Review Article

Stimulation and Production of Erythropoietin and Erythropoietin Gene Expression System

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Accepted on: 12-04-2013; **Finalized on:** 30-06-2013.

ABSTRACT

Erythropoietin, an oxygen regulated glycoprotein hormone is a hematopoietic cytokines that stimulates erythropoiesis by binding to its cellular receptor (Erythropoietin receptor (EPORP)). In humans and other mammals, decreased oxygen tension triggers specific and tightly regulated cellular, vascular and erythropoietic responses. Decreased tissue oxygen tension modulates Epo levels by increasing the expression of the Epo gene. Erythropoietin is produced primarily by the kidney. The expression of Epo is markedly increased in kidneys during hypoxic state, a condition mediated by the transcription factor HIF-1. The ultimate effect is to increase erythropoiesis in an attempt to maintain oxygen delivery to vital organs. Recombinant (r Epo) Epo has been an alternative to blood transfusion for the cancer patients treated with radiation therapy and chemotherapy and is also used for replacement therapy in anemia associated with chronic renal failure to increase hemoglobin levels and to improve the quality of life by decreasing fatigue. Since the cloning of the Epo gene in 1985, considerable progress has been made in understanding the molecular mechanisms by which the Epo gene is regulated by environmental, tissue specific and developmental cells following is the review pertinent to the work carried out by various researchers on regulation of erythropoietin gene.

Keywords: Epo – Erythropoietin, EPOR – Erythropoietin receptor, HIF-1 Hypoxia inducible factor, rEpo – recombinant erythropoietin.

INTRODUCTION

Erythropoietin, a 34.4 – KD glycoprotein hormone, was subsequently identified as the humoral regulator of red blood cell production[1,2]. The human Epo gene has been localized on chromosome 7. The gene encoding human Epo is contained as a single copy in a 5.4 kb region of the genomic DNA. IT contains four introns and five exons for 193 a monoacid peptide[3]. Epo is predominantly synthesized by the kidney, liver accounts for 15% of Epo production in adults and is the major site in utero.

Erythropoietin, which normally proceeds at a low basal level to replace aged red blood cells, is highly induced by loss of red blood cells, decreased ambient oxygen tension, increased oxygen affinity for Hemoglobin, and other stimuli that decrease delivery of oxygen to the tissues. In status of severe hypoxia, production of Epo is increased up to 1,000 fold. The secreted hormone circulates in the blood and binds to receptors expressed specifically on erythroid. Progenitor cells, thereby promoting the viability, proliferation and terminal differentiation of erythroid precursors, resulting in an increase in red blood cell mass. The oxygen carrying capacity of the blood is thus enhanced, increasing tissue oxygen tension, thereby completing the negative feedback loop[4,5]. Epo synthesis is regulated by hypoxia, transcription of Epo gene is regulated by hypoxia inducible factor (HIF)[6] and levels increase with reduction in atmospheric oxygen; blood volume, hemoglobin concentration, reduced oxygen affinity and cardiopulmonary causes of Epo. Increased epo levels stimulate erythropoietin in the bone marrow by accelerating the rate and number of colony forming units, undergoing differentiation into RBCs. The effect is increased RBC mass that leads to a reduction in the stimulus for Epo production. It was found that in brain other metabolic disturbance such as hypoglycemia, raised levels of intracellular calcium or intense neuronal depolarizations generated by mitochondrial reactive oxygen species, can increases cerebral Epo expression through activation of HIF. Anemic stress and insulation release can also lead to increased expression of EPO & EPOR in both neuronal & non neuronal cell populations.

Finally a variety of cytokines, including insulin like growth factor (IGF); tumor necrosis factor (TNFs), interleukin β and interleukin 6 (IL6) can regulate the production and secretion of Epo.

MOLECULAR BIOLOGY OF ERYTHROPOIETIN

Epo gene expression is induced by hypoxia inducible transcription factors (HIF). The principal representative of the HIF family (HIF – 1, 2 & 3) is HIF-1, which is composed of an O_2 labile α sub unit and a constant nuclear sub unit. In normoxia, the– α sub unit of HIF is inactivated following prolyl of asparaginyl hydroxylation by means of α – oxogluterate of Fe^2 dependent HIF specific deoxygenases. While HIF-1 & HIF-2 activate the Epo gene, HFF-3, GATA-2 and NFKB are likely inhibitors of Epo gene transcription. Epo signaling involves tyrosine phosphorylation of the homodimeric EPO receptor and subsequent activation of intracellular antiapoptotic proteins, Kinases and transcription factors. Lack of Epo leads to anemia. Treatment with recombinant human EPO (rHuEPO) is efficient and safe in improving the management of the anemia associated with chronic

neural failure. Evidence suggests that rHuEPO may be a useful neuroprotective agent[7].

STIMULATION AND PRODUCTION OF EPO

Localization of EPO production to the kidneys was first demonstrated by Jacobsen et al.,[8] who showed that, after bilateral nephrectomy rats and rabbits do not respond to hemorrhage with an appropriate increase in plasma EPO levels. Erslev et al.,[9] have proposed that the peritubular region of the renal cortex is the ideal location for Epo production. Oxygen tension at the site of the Epo-producing cells is relatively independent of changes in renal blood flow. At high hematocrit levels, viscosity increases to the point that blood flow to tissues is compromised. If the primary site of EPO production were in an organ other than kidney, the resultant decrease in tissue oxygen tension would lead to a vicious cycle of increasing erythropoisesis causing worsening hypoxia.

When kidney cells were separated into glomerular and tubular fractions, Epo RNA was found only in the tubular fraction that included the peritubular interastitium[10]. Consistent with these findings, in situ hybridization studies with ^{35}S labeled EPO RNA[11, 12] or DNA[13] probes on kidney tissue from anemic mice showed EPO mRNA in peritubular interstitial cells. The number of these cells expressing EPO mRNA increased with a decreasing hematocrit level[14]. In contrast to these studies, other investigators have demonstrated EPO production by renal tubular cells using insitu hybridization for EPO mRNA immunohistochemistry with Epo specific antibodies[15] & detection of β -galactosidase are in transgenic mice bearing a 7-kb fragment of the EPO gene linked to the lacz gene.[16] Human renal tumor cells of tubular origin can express EPO[17]. Immunohistochemistry using EPO specific antibodies is confounded by the reabsorption of circulating EPO by renal tubular cells. Before birth, Epo is primarily produced in the liver. The primary site of Epo production switches from liver to kidney shortly after birth[18,19] but the signals governing this change are poorly understood. In the liver, an oxygen gradient is established as oxygen rich blood from the portal triads becomes depleted of oxygen as it flows towards the central vein. Consequently, in transgenic mice, both the epo transgene and the endogenous epo gene are preferentially expressed near the central vein where oxygen tension is lowest[12]

Two Epo producing cell types were identified in the liver: hepatocytes and a non-parenchyma all type.[12, 20]

Expression and production of both epo and epo receptors have been demonstrated in the brain[21-24]. Oxygen related expression of epo has been observed in astrocytes both in vitro in cultured astrocytes[22-24] and invivo[21,24] in the presence of EPO receptors, the inability of EPO to cross the blood brain barrier, and the regulated expression of EPO in the brain have led researchers to propose a paracrine function for EPO in neural tissue. Recent evidence demonstrates that EPO can protect neurons ischemic damage invivo[25]

The discovery that both EPO mRNA and EPO protein are expressed in erythroid progenitors[26, 27] has raised the intriguing possibility that tonic low-level erythropoiesis may be supported by autocrine stimulation, whereas circulating (hormonal) epo provides a more robust stimulus to erythropoietin during hypoxic stress.

EPO Gene expression System- Regulation

A tissue culture model eluded investigators studying the regulation of EPO. Some cells, such as rat kidney mesangial cells[28]. The renal cell line RC-1[29], and hepatic caricnomas[30] produced EPO at very low levels with minimal induction by hypoxia. Two human hepatoma cell lines, Hep3B, and HepG2 were shown to produce significant amounts of EPO consecutively, with marked induction by hypoxia, which was proved to be an invaluable tool in exploring the molecular basis of EPO gene regulation[31].

EPO gene expression is modulated by a number of physiological and pharmacological agents. Regulation of epo by hypoxia and other stimuli occurs at the mRNA level. In the kidneys of mice anemic made by blood loss, EPO mRNA was increased approximately 200 fold over the level in the kidneys of normal control mice[32]. EPO mRNA was included in the liver as well, but at a lower level of expression. The increase in EPO mRNA reached a maximum at 4 to 8 hours after induction. The magnitude of induction was proportional to the degree of anemia. Similarly, injection of cobalt chloride into rats induced EPO expression in the kidney and to a lesser degree, in the liver[33]. The time course and level of induction of EPO mRNA paralleled with induction of EPO in serum were measured by radioimmuno assay[33].

REGULATORY ELEMENTS IN THE EPO GENE

Tissue specific regulatory domains were mapped in subsequent transgenic experiments by use of constructs with varying lengths of EPO upstream flanking sequence.

EPO3' enhancer

A liver specific DNase I hyper sensitivity site was discovered in the 3' flanking sequence of an EPO transgene[34]. Analysis of this region of the EPO gene by transient transfusions of reporter constructs led to the identification of a hypoxically inducible enchancer[34-37]. In both the mouse and human EPO genes, this enhancer lays in a highly conserved region 120bp 3' to the polyadenylation site and its activity was independent of orientation and distance from the promoter. The enhancer demonstrated the same stimulus specificity as the epo gene with responses to hypoxia, cabaltous chloride and iron chelation, but not to cyanide and 2 deoxyglucose.

Promoter

The EPO promoter does not have consensus TATA or CAAT elements in either the mouse or human genes. The EPO promoter contributes to the hypoxic inducibility of the EPO gene[38]. After deletion of the 3' enhancer,

expression of a stably transfected marked EPO gene was induced approximately 10 fold in response to hypoxia[39]. The minimal promoter acts synergistically with the 3' enhancer to confer a 40 – fold induction in response to hypoxia[37].

The minimal epo promoter capable of induction by hypoxia encompasses 117bp5' to the transcription initiation site[37]. A segment of 17bp is responsible for this up regulation by hypoxia[40]. Addition of antisense oligonucleotides GATA elements increased epo gene expression, whereas the addition of antisense oligonucleotides to CACCC elements decreased epo gene expression, indicating that the promoter is regulated negatively by GATA sites and positively by CACCC sites. By inhibiting the formation of DNA binding complexes and by the binding of inhibitory methyl – CPG binding proteins, methylation may contribute to tissue – specific activity of the EPO promoter.

ENHANCED TRANSCRIPTION

Enhanced transcription accounts for most, but probably not all, of the hypoxic induction of the EPO gene. In Hep3B cells, nuclear run – on experiments showed about a 10 fold increase in transcription of Epo mRNA during exposure to 17% O_2 in the steady state level of EPO mRNA[42]. Two proteins, 70 and 135 KD, which have been designated EPO mRNA binding protein (ERBP), bind to a 120 bp pyrimidine rich region in the 3'UTR of EPO mRNA[43]. This interaction does not seem to be regulated by oxygen tension, but binding is subject to redox control[44]. Heat shock protein 70was participant in a complex with ERBP and EPO mRNA[45]. Deletion of the ERBP binding site prolongs the half life of EPO mRNA and eliminates hypoxically induced stabilization[46]. Deletion of a 50 bp segment lying 70 bp down stream of the binding site for these proteins causes a 7 fold increase in the half life of a transected marked EPO gene[39].

HIF – 1: The transcription factor HIF – 1 mediates hypoxically inducible transcription of O_2 regulated genes. The HIF – 1 site in the EPO 3' enhancer is the primary element in the EPO gene that mediates the transcriptional response to hypoxia. The time course of HIF – 1 activation mimics the induction of the EPO gene[47]. Hypoxia, Cobalt, DFO stimuli that trigger epo gene expression also activate HIF – 1 DNA binding.

Insulin and insulin like growth factor 1 (IGF – 1) activate HIF – 1 DNA – binding activity, HIF – 1 mediated transcriptional activation of a reporter gene[48] and expression of several oxygen regulated genes, including epo[49]. The signaling pathways for hypoxia, insulin and IGF – 1 appear to converge, all leading to stabilization of HFF – 1. Activation of HIF – 1 is modulated by a complex set of mechanisms likely to include not only protein stability, but also phosphroylation, [50, 51] redox chemistry[52-54] and nuclear localization[55] subsequent to binding of HIF – 1 to its cognate cis acting sequence, interaction with adjacent transcription factors and co activator proteins are necessary for hypoxic induction of transcription.

Many experiments have indicated that a decrease in levels of oxygen free radicals after hypoxic stimulus leads to the accumulation of HIF – 1 and the activation of HIF – 1. Addition of H_2O_2 or agents that increase intracellular peroxide concentration block the induction of EPO and the accumulation of HIF – 1[52]

In the nitrogen – fixing bacteria, Rhizobium, oxygen regulated gene expression is mediated by hypoxically inducible phosphorylation of a transcription factor,[56,57] phosphorylation may play a role in signaling the hypoxic stimulus and regulation of HIF – 1 activity in mammalian cells as well.

CLINICAL STATES ASSOCIATED WITH DISREGULATION OF EPO GENE EXPRESSION

In a variety of pathological status, dysregulation of epo gene expression may cause either anemia or polycythemia.

Anemia is a major complication of most forms of renal failure. Because the anemia of renal failure is due primarily to a decrease in epo production.[58,59] Patients are successfully treated by administration of recombinant human epo.[60,61] Inappropriately low levels of erythropoietin have been demonstrated in patients with acquired immuno deficiency syndrome (AIDS),[62] rheumatoid arthritis and other chronic inflammatory disease[63] and cancer.[64].

Uremia is the predominant and prototypical clinical syndrome for EPO replacement therapy but recombinant human EPO (rHuEPO) has been used in a broad range of clinical settings[65].

Primary polycythemia is caused by defects of hematopoietic progenitor cells and is associated with low levels of circulating erythropoietin. Familial congenital polycythemias may be caused by EPO receptor mutations. In contrast, secondary polycythemia is generally associated with an increased EPO production. Elevated levels of plasma EPO are encountered in systemic hypoxemia, in certain neoplasms, and less commonly, in disorders that impair oxygen delivery to tissues.

Excessive activation of EPO gene expression can result from impaired O_2 delivery due to high affinity hemoglobin mutants, methemoglobinemia and 2, 3 biphosphoglycerate dificiency.[66] Chronic arterial hypoxemia often leads to an up regulation of EPO expression, causing a maladaptive erythrocytosis, similarly, patients with right to left cardiac shunts can have extremely high hematocrit levels.

Specific types of neoplasms can also cause over production of EPO. Elevated EPO levels are found most commonly in patients with renal carcinomas. Benign renal tumors can also cause erythrocytosis, possibly due to ischemia of renal EPO-producing cells.

CONCLUSIONS

Regulation of erythropoiesis and red blood cell mass relies on modulating EPO gene expression in response to tissue O_2 tension. Developmental, tissue specific and environmental signals all contribute to the precise regulation of the EPO gene. EPO production and gene expression is restricted to specific subsets of cells; interstitial fibro blast like cells in the kidney and in the liver.

The EPO gene has been a model for the regulation of gene expression by O_2 tension. The magnitude of hypoxically inducible transcription of the EPO gene is greater than only other gene known to be regulated by O_2 tension. Human hepatoma cell lines have provided a useful model system for studying inducible expression of the EPO gene.

The EPO gene has been the portal through which a generalized system of O_2 regulated gene expression was first identified and described. In mammals, HIF-1 of the O_2 sensing mechanism that regulates EPO are critical for the regulation of genes involved in angiogenesis, energy metabolism, respiration, vascular tone and many other processes.

In humans, regulation of the EPO gene provides an elegant and precise mechanism for adjusting red blood cell mass to perturbations in tissue oxygen tension. A more complete understanding of the molecular mechanisms governing induction of the EPO gene may lead to new therapeutic agents to treat patients with anemia or polycythemia due to inappropriate expression of the EPO gene.

Acknowledgement: The authors are thankful to the management of A.V.V.M. Sri Pushpam College (Autonomous), Poondi, for providing them necessary facilities and support to carry out this work.

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Source of Support: Nil, **Conflict of Interest:** None.