Erythropoiesis in Health and Disease – Erythropoietin's In Charge!

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Introduction:

The Dutch naturalist Jan Swammerdam was the first individual to microscopically observe red blood cells (RBCs) or erythrocytes in the year 1658. Credit for the discovery of RBCs, however, often goes to Anton van Leeuwenhoek, another Dutch scientist who made the first recorded illustration of RBCs in 1695, writing, "I have observed, taking some blood out of my hand, that it consists of small round globules driven through a crystalline humidity of water" (Windsor and Rodway, 2007). These observations helped unveil the physiological significance of RBCs, the most common cell type found in blood. In fact, RBCs are the most abundant cell type in the human body, constituting about a quarter of all cells. RBCs serve a critical role as principal transporters of oxygen and carbon dioxide between organs, thus making them pivotal for the existence of all vertebrates (Palis, 2014).

The characteristic oval, biconcave shape of RBCs and their membrane flexibility are critical for their biological function, as they create large surface areas for gas exchange, and allow for repeated passes through narrow capillaries. RBCs are unique amongst vertebrate cells in that they lack nuclei and other organelles such as mitochondria, Golgi apparatus, and endoplasmic reticulum. These differences are important for RBC function enabling maximal cytoplasmic occupation by hemoglobin, an iron-rich metalloprotein, which makes up ~96% of RBCs' dry content. Hemoglobin binds efficiently to oxygen molecules, and somewhat less efficiently to carbon dioxide molecules, thereby functioning in the transport of gases through the bloodstream. Given their critical respiratory function, it is not surprising that RBCs are required at all stages of development, starting from embryogenesis through adulthood, and are indispensable to vertebrate survival and health. A deficiency or surplus in RBC counts is linked to disease states like anemia and polycythemia, respectively, and both conditions severely impact the quality of life of inflicted individuals (Dzierzak and Philipsen, 2013; Palis, 2014).

Erythropoiesis:

In healthy human adults, approximately 2 million RBCs are produced every second, and a mature RBC remains in circulation for about 120 days. RBC counts are maintained at a steady state to ensure proper tissue oxygenation. The 1% of RBCs that are destroyed daily are continually replenished by a process termed erythropoiesis. RBC formation during early embryogenesis is termed primitive erythropoiesis and is initiated in the yolk sac, giving rise to short-lived large, nucleated cells. Subsequently, a second wave of erythropoiesis, called definitive erythropoiesis ensues, occurring in the fetal liver during late stages of embryogenesis. Definitive erythropoiesis occurs all through adulthood, in the bone marrow, and is responsible for producing the small, enucleated RBCs (Palis, 2014).

Erythropoiesis is a tightly regulated process that involves the expansion of hematopoietic stem cells, which then progress to mature erythrocytes through distinct stages of commitment, proliferation, and differentiation. The first committed erythroid precursors are designated the burst forming unit-erythroid (BFU-E) cells. This commitment step is regulated by a host of factors including cytokines like IL-3 and Stem Cell Factor (SCF). These BFU-E cells then expand to form colony forming unit-erythroid (CFU-E) cells, which further differentiate into the first morphologically identifiable erythroid cells called proerythroblasts. During the final stages of erythropoiesis, the proerythroblasts extrude their nuclei to form reticulocytes. The enucleated reticulocytes subsequently expel other sub-cellular organelles, leave the bone marrow, and migrate into circulation where they mature into the characteristic biconcave, circulating erythrocytes (Dzierzak and Philipsen, 2013; Ingley et al., 2004).

EPO-EpoR Signaling Cascade in Erythropoiesis:

The orchestration of a complex, hierarchical process like erythropoiesis requires the precise coordination of a host of cellular factors that work either in concert or at distinct stages of the process, to regulate effective and sufficient formation of RBCs. The principal regulator of erythropoiesis is Erythropoietin (EPO), a heavily glycosylated protein belonging to the class I cytokine family (Jelkmann, 2004). EPO exerts its effects on erythropoiesis by binding to and activating the EPO-receptor (EpoR), a high affinity receptor that is predominantly expressed on the surface of immature erythroid cells. EpoR belongs to the type I cytokine receptor family and exists as a homodimer. Upon ligand binding, EpoR, which lacks intrinsic catalytic activity, undergoes a conformational change and associates with Janus Kinase 2 (JAK2). JAK2 phosphorylates the ligand-activated EpoR, generating multiple docking sites for signaling molecules, and leading to activation of several signal transduction pathways (Watowich, 2011). While one of the earliest studied signaling pathways activated through the EPO-EpoR cascade is the Janus Kinase and Signal Transducer and Activator of Transcription (JAK/STAT) pathway, phosphorylated EpoR induces activation of other cellular signaling cascades including the mitogen-activated protein kinase/extracellular signal-related kinase (MAPK/ERK) and the phosphatidylinositol 3-kinase /protein kinase B (PI-3K/AKT) pathways (Ingley et al., 2004). These pathways impact erythropoiesis by temporally regulating transcription of genes involved in survival, differentiation, and cell proliferation depending on the stage of erythroid maturation. For example, survival signals are provided to CFU-E cells, the most EPO-sensitive cells amongst the erythroid progenitors, as well as to erythroblasts. The importance of EPO-EpoR signaling in erythropoiesis has been demonstrated by EPO-null and EpoR-null mouse models, which are both embryonically lethal, owing to a lack of erythropoiesis in the fetus (Wu et al., 1995). While the EPO-EpoR signaling axis serves as the central hub for erythropoiesis regulation, several other molecular signals, both extrinsic and intrinsic, are involved and serve to further fine-tune the progression of the multi-faceted process of erythropoiesis (Ingley, 2012; Ingley et al., 2004; Kuhrt and Wojchowski, 2015).

Molecular Fine-Tuning of Erythropoiesis by EPO:

More than a century ago, the French scientist Francois-Gilbert Viault observed a rise in RBC numbers at high altitudes when on a journey to the highlands of Peru. This observation, followed by many others, led to the notion that a circulating factor regulates the RBC numbers under conditions of

oxygen depletion (Erslev, 1953). A century of research led to the discovery and molecular cloning of EPO, the premiere cytokine responsible for regulating erythropoiesis (Jelkmann, 2011). Given the physiological relevance of RBCs, significant efforts have been vested in understanding the different regulators of erythropoiesis, with a particular emphasis on exploration of the biomolecular regulation of EPO (Jelkmann, 2007, 2013).

The primary site of EPO production during fetal development is the liver, while the kidney serves as the principal physiological production source postnatally. However, the liver retains its ability to produce EPO, contributing to about 10%-15% of total EPO production in adults, and under certain stress conditions such as oxygen scarcity (hypoxia) produces a third of total circulating EPO in the blood (Jelkmann, 2013).

EPO production is regulated by a negative feedback loop with tissue oxygen tensions being the main determinants of this regulation. Under homeostatic conditions, EPO is present in low amounts in circulation. The body contains no significant reserves of EPO, and fluctuations in oxygen levels serve as a sensor for EPO production. Hypoxic conditions or anemia can serve as triggers, leading to increased EPO production in the kidney, resulting in a rise in circulating EPO levels and a consequent increase in erythropoiesis through activation of the EPO-EpoR signaling cascade. Increase in RBC mass leads to enhanced oxygen carrying capacity in the blood. The restored oxygen levels counteract the hypoxic signals, leading to suppression of EPO production, thereby completing the feedback loop (Fig.1).

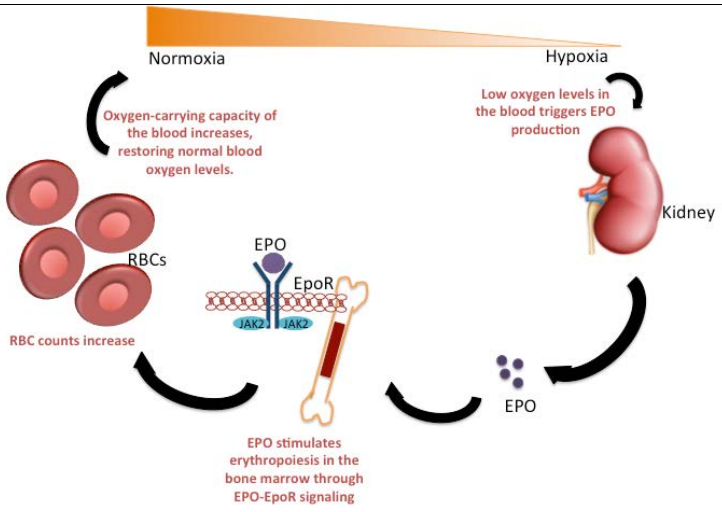

Figure 1: Regulation of Erythropoiesis by a Feedback Loop to Maintain Homeostatic Blood Oxygen Levels

At the molecular level, the feedback loop comprises distinct transcriptional regulators. Under hypoxic conditions, hypoxia-inducible factor (HIF) binds to the EPO enhancer and, in cooperation with other transcriptional co-activators like p300 and cAMP response element-binding protein (CREB)-binding protein (CBP), activates transcription of EPO and activation of erythropoiesis. When oxygen levels are restored to normal, and the demand for erythropoiesis is low, the EPO enhancer is repressed by the transcription factor GATA-2. In addition to the repressive effects of GATA-2, the HIF genes are themselves targeted for degradation in the presence of oxygen, leading to down-regulation of EPO gene transcription and a concomitant block in erythropoiesis (Fig. 2) (Haase, 2013).

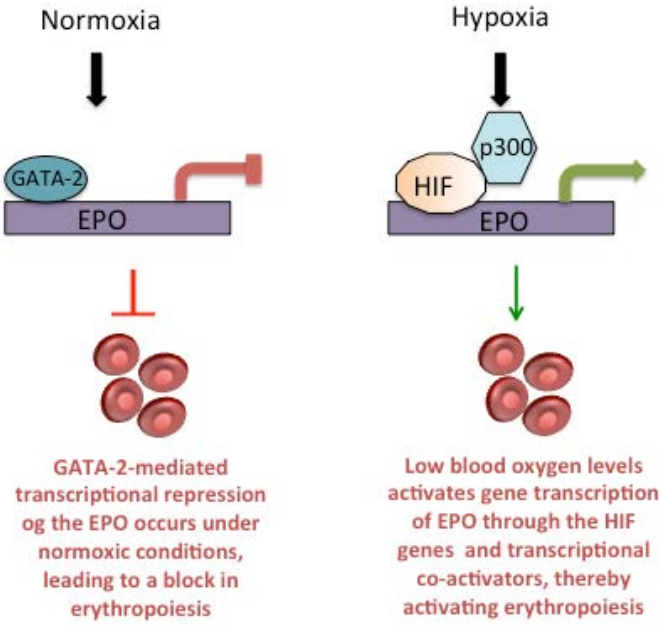

Figure 2: Gene Expression of EPO, the Chief Regulator of Erythropoiesis are Modulated by Oxygen Levels

Unraveling the physiology and molecular mechanisms of EPO regulation paved the way for exploring the administration of EPO as a therapeutic agent in ailments where erythropoiesis goes awry.

Pharmacological Approaches to Increase Erythropoiesis:

Anemia, characterized by decreased numbers of RBCs is major contributor to morbidity and mortality worldwide. Anemia is a common complication associated with ailments like chronic kidney failure, cancer patients on chemotherapy, and other conditions where the body is immune compromised, as is the case with AIDS patients (Sankaran and Weiss, 2015). In these conditions,

anemia is largely associated with a defect in EPO production, leading to a deficiency in RBC counts. Previously, blood transfusions were the mainstay therapeutic option to maintain a steady supply of RBCs. The availability of erythropoiesis-stimulating agents (ESAs), which function like EPO to stimulate RBC production has revolutionized the treatment options for patients suffering from anemia caused by reduced EPO production. In fact, ESAs were the standard of care for treatment of anemia, reducing the dependence on blood transfusions and increasing the overall quality of life for patients (Hedley et al., 2011; Horl, 2013).

The approval of the first commercially available recombinant human EPO (rHuEPO), one of the earliest successes of recombinant DNA technology serves as a poster child for bench-to-bedside science (Jacobs et al., 1985; Lin et al., 1985). rHuEPO is the standard of care for anemia patients and is by far the most commonly used ESA clinically. rHuEPO is widely administered to patients suffering from anemia due to chronic kidney disease (CKD). As the kidney is the primary organ for EPO production, CKD is associated with decreased EPO production, consequently leading to anemia due to a deficiency in RBC counts. Apart from its use to treat renal anemias, rHuEPO is also used in non-renal indications. rHuEPO has been approved for use in patients with other anemias, including cancer patients undergoing chemotherapy (Sankaran and Weiss, 2015).

Veterinary uses for EPO

Apart from being administered in humans, recombinant EPO has found its way in veterinary medicine to treat renal and non-renal anemias alike (Henry, 1994; Woo and Jusko, 2007). Recombinant EPO is used to treat anemia in dogs and cats suffering from CKD and some forms of cancer. Currently there are no species-specific EPO molecules available clinically to treat animals with anemia. However given the overall amino acid identity between the human EPO protein, and the dog and cat proteins (canine EPO has 81.3% identity with human EPO, and feline EPO has 83.3% identity), rHuEPO has been approved for treatment in cats and dogs (MacLeod et al., 1998). While the use of rHuEPO has been successful in resolving anemia and improving overall quality of life, there are some adverse side effects associated with its consumption. One of the most serious complications is the production of anti-rHuEPO antibodies. These *de novo* generated antibodies not only react against rHuEPO, but also cross-react with the native EPO antigens leading to a complete block in red blood cell formation, which could potentially be life-threatening (Maiese et al., 2008). Currently, researchers are investigating the effects of using canine- and feline-specific recombinant EPO in dogs and cats, respectively. While the canine and feline versions of rEPO have been generated, they are not available commercially and studies are underway to determine the safety and efficacy of species-specific recombinant proteins.

Perspectives for the future:

While the use of ESAs remains the mainstay for management of anemia, alternate approaches to stimulate erythropoiesis are also being actively evaluated (Macdougall, 2012). For example, a group of researchers reported promising results with EPO gene therapy in a mouse model. Gene therapy with feline EPO has also been investigated and the evaluation of this novel technique in human subjects is currently ongoing (Beall et al., 2000). By utilizing the knowledge researchers have amassed about

RBCs production, generation of new reagents and novel therapies could be used to stimulate erythropoiesis and treat anemia in animals and their human counterparts in the near future.

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