Blood Reviews 27 (2013) 41–53

Contents lists available at SciVerse ScienceDirect

Blood Reviews

journal homepage: www.elsevier.com/locate/blre

REVIEW

# Regulation of erythropoiesis by hypoxia-inducible factors

Volker H. Haase *

*Department of Medicine, Vanderbilt School of Medicine, Nashville, TN, USA*
*Department of Cancer Biology, Vanderbilt School of Medicine, Nashville, TN, USA*
*Department of Molecular Physiology and Biophysics, Vanderbilt School of Medicine, Nashville, TN, USA*

ARTICLE INFO

*Keywords:*
Anemia
Chronic mountain sickness
Erythropoiesis
Erythrocytosis
EPO
Hepcidin
Hypoxia-inducible factors
Iron metabolism

ABSTRACT

A classic physiologic response to systemic hypoxia is the increase in red blood cell production. Hypoxia-inducible factors (HIFs) orchestrate this response by inducing cell-type specific gene expression changes that result in increased erythropoietin (EPO) production in kidney and liver, in enhanced iron uptake and utilization and in adjustments of the bone marrow microenvironment that facilitate erythroid progenitor maturation and proliferation. In particular HIF-2 has emerged as the transcription factor that regulates EPO synthesis in the kidney and liver and plays a critical role in the regulation of intestinal iron uptake. Its key function in the hypoxic regulation of erythropoiesis is underscored by genetic studies in human populations that live at high-altitude and by mutational analysis of patients with familial erythrocytosis. This review provides a perspective on recent insights into HIF-controlled erythropoiesis and iron metabolism, and examines cell types that have EPO-producing capability. Furthermore, the review summarizes clinical syndromes associated with mutations in the $O_2$-sensing pathway and the genetic changes that occur in high altitude natives. The therapeutic potential of pharmacologic HIF activation for the treatment of anemia is discussed.



## 1. Introduction

Over 100 years ago Paul Bert and Denis Jourdanet described the association between reduced atmospheric $O_2$ pressure and elevated rbc numbers in humans and in animals,[1–3] which in 1890, during a high-altitude expedition to the Peruvian Andes led by Francois-Gilbert Viault, was shown to result from an acute physiologic response rather than being an inherited condition.[4] It was the interest in understanding the molecular basis of this erythropoietic response that first led to the discovery of erythropoietin (EPO) and later on to the identification of the molecular machinery that senses $pO_2$. The hypoxic induction of *EPO* serves as a paradigm of $O_2$-dependent gene regulation and the search for the transcription factor that regulates *EPO* resulted in the identification of hypoxia-inducible factor (HIF), which controls a wide spectrum of tissue-specific and systemic hypoxia responses.

Recent experimental data indicate that HIF promotes erythropoiesis at multiple levels and coordinates cell type-specific hypoxia responses. These include renal and hepatic EPO synthesis, enhanced iron uptake and utilization, as well as changes in the bone marrow microenvironment that facilitate erythroid progenitor maturation and proliferation. Because of its central role in the hypoxic regulation of erythropoiesis, pharmacological targeting of the HIF $O_2$-sensing pathway has therapeutic potential for the treatment of anemia, in particular anemia associated with inadequate EPO production, e.g. in patients with chronic kidney disease (CKD). This review discusses recent insights into the cellular and molecular mechanisms that underlie $O_2$-dependent regulation of EPO synthesis, iron metabolism and erythroid progenitor maturation, and examines their relevance to clinical disorders and anemia therapy.

## 2. EPO-producing cell types

Surgical organ removal in animals identified the kidney as the major site of EPO synthesis in adults.[5] Although initially debated, EPO is produced by peritubular interstitial fibroblasts and not by renal tubular epithelial cells or peritubular endothelial cells.[6–12] Renal EPO-producing cells (REPC) can be typically found in the renal cortex (predominantly juxtamedullary region) and outer medulla (Fig. 1). REPC express ecto-5′-nucleotidase (CD73) and platelet-derived growth factor receptor β-polypeptide (PDGFRB),[9,13] both are also markers of pericytes and EPO-negative interstitial fibroblasts.[14] *Epo* expression in tubular epithelial cells appears to be suppressed by GATA transcription factors, in particular GATA-2 and GATA-3, and can be reactivated under normoxic or hypoxic conditions when the GATA core consensus binding sequence upstream of the *Epo* transcription start site is mutated.[11] The kidney responds to hypoxia by increasing the number of REPC in an $O_2$-dependent manner and therefore regulates EPO output through adjustments in REPC number.[8,11] $O_2$-dependent *Epo* transcription is controlled by distinct regulatory DNA sequences. These flank the *Epo* coding sequence on both sides, the kidney-inducibility element in the 5′-region and the liver-inducibility

* Department of Medicine, Division of Nephrology and Hypertension, Vanderbilt University Medical Center, C-3119A, MCN, 1161 21st Avenue South, Nashville TN 37232, USA. Tel.: +1 615 343 7254; fax: +1 615 322 6854.
*E-mail address:* volker.haase@vanderbilt.edu.

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http://dx.doi.org/10.1016/j.blre.2012.12.003

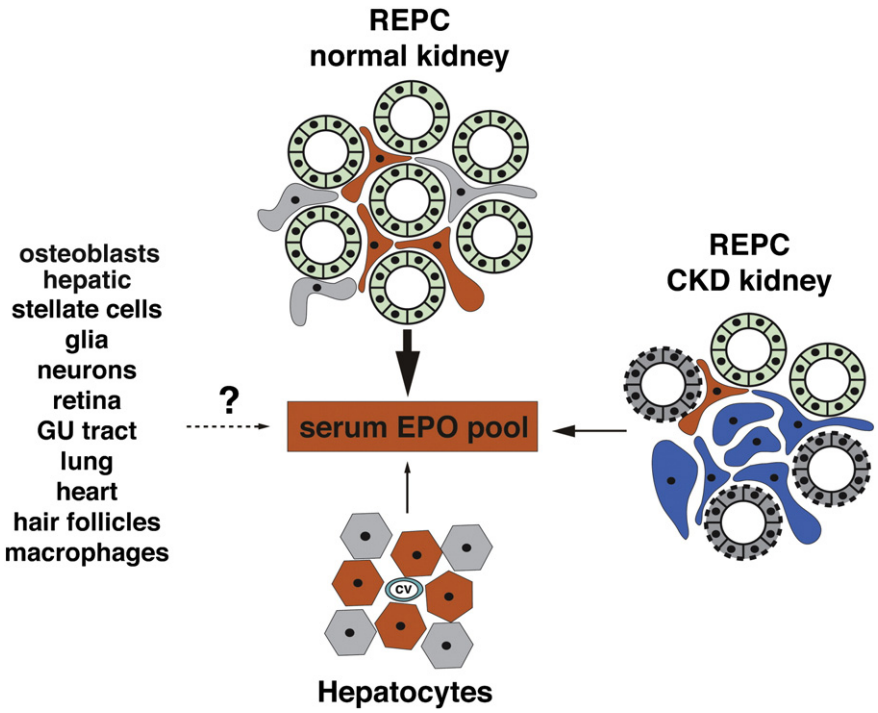


**Fig. 1.** Cellular sources of EPO. Shown is a schematic overview of cell types and tissues with EPO-producing capacity. In adults, the kidney and liver are the two major contributors to the serum EPO pool, with the kidney being the main physiologic site of EPO synthesis. While *EPO* transcripts are not detectable at baseline, the liver produces EPO when stimulated with either moderate to severe hypoxia or pharmacologically. The contribution of other cell types to erythropoiesis under stress conditions is not clear. A list of cell types and tissues in which *EPO* transcripts have been detected under various experimental conditions is shown on the left. Renal EPO-producing cells (REPC) are peritubular interstitial fibroblasts (shown in orange). Tubular epithelial cells do not produce EPO and are shown in green. Under chronic injury conditions, REPC undergo transdifferentiation into collagen-producing myofibroblasts (blue) and loose their ability to produce EPO; injured renal tubuli are shown in gray color. In CKD kidneys, the number of REPC is reduced, which results in inadequate EPO production in response to hypoxic stimuli and leads to the development of anemia. Abb.: cv, central vein.

element in the 3′-region.[15–17] The 3′-hypoxia enhancer region is absolutely required for the hypoxic induction of *Epo* in the liver, as shown by genetic studies in mice.[18]

REPC have been visualized in BAC transgenic mice through the use of green fluorescent protein (GFP). In this transgenic model the *Epo* coding sequence was replaced by GFP cDNA, which brings GFP under the control of *Epo* regulatory elements.[11] GFP expression was found in renal peritubular interstitial cells and in a subpopulation of hepatocytes that were localized around the central vein, supporting the notion that these two cell types represent the major sites of physiologic EPO production under conditions of systemic hypoxia. In the kidney, GFP-positive interstitial cells were unique in their morphologic appearance, as they displayed dendrite-like processes and expressed neuronal-specific markers, such as microtubule-associated protein 2 (MAP2) and neurofilament protein light polypeptide (NFL), indicating that REPC may be derived from progenitor cells of neuronal origin. This notion is furthermore supported by lineage tracing studies that utilized myelin protein zero (P0)-Cre transgenic mice, which express Cre-recombinase in neural crest-derived cells.[13] In keeping with this observation, Frede and colleagues established an EPO-producing renal tumor cell line with similar morphologic and molecular characteristics.[19] Although the hypoxic induction of *Epo* was reported in 4E cells, a mesenchymal cell clone with characteristics of embryonic kidney stromal cells,[20] primary REPC that retain their EPO-producing ability are difficult to culture. The molecular mechanisms underlying this phenomenon are unclear. Transdifferentiation of REPC into myofibroblasts, which are a main source of collagen in fibrotic kidneys, has been proposed as a potential mechanism by which REPC loose their ability to synthesize EPO in CKD (Fig. 1).[13] Myofibroblasts appear to be derived from pericytes,[21,22] which express cellular markers that are also found on REPC (e.g. CD73 and PDGFRB). To what degree these two cell populations overlap remains to be determined.

While the kidney is the primary physiologic source of EPO synthesis in adults, the liver is the main site of EPO production during embryonic development. However, in adults, the liver retains its ability to produce EPO in response to moderate/severe hypoxia or to pharmacologic HIF activation.[23–25] Similar to the kidney, the liver responds to severe hypoxia by increasing the number of EPO-producing hepatocytes that localize around the central vein.[11] *Epo* has also been detected in hepatic stellate cells, which have been previously referred to as ITO cells.[26,27] The timing of transition from liver to the kidney as the primary site of EPO production is species-dependent and usually occurs during late gestation or at around birth.[28–31] The molecular mechanisms that underlie this switch are poorly understood, but may involve transcriptional repression and/or reduced expression of certain transcriptional activators, such as GATA-4.[32]

In the adult liver, *Epo* mRNA levels, which are very difficult to detect at baseline, rise substantially under conditions of moderate to severe hypoxia, and account for most, if not all, physiologically relevant systemic EPO of extra-renal origin.[23,33] While hypoxia-induced EPO production in the liver does not normalize Hgb values in CKD, hepatic HIF can be sufficiently stimulated by pharmacologic means to correct anemia that results from inadequate EPO production or from inflammatory conditions.[24,34]

Aside from kidney and liver as the two major sources of EPO synthesis, *EPO* mRNA expression has also been detected in the brain (neurons and glial cells), lung, heart, bone marrow, spleen, hair follicles, the reproductive tract and in osteoblasts.[31,35–46] While the role of these cell types in erythropoiesis under baseline conditions has not been demonstrated, they may, to a certain degree, contribute to stress-induced erythropoiesis (Fig. 1).[45,47] EPO synthesized by these cells is more likely to act locally, modulating, for example, regional angiogenesis and cellular viability (for an overview of the non-hematopoietic actions of EPO see Jelkmann[48]).

While $pO_2$ is critical for the regulation of renal EPO synthesis, some studies have investigated the role of extrinsic signals in the regulation