81). In this way, EPO plays a central role in the regulation of erythropoiesis by blocking apoptosis in erythroid progenitors and enhancing proliferation and differentiation of their progenies. Additionally, it has been reported that EPO contributes to the expansion of multipotent hematopoietic progenitors (82). Mice lacking EPO or EpoR are embryonic lethal *in utero* due to severe anemia, suggesting EPO primary role in the regulation of red cell production (81).

After birth, EPO is mainly produced by peritubular interstitial fibroblasts in the renal cortex (83-85), although its mRNA may be detectable in the liver, spleen, bone marrow, lung and brain (86). Hepatic EPO is produced by hepatocytes and contributes about 10% of the plasma EPO (87). The secretion of EPO into the circulation and its plasma concentrations (6-32 UI/L) are determined by the transcription rate of the EPO gene (in chromosome 7), which plays a key role in controlling cellular oxygen concentration (78,88, 89). In this way, plasmatic EPO is inversely related to the oxygen content in the blood.

Hypoxia is the main regulator of EPO levels (90). Although several transcription factors are involved, hypoxia inducible factors (HIFs) are key regulators of the hypoxic response pathway. The three members of the HIF transcription factor family are known as HIF-1, -2 and -3. HIF-1a was first identified as a mediator of EPO induction in response to hypoxia *in vitro*. However, HIF-2 has now been identified as the primary transcription factor that controls EPO synthesis. All HIF transcription factors interact with HIF-1b to regulate the genes involved in erythropoiesis, including the Epo gene, as well as in iron metabolism, which is essential for tissue oxygen delivery (91-93). The HIFs are inactivated in normoxia by enzymatic hydroxylation of their α-subunits (94-96). Acute exposure of mammals - including humans - to hypoxia conditions, such as those found at high altitudes, enhances plasma EPO in more than 10-fold (90). Although the ratio of oxygen delivery to oxygen requirements is the primary physiologic regulator of EPO production, anabolic and androgenic steroids also can stimulate EPO production by an unknown mechanism (97-100).

HIFs play a central role in control of both iron metabolism and erythropoiesis. Interestingly, hepatocyte-derived HIF-2 is also involved in the regulation of iron metabolism genes, supporting a role for HIF-2 in the coordination of EPO synthesis with iron homeostasis (92).

Erythropoietic tissue is the major body consumer of iron, which is essential for erythropoiesis especially for heme and Hb synthesis by maturing erythroblasts (101,102). Most of the iron in the body is in Hb of red cells, which contain about 1 mg of iron per mL of erythrocytes. In contrast, transferrin, the plasma iron carrier that is the exclusive source of iron for erythropoiesis, carries 2–3 mg of iron in all blood plasma (103,104).

Approximately 20%-30% of body iron is stored within polymers of ferritin in hepatocytes and in macrophages (105). Erythropoiesis requires approximately 30 mg of iron per day, mainly provided by the recycled iron from macrophages, which ingest senescent erythrocytes and release iron (105). A minimal amount of iron is provided by intestinal iron absorption through the mediation of specific transport proteins (101). The most important transport protein for iron systemic regulation is ferroportin-1 (FPN1), which in association with the plasma ferroxidase ceruloplasmin, allows the transport of iron into the plasma across the membrane of absorptive enterocytes, macrophages and hepatocytes (105-108). Cellular iron uptake primarily occurs by the endocytic pathway, in which iron is transported into the circulation by transferrin and released to erythroblasts by the interaction of diferric transferrin with the transferrin receptor (109-111).

The key regulator of systemic iron homeostasis is hepcidin, a small antimicrobial peptide synthesized by the liver, which regulates intestinal iron absorption, plasma iron concentrations and tissue iron distribution due its ability to bind FPN1 on cellular surface blocking its iron transport activity, and to increase FPN1 degradation (103,112). Hepcidin is upregulated by both increased iron stores and inflammation. On the other hand, hepcidin secretion is reduced in response to signals that cause an increase in iron release from cells, such as iron deprivation, and stimulus to erythropoiesis (105,113-115).

A large amount of literature is available regarding iron transporting through membranes, intracellular iron trafficking and the relationship between iron and erythropoiesis (105-113).

3.4. Clinical relevance of erythropoiesis disorders

Erythroid development is a refined regulated process that requires a continual replacement of cells as mature blood cells have limited lifespans. Dysregulation of this process may cause hematological malignancies (116).

Several diseases that are related to erythropoietic disorders have been documented over the years. Among them, anemia appears in higher frequency in consequence of diverse pathologic states such as neoplasias, inflammation, hemolysis, acute bleeding, marrow damage and cell maturation disorders. Anemia may be developed as a consequence of three main factors: 1) blood loss, 2) decrease in RBCs production and 3) increase of RBCs breakdown (117). Blood loss usually involves a bleeding trauma. Whereas the decrease in RBCs production often occurs in response to iron deficiency, lack of folate and/or vitamin B12, thalassemia, bone marrow neoplasms and others, the increase RBCs breakdown is a consequence of genetic disorders and environmental conditions that may lead to infections (118,119).

Megaloblastic anemia is usually associated with lack of vitamin B12 and results in the inhibition of DNA synthesis during RBCs production and can cause important neurological manifestations (120). Another type of megaloblastic anemia is derived from folate deficiency, essential for RBCs formation and to growth (121). However, among the micronutrients-related anemia, iron deficiency is the most common (122). Iron anemia is a result of a prolonged negative iron balance due a chronic and prolonged iron loss or by inefficient iron absorption or by a nutrition iron deficiency. Iron anemia results in body iron storage depletion and can be distinguished from functional or relative iron deficiency, which are defined as a response to intravenous iron with an increase in Hb or a decrease in erythropoiesis-stimulating agent requirement, respectively (123).

Thalassemia is another erythropoietic syndrome that results from the imbalance of α- or β-globin chain production. This imbalance leads to an impaired RBC synthesis and more erythroid progenitors in the bloodstream, correlating to an imbalance of the proper erythropoiesis and the iron metabolism (124). The neoplasms, such as bone marrow neoplasms and solid tumors, particularly gastric, breast, and lung cancers are related to the decreased production of RBCs (125), and also the aplastic anemia which is characterized by the by peripheral blood pancytopenia in response to the fatty replacement and decreased hematopoietic precursors of the bone marrow immune-mediated (126). In addition to bone marrow neoplasms, acute erythroid leukemias may be derived from abnormal erythroid hyperplasia in bone marrow (127).

As previously mentioned, RBCs breakdown is usually caused by genetic disorders and detrimental environmental conditions, causing hemolytic anemia, such as sickle cell anemia, G6PD deficiency, abnormalities of the erythrocyte membrane, autoimmune anemia, exposure to certain chemicals, drugs, and toxins as well as infections. Other causes are blood clots in small blood vessels and transfusion of blood from a donor with a blood type that does not match yours (130-132).

Iron deficiency anemia may be related to nutritional deficiency and/or blood loss. Nevertheless, this type of anemia remains the most common treatable anemia in the world, and despite of the low frequency of inherited erythropoietic syndromes, anemia as a blood disorder still deserves attention, due to its incidence in patients with cancer, as a result of the malignity nature of the tumor and/or the cytotoxic effects of chemotherapy on the hematopoietic cells. Therefore, the management of cancer-associated anemia is one of the key issues to improve life quality and reducing morbidity in cancer patients (128).

4. SUMMARY

Although much is known about the erythropoiesis regulation as several factors involved in this process much still remains to be studied. However, the current knowledge ranging from the generation of RBCs and factors involved in this process can act in preventive and reparative ways providing benefits to those whom are committed by some RBC disorder.

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6. REFERENCES

1. J. C. Vandekerckhove, G.; Coulon, S.; Ribeil, J.-A.; Hermine, O.: Regulation of erythropoiesis. In: *ESH Handbook on Disorders of Iron Metabolism*. European School of Hematology, (2009)
2. J. L. Haar and G. A. Ackerman: A phase and electron microscopic study of vasculogenesis and erythropoiesis in the yolk sac of the mouse. *Anat Rec*, 170(2), 199-223 (1971) DOI: 10.1002/ar.1091700206
3. J. Palis: Primitive and definitive erythropoiesis in mammals. *Front Physiol*, 5, 3 (2014) DOI: 10.3389/fphys.2014.00003
4. M. Tavian and B. Peault: Embryonic development of the human hematopoietic system. *Int J Dev Biol*, 49(2-3), 243-50 (2005) DOI: 10.1387/ijdb.041957mt
5. E. Dzierzak and N. A. Speck: Of lineage and legacy: the development of mammalian hematopoietic stem cells. *Nat Immunol*, 9(2), 129-36 (2008) DOI: 10.1038/ni1560
6. S. H. Orkin and L. I. Zon: Hematopoiesis: an evolving paradigm for stem cell biology. *Cell*, 132(4), 631-44 (2008) DOI: 10.1016/j.cell.2008.01.025
7. M. H. Baron, A. Vacaru and J. Nieves: Erythroid development in the mammalian embryo. *Blood Cells Mol Dis*, 51(4), 213-9 (2013) DOI: 10.1016/j.bcmd.2013.07.006
8. L. K. Lee, M. Ueno, B. Van Handel and H. K. Mikkola: Placenta as a newly identified source of hematopoietic stem cells. *Curr Opin Hematol*, 17(4), 313-8 (2010) DOI: 10.1097/moh.0b013e328339f295
9. F. M. Wolber, E. Leonard, S. Michael, C. M. Orschell-Traycoff, M. C. Yoder and E. F. Srour: Roles of spleen and liver in development of the murine hematopoietic system. *Exp Hematol*, 30(9), 1010-9 (2002) DOI: 10.1016/s0301-472x(02)00881-0
10. A. Cumano and I. Godin: Ontogeny of the hematopoietic system. *Annu Rev Immunol*, 25, 745-85 (2007) DOI: 10.1146/annurev.immunol.25.022106.141538
11. P. Kumaravelu, L. Hook, A. M. Morrison, J. Ure, S. Zhao, S. Zuyev, J. Ansell and A. Medvinsky: Quantitative developmental anatomy of definitive haematopoietic stem cells/long-term repopulating units (HSC/RUs): role of the aorta-gonad-mesonephros (AGM) region and the yolk sac in colonisation of the mouse embryonic liver. *Development*, 129(21), 4891-9 (2002)
12. K. Choi, M. Kennedy, A. Kazarov, J. C. Papadimitriou and G. Keller: A common precursor for hematopoietic and endothelial cells. *Development*, 125(4), 725-32 (1998) DOI: 10.1038/386488a0
13. T. L. Huber, V. Kouskoff, H. J. Fehling, J. Palis and G. Keller: Haemangioblast commitment is initiated in the primitive streak of the mouse embryo. *Nature*, 432(7017), 625-30 (2004) DOI: 10.1038/nature03122
14. A. A. Maximow: Untersuchungenuberblut und bindegewebe 1. Die fruhestenentwicklungsstadien der blut- und binde- gewebszellanbeinsaugetierembryo, biszumanfang der blutbildingundenleber. *Arch. Mikroskop. Anat.*, 73, 444-561 (1909)
15. F. R. Sabin: Studies on the origin of blood vessels and red blood corpuscles as seen in the living blastoderm of chicks during the second day of incubation. *Contrib. Embryol.*, 9, 213–262 (1920)
16. E. T. Zambidis, B. Peault, T. S. Park, F. Bunz and C. I. Civin: Hematopoietic differentiation of human embryonic stem cells progresses through sequential hematoendothelial, primitive, and definitive stages resembling human yolk sac development. *Blood*, 106(3), 860-70 (2005) DOI: 10.1182/blood-2004-11-4522

17. P. M. Wong, S. W. Chung, D. H. Chui and C. J. Eaves: Properties of the earliest clonogenic hemopoietic precursors to appear in the developing murine yolk sac. *Proc Natl Acad Sci U S A*, 83(11), 3851-4 (1986)
DOI: 10.1073/pnas.83.11.3851

18. J. Flygare, V. Rayon Estrada, C. Shin, S. Gupta and H. F. Lodish: HIF1alpha synergizes with glucocorticoids to promote BFU-E progenitor self-renewal. *Blood*, 117(12), 3435-44 (2011)
DOI: 10.1182/blood-2010-07-295550

19. S. J. England, K. E. McGrath, J. M. Frame and J. Palis: Immature erythroblasts with extensive *ex vivo* self-renewal capacity emerge from the early mammalian fetus. *Blood*, 117(9), 2708-17 (2011)
DOI: 10.1182/blood-2010-07-299743

20. M. J. Ferkowicz and M. C. Yoder: Blood island formation: longstanding observations and modern interpretations. *Exp Hematol*, 33(9), 1041-7 (2005)
DOI: 10.1016/j.exphem.2005.06.006

21. M. Kennedy, S. L. D'Souza, M. Lynch-Kattman, S. Schwantz and G. Keller: Development of the hemangioblast defines the onset of hematopoiesis in human ES cell differentiation cultures. *Blood*, 109(7), 2679-87 (2007)
DOI: 10.1182/blood-2006-09-047704

22. G. Migliaccio, A. R. Migliaccio, S. Petti, F. Mavilio, G. Russo, D. Lazzaro, U. Testa, M. Marinucci and C. Peschle: Human embryonic hemopoiesis. Kinetics of progenitors and precursors underlying the yolk sac----liver transition. *J Clin Invest*, 78(1), 51-60 (1986)
DOI: 10.1172/jci112572

23. J. S. Blatnik, G. W. Schmid-Schonbein and L. A. Sung: The influence of fluid shear stress on the remodeling of the embryonic primary capillary plexus. *Biomech Model Mechanobiol*, 4(4), 211-20 (2005)
DOI: 10.1007/s10237-005-0001-2

24. P. D. Kingsley, E. Greenfest-Allen, J. M. Frame, T. P. Bushnell, J. Malik, K. E. McGrath, C. J. Stoeckert and J. Palis: Ontogeny of erythroid gene expression. *Blood*, 121(6), e5-e13 (2013)
DOI: 10.1182/blood-2012-04-422394

25. J. Isern, S. T. Fraser, Z. He and M. H. Baron: The fetal liver is a niche for maturation of primitive erythroid cells. *Proc Natl Acad Sci U S A*, 105(18), 6662-7 (2008)
DOI: 10.1073/pnas.0802032105

26. K. E. McGrath, A. D. Koniski, J. Malik and J. Palis: Circulation is established in a stepwise pattern in the mammalian embryo. *Blood*, 101(5), 1669-76 (2003)
DOI: 10.1182/blood-2002-08-2531

27. M.-T. R. Morioka K: Temporal characteristics of the differentiation of embryonic erythroid cells in fetal peripheral blood of the Syrian hamster. *Dev Growth Differ*, 35, 569- 582. (1993)
DOI: 10.1111/j.1440-169x.1993.00569.x

28. J. Palis, S. Robertson, M. Kennedy, C. Wall and G. Keller: Development of erythroid and myeloid progenitors in the yolk sac and embryo proper of the mouse. *Development*, 126(22), 5073-84 (1999)

29. J. A. Chasis, M. Prenant, A. Leung and N. Mohandas: Membrane assembly and remodeling during reticulocyte maturation. *Blood*, 74(3), 1112-20 (1989)

30. A. Chow, M. Huggins, J. Ahmed, D. Hashimoto, D. Lucas, Y. Kunisaki, S. Pinho, M. Leboeuf, C. Noizat, N. van Rooijen, M. Tanaka, Z. J. Zhao, A. Bergman, M. Merad and P. S. Frenette: CD169(+) macrophages provide a niche promoting erythropoiesis under homeostasis and stress. *Nat Med*, 19(4), 429-36 (2013)
DOI: 10.1038/nm.3057

31. J. Malik, A. R. Kim, K. A. Tyre, A. R. Cherukuri and J. Palis: Erythropoietin critically regulates the terminal maturation of murine and human primitive erythroblasts. *Haematologica*, 98(11), 1778-87 (2013)
DOI: 10.3324/haematol.2013.087361

32. P. Ramos, C. Casu, S. Gardenghi, L. Breda, B. J. Crielaard, E. Guy, M. F. Marongiu, R. Gupta, R. L. Levine, O. Abdel-Wahab, B. L. Ebert, N. Van Rooijen, S. Ghaffari, R. W. Grady, P. J. Giardina and S. Rivella: Macrophages support pathological erythropoiesis in polycythemia vera and beta-thalassemia. *Nat Med*, 19(4), 437-45 (2013)
DOI: 10.1038/nm.3126

33. K. McGrath and J. Palis: Ontogeny of erythropoiesis in the mammalian embryo. *Curr Top Dev Biol*, 82, 1-22 (2008)
DOI: 10.1016/s0070-2153(07)00001-4

34. K. Chen, J. Liu, S. Heck, J. A. Chasis, X. An and N. Mohandas: Resolving the distinct stages in erythroid differentiation based on dynamic changes in membrane protein expression during erythropoiesis. *Proc Natl Acad Sci U S A*, 106(41), 17413-8 (2009)
DOI: 10.1073/pnas.0909296106

35. R. E. Griffiths, S. Kupzig, N. Cogan, T. J. Mankelow, V. M. Betin, K. Trakarnsanga, E. J. Massey, S. F. Parsons, D. J. Anstee and J. D. Lane: The ins and outs of human reticulocyte maturation: autophagy and the endosome/exosome pathway. *Autophagy*, 8(7), 1150-1 (2012)
DOI: 10.4161/auto.20648

36. M. Koulnis, R. Pop, E. Porpiglia, J. R. Shearstone, D. Hidalgo and M. Socolovsky: Identification and analysis of mouse erythroid progenitors using the CD71/TER119 flow-cytometric assay. *J Vis Exp*(54) (2011)
DOI: 10.3791/2809

37. J. Liu, J. Zhang, Y. Ginzburg, H. Li, F. Xue, L. De Franceschi, J. A. Chasis, N. Mohandas and X. An: Quantitative analysis of murine terminal erythroid differentiation *in vivo*: novel method to study normal and disordered erythropoiesis. *Blood*, 121(8), e43-9 (2013)
DOI: 10.1182/blood-2012-09-456079

38. B. Van Handel, S. L. Prashad, N. Hassanzadeh-Kiabi, A. Huang, M. Magnusson, B. Atanassova, A. Chen, E. I. Hamalainen and H. K. Mikkola: The first trimester human placenta is a site for terminal maturation of primitive erythroid cells. *Blood*, 116(17), 3321-30 (2010)
DOI: 10.1182/blood-2010-04-279489

39. J. B. Geiduschek and S. J. Singer: Molecular changes in the membranes of mouse erythroid cells accompanying differentiation. *Cell*, 16(1), 149-63 (1979)
DOI: 10.1016/0092-8674(79)90196-x

40. S. T. Koury, M. J. Koury and M. C. Bondurant: Cytoskeletal distribution and function during the maturation and enucleation of mammalian erythroblasts. *J Cell Biol*, 109(6 Pt 1), 3005-13 (1989)
DOI: 10.1083/jcb.109.6.3005

41. K. E. McGrath, T. P. Bushnell and J. Palis: Multispectral imaging of hematopoietic cells: where flow meets morphology. *J Immunol Methods*, 336(2), 91-7 (2008)
DOI: 10.1016/j.jim.2008.04.012

42. R. E. Waugh, A. Mantalaris, R. G. Bauserman, W. C. Hwang and J. H. Wu: Membrane instability in late-stage erythropoiesis. *Blood*, 97(6), 1869-75 (2001)
DOI: 10.1182/blood.v97.6.1869

43. G. D. Bennett and M. M. Kay: Homeostatic removal of senescent murine erythrocytes by splenic macrophages. *Exp Hematol*, 9(3), 297-307 (1981)
DOI: 10.1073/pnas.72.9.3521

44. K. E. McGrath, J. M. Frame, G. J. Fromm, A. D. Koniski, P. D. Kingsley, J. Little, M. Bulger and J. Palis: A transient definitive erythroid lineage with unique regulation of the beta-globin locus in the mammalian embryo. *Blood*, 117(17), 4600-8 (2011)
DOI: 10.1182/blood-2010-12-325357

45. V. G. Sankaran, J. Xu and S. H. Orkin: Advances in the understanding of haemoglobin switching. *Br J Haematol*, 149(2), 181-94 (2010)
DOI: 10.1111/j.1365-2141.2010.08105.x

46. S. Addya, M. A. Keller, K. Delgrosso, C. M. Ponte, R. Vadigepalli, G. E. Gonye and S. Surrey: Erythroid-induced commitment of K562 cells results in clusters of differentially expressed genes enriched for specific transcription regulatory elements. *Physiol Genomics*, 19(1), 117-30 (2004)
DOI: 10.1152/physiolgenomics.00028.2004

47. S. M. Chambers, N. C. Boles, K. Y. Lin, M. P. Tierney, T. V. Bowman, S. B. Bradfute, A. J. Chen, A. A. Merchant, O. Sirin, D. C. Weksberg, M. G. Merchant, C. J. Fisk, C. A. Shaw and M. A. Goodell: Hematopoietic fingerprints: an expression database of stem cells and their progeny. *Cell Stem Cell*, 1(5), 578-91 (2007)
DOI: 10.1016/j.stem.2007.10.003

48. S. I. Kim and E. H. Bresnick: Transcriptional control of erythropoiesis: emerging mechanisms and principles. *Oncogene*, 26(47), 6777-94 (2007)
DOI: 10.1038/sj.onc.1210761

49. A. T. Merryweather-Clarke, A. Atzberger, S. Soneji, N. Gray, K. Clark, C. Waugh, S. J. McGowan, S. Taylor, A. K. Nandi, W. G. Wood, D. J. Roberts, D. R. Higgs, V. J. Buckle and K. J. Robson: Global gene expression analysis of human erythroid progenitors. *Blood*, 117(13), e96-108 (2011)

DOI: 10.1182/blood-2010-07-290825

50. L. C. Redmond, J. L. Haar, M. L. Giebel, C. I. Dumur, P. Basu, J. L. Ware and J. A. Lloyd: Isolation of erythroid cells from the mouse embryonic yolk sac by laser capture microdissection and subsequent microarray hybridization. *Blood Cells Mol Dis*, 37(1), 27-32 (2006)
DOI: 10.1016/j.bcmd.2006.02.006

51. A. S. Tsiftsoglou, I. S. Vizirianakis and J. Strouboulis: Erythropoiesis: model systems, molecular regulators, and developmental programs. *IUBMB Life*, 61(8), 800-30 (2009)
DOI: 10.1002/iub.226

52. H. Kaneko, R. Shimizu and M. Yamamoto: GATA factor switching during erythroid differentiation. *Curr Opin Hematol*, 17(3), 163-8 (2010)
DOI: 10.1097/moh.0b013e32833800b8

53. K. J. Hewitt, R. Sanalkumar, K. D. Johnson, S. Keles and E. H. Bresnick: Epigenetic and genetic mechanisms in red cell biology. *Curr Opin Hematol*, 21(3), 155-64 (2014)
DOI: 10.1097/moh.0000000000000034

54. J. Li, J. Hale, P. Bhagia, F. Xue, L. Chen, J. Jaffray, H. Yan, J. Lane, P. G. Gallagher, N. Mohandas, J. Liu and X. An: Isolation and transcriptome analyses of human erythroid progenitors: BFU-E and CFU-E. *Blood* (2014)
DOI: 10.1182/blood-2014-07-588806

55. G. Gronowicz, H. Swift and T. L. Steck: Maturation of the reticulocyte *in vitro*. *J Cell Sci*, 71, 177-97 (1984)

56. M. Kundu, T. Lindsten, C. Y. Yang, J. Wu, F. Zhao, J. Zhang, M. A. Selak, P. A. Ney and C. B. Thompson: Ulk1 plays a critical role in the autophagic clearance of mitochondria and ribosomes during reticulocyte maturation. *Blood*, 112(4), 1493-502 (2008)
DOI: 10.1182/blood-2008-02-137398

57. C. J. Gregory and A. C. Eaves: Three stages of erythropoietic progenitor cell differentiation distinguished by a number of physical and biologic properties. *Blood*, 51(3), 527-37 (1978)

58. J. R. Stephenson, A. A. Axelrad, D. L. McLeod and M. M. Shreeve: Induction of colonies of hemoglobin-synthesizing cells by erythropoietin *in vitro*. *Proc Natl Acad Sci U S A*, 68(7), 1542-6 (1971)
DOI: 10.1073/pnas.68.7.1542

59. J. A. Lowry and W. R. Atchley: Molecular evolution of the GATA family of transcription factors: conservation within the DNA-binding domain. *J Mol Evol*, 50(2), 103-15 (2000)
DOI: 10.1007/pl00006285

60. R. K. Patient and J. D. McGhee: The GATA family (vertebrates and invertebrates). *Curr Opin Genet Dev*, 12(4), 416-22 (2002)
DOI: 10.1016/s0959-437x(02)00319-2

61. A. H. Fox, C. Liew, M. Holmes, K. Kowalski, J. Mackay and M. Crossley: Transcriptional cofactors of the FOG family interact with GATA proteins by means of multiple zinc fingers. *EMBO J*, 18(10), 2812-22 (1999)
DOI: 10.1093/emboj/18.10.2812

62. A. P. Tsang, Y. Fujiwara, D. B. Hom and S. H. Orkin: Failure of megakaryopoiesis and arrested erythropoiesis in mice lacking the GATA-1 transcriptional cofactor FOG. *Genes Dev*, 12(8), 1176-88 (1998)
DOI: 10.1101/gad.12.8.1176

63. A. P. Tsang, J. E. Visvader, C. A. Turner, Y. Fujiwara, C. Yu, M. J. Weiss, M. Crossley and S. H. Orkin: FOG, a multitype zinc finger protein, acts as a cofactor for transcription factor GATA-1 in erythroid and megakaryocytic differentiation. *Cell*, 90(1), 109-19 (1997)
DOI: 10.1016/s0092-8674(00)80318-9

64. K. D. Johnson, M. E. Boyer, J. A. Kang, A. Wickrema, A. B. Cantor and E. H. Bresnick: Friend of GATA-1-independent transcriptional repression: a novel mode of GATA-1 function. *Blood*, 109(12), 5230-3 (2007)
DOI: 10.1182/blood-2007-02-072983

65. H. Yoo, S. K. Ku, S. W. Kim and J. S. Bae: Early Diagnosis of Sepsis Using Serum Hemoglobin Subunit Beta. *Inflammation* (2014)
DOI: 10.1007/s10753-014-0043-7

66. J. M. Manning, A. M. Popowicz, J. C. Padovan, B. T. Chait and L. R. Manning: Intrinsic regulation of hemoglobin expression by variable subunit interface strengths. *FEBS J*, 279(3), 361-9 (2012)
DOI: 10.1111/j.1742-4658.2011.08437.x

67. E. R. D. Huens, N; Beaven, G.: Human embryonic hemoglobins. *Nature*, 201, 1095,1964. (1964)
DOI: 10.1038/2011095a0

68. Z. He and J. E. Russell: Expression, purification, and characterization of human hemoglobins Gower-1 (zeta(2)epsilon(2)), Gower-2 (alpha(2)epsilon(2)), and Portland-2 (zeta(2)beta(2)) assembled in complex transgenic-knockout mice. *Blood*, 97(4), 1099-105 (2001)
DOI: 10.1182/blood.v97.4.1099

69. C. L. Bauer, I. & Ludwing, M.: Different effects of 2, 3 diphosphoglicerato and adenosine triphosphate on oxygen affinity of adult and fetal humanhemoglobin. *Life Sci.*, 7, 1339,1968. (1968)

70. W. G. Wood and D. J. Weatherall: Haemoglobin synthesis during human foetal development. *Nature*, 244(5412), 162-5 (1973)
DOI: 10.1038/244162a0

71. H. Bard, J. C. Fouron, J. Prosmanne and J. Gagnon: Effect of hypoxemia on fetal hemoglobin synthesis during late gestation. *Pediatr Res*, 31(5), 483-5 (1992)
DOI: 10.1203/00006450-199205000-00015

72. H. Bard and F. Teasdale: Red cell oxygen affinity, hemoglobin type, 2,3-diphosphoglycerate, and pH as a function of fetal development. *Pediatrics*, 64(4), 483-7 (1979)

73. M. Heikinheimo and M. A. Siimes: Regulation of erythropoiesis in the newborn: a complex system. *Ann Med*, 24(5), 309-11 (1992)
DOI: 10.3109/07853899209147828

74. I. Cantu and S. Philipsen: Flicking the switch: adult hemoglobin expression in erythroid cells derived from cord blood and human induced pluripotent stem cells. *Haematologica*, 99(11), 1647-9 (2014)
DOI: 10.3324/haematol.2014.116483

75. C. E. Edling and B. Hallberg: c-Kit--a hematopoietic cell essential receptor tyrosine kinase. *Int J Biochem Cell Biol*, 39(11), 1995-8 (2007)
DOI: 10.1016/j.biocel.2006.12.005

76. H. E. Broxmeyer: Erythropoietin: multiple targets, actions, and modifying influences for biological and clinical consideration. *J Exp Med*, 210(2), 205-8 (2013)
DOI: 10.1084/jem.20122760

77. T. Miyake, C. K. Kung and E. Goldwasser: Purification of human erythropoietin. *J Biol Chem*, 252(15), 5558-64 (1977)
DOI: 10.2172/4675513

78. W. Jelkmann: Physiology and pharmacology of erythropoietin. *Transfus Med Hemother*, 40(5), 302-9 (2013)
DOI: 10.1159/000356193

79. M. Buemi, V. Donato and D. Bolignano: (Erythropoietin: pleiotropic actions). *Recenti Prog Med*, 101(6), 253-67 (2010)

80. S. B. Krantz and E. Goldwasser: Specific binding of erythropoietin to spleen cells infected with the anemia strain of Friend virus. *Proc Natl Acad Sci U S A*, 81(23), 7574-8 (1984)
DOI: 10.1073/pnas.81.23.7574

81. L. Wang, L. Di and C. T. Noguchi: Erythropoietin, a novel versatile player regulating energy metabolism beyond the erythroid system. *Int J Biol Sci*, 10(8), 921-39 (2014)
DOI: 10.7150/ijbs.9518

82. S. B. Krantz: Erythropoietin. *Blood*, 77(3), 419-34 (1991)
DOI: 10.1016/b978-0-12-595705-2.50022-7

83. S. W. Smith, S. Chand and C. O. Savage: Biology of the renal pericyte. *Nephrol Dial Transplant*, 27(6), 2149-55 (2012)
DOI: 10.1093/ndt/gfs134

84. S. Bachmann, M. Le Hir and K. U. Eckardt: Co-localization of erythropoietin mRNA and ecto-5'-nucleotidase immunoreactivity in peritubular cells of rat renal cortex indicates that fibroblasts produce erythropoietin. *J Histochem Cytochem*, 41(3), 335-41 (1993)
DOI: 10.1177/41.3.8429197

85. P. H. Maxwell, M. K. Osmond, C. W. Pugh, A. Heryet, L. G. Nicholls, C. C. Tan, B. G. Doe, D. J. Ferguson, M. H. Johnson and P. J. Ratcliffe: Identification of the renal erythropoietin-producing cells using transgenic mice. *Kidney Int*, 44(5), 1149-62 (1993)
DOI: 10.1038/ki.1993.362

86. M. Bernaudin, A. Bellail, H. H. Marti, A. Yvon, D. Vivien, I. Duchatelle, E. T. Mackenzie and E. Petit: Neurons and astrocytes express EPO mRNA: oxygen-sensing mechanisms that involve the redox-state of the brain. *Glia*, 30(3), 271-8 (2000)
DOI: 10.1002/(sici)1098-1136(200005)30:3<271:aid-glia6>3.3.co;2-8

87. H. H. Marti, R. H. Wenger, L. A. Rivas, U. Straumann, M. Digicaylioglu, V. Henn, Y.

Yonekawa, C. Bauer and M. Gassmann: Erythropoietin gene expression in human, monkey and murine brain. *Eur J Neurosci*, 8(4), 666-76 (1996)
DOI: 10.1111/j.1460-9568.1996.tb01252.x

88. S. T. Koury, M. J. Koury, M. C. Bondurant, J. Caro and S. E. Graber: Quantitation of erythropoietin-producing cells in kidneys of mice by *in situ* hybridization: correlation with hematocrit, renal erythropoietin mRNA, and serum erythropoietin concentration. *Blood*, 74(2), 645-51 (1989)

89. M. E. Miller, E. P. Cronkite and J. F. Garcia: Plasma levels of immunoreactive erythropoietin after acute blood loss in man. *Br J Haematol*, 52(4), 545-9 (1982)
DOI: 10.1111/j.1365-2141.1982.tb03929.x

90. P. H. Abbrecht and J. K. Littell: Plasma erythropoietin in men and mice during acclimatization to different altitudes. *J Appl Physiol*, 32(1), 54-8 (1972)

91. M. Scortegagna, K. Ding, Q. Zhang, Y. Oktay, M. J. Bennett, M. Bennett, J. M. Shelton, J. A. Richardson, O. Moe and J. A. Garcia: HIF-2alpha regulates murine hematopoietic development in an erythropoietin-dependent manner. *Blood*, 105(8), 3133-40 (2005)
DOI: 10.1182/blood-2004-05-1695

92. P. P. Kapitsinou, Q. Liu, T. L. Unger, J. Rha, O. Davidoff, B. Keith, J. A. Epstein, S. L. Moores, C. L. Erickson-Miller and V. H. Haase: Hepatic HIF-2 regulates erythropoietic responses to hypoxia in renal anemia. *Blood*, 116(16), 3039-48 (2010)
DOI: 10.1182/blood-2010-02-270322

93. C. Warnecke, Z. Zaborowska, J. Kurreck, V. A. Erdmann, U. Frei, M. Wiesener and K. U. Eckardt: Differentiating the functional role of hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha (EPAS-1) by the use of RNA interference: erythropoietin is a HIF-2alpha target gene in Hep3B and Kelly cells. *FASEB J*, 18(12), 1462-4 (2004)
DOI: 10.1096/fj.04-1640fje

94. G. L. Wang and G. L. Semenza: General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. *Proc Natl Acad Sci U S A*, 90(9), 4304-8 (1993)
DOI: 10.1073/pnas.90.9.4304

95. L. E. Huang, V. Ho, Z. Arany, D. Krainc, D. Galson, D. Tendler, D. M. Livingston and H. F. Bunn: Erythropoietin gene regulation depends on heme-dependent oxygen sensing and assembly of interacting transcription factors. *Kidney Int*, 51(2), 548-52 (1997)
DOI: 10.1038/ki.1997.76

96. K. La Ferla, C. Reimann, W. Jelkmann and T. Hellwig-Burgel: Inhibition of erythropoietin gene expression signaling involves the transcription factors GATA-2 and NF-kappaB. *FASEB J*, 16(13), 1811-3 (2002)
DOI: 10.1096/fj.02-0168fje

97. E. A. Mirand, A. S. Gordon and J. Wenig: Mechanism of testosterone action in erythropoiesis. *Nature*, 206(981), 270-2 (1965)
DOI: 10.1038/206270a0

98. R. Alexanian, W. K. Vaughn and M. W. Ruchelman: Erythropoietin excretion in man following androgens. *J Lab Clin Med*, 70(5), 777-85 (1967)

99. B. D. Doane, W. Fried and F. Schwartz: Response of uremic patients to nandrolone decanoate. *Arch Intern Med*, 135(7), 972-5 (1975)
DOI:10.1001/archinte.1975.00330070094015

100. W. Fried and C. W. Gurney: Erythropoietic effect of plasma from mice receiving testosterone. *Nature*, 206(989), 1160-1 (1965)
DOI: 10.1038/2061160a0

101. C. Camaschella and A. Pagani: Iron and erythropoiesis: a dual relationship. *Int J Hematol*, 93(1), 21-6 (2011)
DOI: 10.1007/s12185-010-0743-1

102. H. Ye, S. Y. Jeong, M. C. Ghosh, G. Kovtunovych, L. Silvestri, D. Ortillo, N. Uchida, J. Tisdale, C. Camaschella and T. A. Rouault: Glutaredoxin 5 deficiency causes sideroblastic anemia by specifically impairing heme biosynthesis and depleting cytosolic iron in human erythroblasts. *J Clin Invest*, 120(5), 1749-61 (2010)
DOI: 10.1172/jci40372

103. T. Ganz and E. Nemeth: Hepcidin and iron homeostasis. *Biochim Biophys Acta*, 1823(9), 1434-43 (2012)
DOI: 10.1016/j.bbamcr.2012.01.014

104. M. W. Hentze, M. U. Muckenthaler and N. C. Andrews: Balancing acts: molecular control of mammalian iron metabolism. *Cell*, 117(3),

285-97 (2004)
DOI: 10.1016/s0092-8674(04)00343-5

105. E. Canavesi, C. Alfieri, S. Pelusi and L. Valenti: Hepcidin and HFE protein: Iron metabolism as a target for the anemia of chronic kidney disease. *World J Nephrol*, 1(6), 166-76 (2012) DOI: 10.5527/wjn.v1.i6.166

106. S. Kono and H. Miyajima: Molecular and pathological basis of aceruloplasminemia. *Biol Res*, 39(1), 15-23 (2006) DOI: 10.4067/s0716-97602006000100003

107. I. De Domenico, D. M. Ward, M. C. di Patti, S. Y. Jeong, S. David, G. Musci and J. Kaplan: Ferroxidase activity is required for the stability of cell surface ferroportin in cells expressing GPI-ceruloplasmin. *EMBO J*, 26(12), 2823-31 (2007) DOI: 10.1038/sj.emboj.7601735

108. D. L. Zhang, R. M. Hughes, H. Ollivierre-Wilson, M. C. Ghosh and T. A. Rouault: A ferroportin transcript that lacks an iron-responsive element enables duodenal and erythroid precursor cells to evade translational repression. *Cell Metab*, 9(5), 461-73 (2009) DOI: 10.1016/j.cmet.2009.03.006

109. J. E. Levy, O. Jin, Y. Fujiwara, F. Kuo and N. C. Andrews: Transferrin receptor is necessary for development of erythrocytes and the nervous system. *Nat Genet*, 21(4), 396-9 (1999) DOI: 10.1038/7727

110. R. S. Ohgami, D. R. Campagna, E. L. Greer, B. Antiochos, A. McDonald, J. Chen, J. J. Sharp, Y. Fujiwara, J. E. Barker and M. D. Fleming: Identification of a ferrireductase required for efficient transferrin-dependent iron uptake in erythroid cells. *Nat Genet*, 37(11), 1264-9 (2005) DOI: 10.1038/ng1658

111. L. Li, C. J. Fang, J. C. Ryan, E. C. Niemi, J. A. Lebron, P. J. Bjorkman, H. Arase, F. M. Torti, S. V. Torti, M. C. Nakamura and W. E. Seaman: Binding and uptake of H-ferritin are mediated by human transferrin receptor-1. *Proc Natl Acad Sci U S A*, 107(8), 3505-10 (2010) DOI: 10.1073/pnas.0913192107

112. E. Nemeth, M. S. Tuttle, J. Powelson, M. B. Vaughn, A. Donovan, D. M. Ward, T. Ganz and J. Kaplan: Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science*, 306(5704), 2090-3 (2004) DOI: 10.1126/science.1104742

113. A. Pagani, A. Nai, G. Corna, L. Bosurgi, P. Rovere-Querini, C. Camaschella and L. Silvestri: Low hepcidin accounts for the proinflammatory status associated with iron deficiency. *Blood*, 118(3), 736-46 (2011) DOI: 10.1182/blood-2011-02-337212

114. J. L. Babitt and H. Y. Lin: Molecular mechanisms of hepcidin regulation: implications for the anemia of CKD. *Am J Kidney Dis*, 55(4), 726-41 (2010) DOI: 10.1053/j.ajkd.2009.12.030

115. G. Dallalio, E. Law and R. T. Means, Jr.: Hepcidin inhibits *in vitro* erythroid colony formation at reduced erythropoietin concentrations. *Blood*, 107(7), 2702-4 (2006) DOI: 10.1182/blood-2005-07-2854

116. A. B. Cantor and S. H. Orkin: Transcriptional regulation of erythropoiesis: an affair involving multiple partners. *Oncogene*, 21(21), 3368-76 (2002) DOI: 10.1038/sj.onc.1205326

117. R. S. F. Hillman, C.A.: Red Cell Manual. *7th ed. Philadelphia, PA: FA Davis;*, 1-38 (1996)

118. T. G. Janz, R. L. Johnson and S. D. Rubenstein: Anemia in the emergency department: evaluation and treatment. *Emerg Med Pract*, 15(11), 1-15; quiz 15-6 (2013)

119. R. E. Smith, Jr.: The clinical and economic burden of anemia. *Am J Manag Care*, 16 Suppl Issues, S59-66 (2010) DOI: 10.1016/j.hfc.2010.02.003

120. P. G. Divate and R. Patanwala: Neurological manifestations of B12 deficiency witi emphasis on its aetiology. *J Assoc Physicians India*, 62(5), 400-5 (2014)

121. A. C. Antony: Megaloblastic anemias. In: *In: Goldman L, Schafer AI, eds. Goldman's Cecil Medicine*. Saunders Elsevier, Philadelphia, PA (2011) DOI: 10.1016/b978-1-4377-1604-7.00167-6

122. G. Weiss and L. T. Goodnough: Anemia of chronic disease. *N Engl J Med*, 352(10), 1011-23 (2005) DOI: 10.1056/nejmra041809

123. J. B. Wish: Assessing iron status: beyond serum ferritin and transferrin saturation. *Clin J Am Soc Nephrol*, 1 Suppl 1, S4-8 (2006) DOI: 10.2215/cjn.01490506

124. J. S. Guimaraes, J. G. Cominal, A. C. Silva-Pinto, G. Olbina, Y. Z. Ginzburg, V. Nandi, M. Westerman, S. Rivella and A. M. de Souza: Altered erythropoiesis and iron metabolism in carriers of thalassemia. *Eur J Haematol* (2014)
DOI: 10.1111/ejh.12464

125. S. Y. Shin, H. Park, S. W. Chae and H. Y. Woo: Microangiopathic hemolytic anemia as the first manifestation of metastatic signet ring cell carcinoma of unknown origin: a case report and review of literature. *Korean J Lab Med*, 31(3), 157-61 (2011)
DOI: 10.3343/kjlm.2011.31.3.157

126. S. H. Shin, S. E. Lee and J. W. Lee: Recent advances in treatment of aplastic anemia. *Korean J Intern Med*, 29(6), 713-26 (2014)
DOI: 10.3904/kjim.2014.29.6.713

127. D. Z. Mihova, L.: Acute Erythroid Leukemia: A Review. *North American Journal of Medicine and Science.*, 5(2), 110-118 (2012)
DOI: 10.7156/v5i2p110

128. X. Li, Z. Yan, D. Kong, W. Zou, J. Wang, D. Sun, Y. Jiang and C. Zheng: Erythropoiesis-stimulating agents in the management of cancer patients with anemia: a meta-analysis. *Chin J Cancer Res*, 26(3), 268-76 (2014)
DOI: 10.3978/j.issn.1000-9604.2014.05.03

Abbreviations: RBCs: red blood cells; EPO: erythropoietin; HSCs: hematopoietic stem cells; E: embryonic days; BFU-E: burst forming unit-erythroid; CFU-E: colony forming unit-erythroid; Hb: haemoglobin; CFU-E: colony forming unit-erythroid; SCF: stem cells factor; KLF1: Kruppel-like factor 1; IL-3: Interleukin-3; EpoR: erythropoetin receptor; FPN1: ferroportin-1; HIFs: hypoxia inducible factors

Key Words: Fetus, Adult, Erythropoiesis, Hemoglobin, Erythropoietin, Iron metabolism, Erythropoietic disorders, Review

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