Erythropoietin: A Hormone with Multiple Functions

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Abstract

Erythropoietin (EPO), the main hemopoietic hormone synthesized by the kidney as well as by the liver in fetal life, is implicated in mammalian erythropoiesis. Production and secretion of EPO and the expression of its receptor (EPO-R) are regulated by tissue oxygenation. EPO and EPO-R, expressed in several tissues, exert pleiotropic activities and have different effects on nonhemopoietic cells. EPO is a cytokine with antiapoptotic activity and plays a potential neuroprotective and cardioprotective role against ischemia. EPO is also involved in angiogenesis, neurogenesis, and the immune response. EPO can prevent metabolic alterations, neuronal and vascular degeneration, and inflammatory cell activation. Consequently, EPO may be of therapeutic use for a variety of disorders. Many tumors express EPO and/or EPO-R, but the action of EPO on tumor cells remains controversial. It has been suggested that EPO promotes the proliferation and survival of cancer cells expressing EPO-R. On the other hand, other reports have concluded that EPO-R plays no role in tumor progression. This review provides a detailed insight into the nonhemopoietic role of EPO and its mechanism(s) of action which may lead to a better understanding of its potential therapeutic value in diverse clinical settings.

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Introduction

Erythropoietin (EPO) acts as a major regulator of erythropoiesis by promoting the survival, proliferation, and differentiation of erythroid progenitor cells and regulating the number of erythrocytes in peripheral blood. The kidneys are the major site of EPO production in adults, while in the fetuses the liver is the principal site of EPO gene expression. The kidneys produce EPO in response to hypoxia, which in turn increases the red cell mass, thereby improving tissue oxygenation. Within the kidney, EPO is expressed in the cortical peritubular cells. The microanatomical complexity underlying renal oxygenation makes the renal interstitium a suitable site for sensing changes in oxygen availability [1].

Biology of EPO

EPO is a 30.4-kDa glycoprotein. Its gene, in humans, is located on chromosome 7 and occupies a 5.4-kb region of genomic DNA. It encodes a 193-amino acid polypeptide chain [2, 3]. The mature circulating protein consists of 165 amino acids resulting from cleavage of the 27 N-terminal amino acid leader sequence and loss of the C-terminal arginine during posttranslational modification. The EPO molecule encloses 2 disulfide bonds between amino acids 7 and 161 and between amino acids 29 and 33 that stabilize and preserve the molecular structure of

EPO; their reduction results in loss of bioactivity. The disulphide bond between the cysteine at position 7 and that at position 161 is functionally more important because it acts as a tether ensuring that the molecule maintains the configuration necessary for binding to the EPO receptor (EPO-R) [4].

Thirty-nine percent of EPO consists of carbohydrate moieties, 3 of which are N-linked sugars at positions 24, 38, and 53 (fully sialylated) and 1 which is an O-linked sugar at position 126 [2]. Deglycosylated EPO is biologically active but very short-lived [5]. Sialylation slows the hepatic clearance of EPO, thus prolonging the time EPO remains in circulation to stimulate erythrocyte progenitor cells in bone marrow [4]. The tertiary structure of EPO consists of 4 antiparallel α -helices with adjoining loops.

A single EPO molecule binds to 2 adjacent EPO-R on the target cell membrane and begins an intracellular signaling cascade. Having docked on the cell surface, nuclear messaging outcomes include survival, proliferation, and differentiation of erythrocyte precursors [4].

EPO-R, also known as p66, belongs to the cytokine receptor superfamily and consists of an extracellular domain, a transmembrane domain, and an intracellular domain [4, 6, 7]. Like other hemopoietic growth factor receptors, including those of growth hormone, prolactin, thrombopoietin, oncostatin M, and several interleukins, EPO-R has no endogenous tyrosine kinase activity by which to activate receptor signaling. As previously stated, a single EPO molecule binds to 2 adjacent receptors on the cell surface. The resultant dimerization of EPO-R activates a receptor-associated tyrosine kinase (Janus Kinase 2 or Jak2) by transphosphorylation. The specific tyrosines in the intracellular portion of EPO-R that have been phosphorylated serve as docking sites for intracellular proteins including STAT5, a signal transducer and transcription activator of several cascades. Once activated, STAT5 is translocated to the nucleus where it recognizes a specific base sequence in the promoter region of its target gene and initiates the transcription of erythroid genes. Thereafter, phosphatases dephosphorylate Jak2 and downregulate the receptor [7–10].

EPO-R has been investigated in a variety of erythroid cell types. The receptor numbers range from 34 to approximately 3,000/cell [11, 12]. It is of note that the molecular weight of EPO-R found in diverse tissues differs from that found in cells wherein it exerts a differentiation/maturation effect [13]. In addition, studies of the thermodynamics of binding revealed 2 different affinity classes of EPO-R, i.e. high and lower affinity class bind-

ing sites [14, 15]. It has been speculated that high-affinity receptors are necessary for differentiating effects on hemopoietic cells, whereas low-affinity receptors simply promote the proliferative action of EPO [13]. However, other studies have identified only 1 class of receptors [16]. There is no clear molecular explanation for these differing observations.

EPO Production

Research on the regulation of EPO production has led to the identification of a broadly distributed cellular oxygen-sensing mechanism. The production and secretion of EPO and the expression of EPO-R are regulated by the tissue oxygen supply. Hypoxia results in an increase in the level of gene transcription and the production of both EPO [17–19] and EPO-R [3] via activation of the hypoxia-inducible factor 1 (HIF-1) pathway [18, 20]. All HIF family members (HIF-1 α , HIF-1 β , and HIF-3 α) play a significant role in regulating EPO and EPO-R expression. The physiological concentration of EPO in healthy animals and humans is in the range of 4–30 mU/ml of plasma; however, in severe anemia 100-fold increases can be seen [13]. There are no preformed stores of EPO [6].

It remains to be established which renal cell(s) produces EPO. The list includes proximal tubular cells [21] and glomerular [22], mesangial [23] and interstitial cells of the renal cortex [24–28]. This lack of agreement is probably due to low sensitivity in the detection of EPO. Recently, Obara et al. [29] identified EPO-producing cells as peritubular interstitial cells expressing neuronal markers showing a unique stellar or arborizing configuration with long multidirectional projections. Such cells appear to form a reticular network between renal tubules and capillaries.

Koury et al. [30] estimated that 20–30% of interstitial cells in the inner cortex, and less than 10% in the subcapsular cortex produce EPO. When the number of interstitial cells exhibiting EPO mRNA was calculated in relation to anemia it was noted that they exponentially increase in number, paralleling both total EPO mRNA and serum EPO levels. Thus, EPO production correlated with increased numbers of interstitial cells producing EPO mRNA.

The liver is the primary site of EPO production in the fetus [31, 32]. Hepatocytes surrounding central veins contain EPO mRNA [33]. Hepatic Ito cells, which show many similarities to the EPO-producing renal fibroblast-like interstitial cells, also appear to be EPO producers [34].

In adults, the kidney is the source of the circulating EPO that maintains the erythrocyte mass. It is transported in the plasma to target tissues wherein it inhibits apoptosis of erythrocyte precursors in a negative feedback fashion. EPO expression by some cells which bind to EPO-R in the brain and endothelial cells have a paracrine function. The ability of some astrocytes to synthesize EPO is an example of an autocrine process involving this hormone [4, 35].

EPO and Erythropoiesis

Erythropoietin is essential for the survival, proliferation, and differentiation of erythrocyte progenitors in bone marrow. Erythrocyte production is continuously adjusted to regulate the loss of senescent red blood cells and to guarantee optimal tissue oxygenation.

Erythropoiesis is regulated by several cytokines. Growth factors involved include granulocyte colony-stimulating factor (G-CSF), interleukin (IL)-6, stem cell factor (SCF), IL-1, IL-3, IL-4, IL-9, IL-11, granulocyte-macrophage colony-stimulating factor (GM-CSF), insulin-like growth factor (IGF-1), and, of course, EPO [36]. EPO acts in later stages of the maturation of erythroid progenitor cells. Its primary target cells in bone marrow are colony-forming unit erythroid (CFU-E) cells. EPO prompts these cells to proliferate and mature from normoblasts into reticulocytes and onto mature erythrocytes [37]. Actually, CFU-E are the most EPO-sensitive cells with the highest density of EPO-R on their surfaces [38].

The normally low concentration of EPO enables only a small percentage of progenitors to survive and proliferate, whereas the remaining progenitors undergo apoptosis. In contrast, when the concentration of EPO rises in blood (either endogenously or exogenously), many more burst-forming unit erythroid (BFU-E) and CFU-E escape from apoptosis and proliferate to result in the growth and maturation of proerythroblasts and normoblasts [38].

EPO acts synergistically with SCF, GM-CSF, IL-3, IL-4, IL-9, and IGF-1 to induce red cell precursors to proliferate and mature from the stage of BFU-E and CFU-E cells to the normoblast stage [39, 40]. Thus, EPO is the critical growth factor that acts on bone marrow erythroid progenitor cells to prevent them from undergoing apoptosis [36].

In bone marrow, erythroid progenitors express both EPO and EPO-R. In contrast, only EPO-R is present in megakaryocytes, myeloid cells, and endothelial progenitors [3].

A reduction in the number of red blood cells does not directly stimulate EPO synthesis and release. Instead, EPO production is controlled by the systemic availability of oxygen in a tightly regulated feedback loop. The oxygen-dependent control of EPO formation requires sensing mechanisms that perceive changes in the oxygen supply and translate them into alterations of EPO gene activity in the liver and kidneys. These mechanisms are the key element in the feedback control of erythropoiesis and are sensitive to conditions that affect arterial pO_2 and tissue and venous pO_2 [41]. Because renal cell preparations producing EPO in vitro are not available yet, studies on the molecular control of EPO gene activity have been performed in hepatoma cells and hepatocytes. In situ hybridization has shown that EPO is mainly expressed in pericentral areas of the hepatic lobules. This is consistent with local oxygen gradients because tissue oxygen tension in the pericentral part of the hepatic lobule is lower than in the periportal area. In vitro experiments have also shown that isolated hepatocytes can directly change the rate of EPO production in response to changes in their oxygenation [41]. Therefore, EPO levels remain constant under physiological conditions, whereas in anemias and reduced renal oxygen supply EPO secretion increases with a resultant enhancement of erythrocyte production [42]. In contrast, decreased EPO concentrations are typical of conditions with an increased oxygen supply or a reduced oxygen demand [41]. Figure 1 illustrates the EPO endocrine feedback loop involved in the regulation of erythropoiesis.

It is of note that recombinant human EPO (rHuEPO) is illegally used as a performance-enhancing agent in endurance sports. According to Robinson et al. [43], blood tests used to detect such 'doping' are essential and may succeed in limiting the misuse of rHuEPO. Interlaboratory standardization of methods and quality control in all doping analyses undertaken by World Anti-Doping Agency-accredited laboratories is mandatory [44].

EPO and EPO-R Expression in Nonerythroid Tissues

Aside from the kidney and liver, EPO and EPO-R are expressed in the brain and in the cardiovascular, digestive, endocrine, female and male reproductive, and respiratory systems.

EPO possesses novel physiological functions in the brain. Neurons express EPO-R [45, 46] and astrocytes produce EPO [47, 48]. Thus, the central nervous system can be regarded as a paracrine EPO/EPO-R system unre-

Fig. 1. EPO endocrine feedback loop. Regulation of adult erythropoiesis by renal EPO based on the O_2 supply to the tissues.

lated to erythropoiesis. In peripheral nerves, EPO-R is expressed only in myelin sheaths (Schwann cells) [49].

In the cardiovascular system, EPO and EPO-R are expressed in endothelial cells [50, 51] as well as in smooth muscle cells [52–54]. EPO-R is also expressed in cardiac myocytes [55, 56]. Recent studies conclude that functional EPO-R is undetectable in endothelial, cardiac, neuronal, and renal cells [57], thus challenging the notion that the receptor plays a physiological role in nonhematopoietic cells.

With respect to the digestive tract, cells in the gastric mucosa [58] and enterocytes in the fetal and neonatal small intestine of the rat [59, 60] express EPO-R.

In the endocrine system, expression of EPO-R has been demonstrated in insulin-producing cells of the pancreatic islets [61] as well as in parathyroid cells [62]. EPO and EPO-R are also expressed in the pituitary gland [63].

In the female reproductive tract of humans, EPO is expressed in the cervix, endometrium, ovary [64] and oviduct [65]. EPO-R is present in vascular endothelial and smooth muscle cells, endometrial decidual cells, glandular epithelial cells, and ovarian follicles at various

stages of development, including oocytes, granulosa, theca interna, and lutein cells [64]. EPO plays an important angiogenic role in female reproductive organs via its receptor, which is expressed in vascular endothelial cells of the endometrium [66]. Whereas EPO production in the kidney, liver, and brain is hypoxia-inducible, in the oviduct and endometrium it is modulated by estrogen 17β (E2) and the oxygen supply [65, 66].

With respect to the male reproductive system, the testis produces both EPO and EPO-R. Sertoli and peritubular myoid cells in particular express EPO mRNA [67], whereas Leydig cells express only EPO-R [68]. EPO production has also been demonstrated in the epididymis [69].

Zhang et al. [70] found EPO-R protein in the maturing lungs of dogs, suggesting a role for paracrine EPO signaling in growth and remodeling.

High concentrations of EPO are present in human milk [71–73] and milk from other species, such as the rat [74], suggesting that it plays a role in the erythropoiesis, neurodevelopment, and gut maturation of neonates.

Tissue-Protective Effects of EPO

EPO is a tissue-protective hormone with more pleiotropic potential than had previously been thought. It can prevent the tissue destruction surrounding a site of injury by signaling via a nonhemopoietic receptor [75]. EPO also prevents apoptosis in reduced or absent oxygen tension, excitotoxicity, and free radical exposure [76, 77]. The latter are major mediators of injuries produced as by-products under hypoxic conditions. Endogenous, locally produced EPO can prevent injury under these circumstances. Hypoxia activates the HIF family which, in turn, activates a number of protective genes, such as those encoding EPO [78].

The development of an infarct is a complex process involving apoptotic and necrotic cell death, neutrophil infiltration and inflammation, changes in the extracellular matrix, and numerous other factors [79]. Stroke and myocardial infarction are due to ischemic injury characterized by a central zone of rapid cell death surrounded by 'at risk' tissues. It seems that when a stroke/infarct takes place, the primary focus undergoes necrosis and the cells in the surrounding tissue, affected but not rapidly dead, undergo apoptosis [79, 80]. EPO has an effect on this surrounding tissue, lessening the apoptosis ratio compared with nontreated animals [79]. Treatments preserving the brain and myocardium following infarction aim to eliminate or abbreviate the ischemic episode and reduce its effects.

Several *in vivo* studies have confirmed the beneficial neuroprotective effect of EPO in traumatic brain or spinal cord injuries as well as in infarction. Cells possess a remarkable ability to adapt to stress by developing resistance to subsequent injury. Termed 'preconditioning', this powerful adaptive phenomenon apparent in ischemia enhances the tolerance to subsequent lethal stress. Prass et al. [81] were the first to present evidence that EPO is protective and essential in hypoxia. When preventively administered, EPO mimicked ischemic preconditioning, protecting neuronal and cardiac cells against diverse stresses, including lethal ischemia and cytotoxic drugs [82]. Exogenous EPO administered after such stress as cerebral or cardiac ischemia was also capable of protecting affected cells against deleterious effects, thus suggesting that EPO can be of therapeutic use in ischemic episodes [83]. Dawson provided further evidence for the role of EPO in ischemic preconditioning [84]. As shown by Rusher et al. [85] in an *in vitro* model of ischemic preconditioning, EPO released by astrocytes acts as a paracrine mediator resulting in neuroprotection. Although circu-

lating EPO does not cross the blood-brain barrier, after cerebral infarction (stroke) the blood-brain barrier becomes permeable, thus making brain cells accessible to blood-born EPO [86].

Both *in vivo* and *in vitro*, EPO has potent neuroprotective properties and appears to act by directly protecting neurons from acute ischemic damage and by stimulating endothelial cells to induce protracted angiogenesis [86, 87].

In the rat, EPO also exerts a neuroprotective effect following (a) spinal cord injury [88] and acute traumatic brain injury, probably improving energy metabolism in the acute phase [89], (b) subacute traumatic brain injury after a 10-day interval, likely reducing the lesion's volume and cell loss as well as enhancing neurogenesis [90, 91], and (c) ischemic injury.

In addition to preventing apoptosis, EPO was also involved in neuronal progenitor cell development through the activation of nuclear factor- κ B which stimulates the production of neural stem cells [92]. EPO also significantly enhanced the survival of neural stem cells but did not affect neuronal differentiation or migration [93].

It is of note that EPO can reduce cognitive and behavioral symptoms after mechanical injury to the hippocampus [94]. It may also be useful in the treatment of Alzheimer's disease since, as a cytoprotective agent, EPO can modulate several cellular pathways. Thus, it may provide protection against amyloid toxicity [95, 96].

With regard to its cardiovascular protective effects, EPO minimizes myocardial cell apoptosis and decreases infarct size with a resultant improvement in left ventricular contractility. In experimental animals subjected to coronary ligation, EPO not only reduces myocardial infarct size but also prevents ischemia/reperfusion injury and it promotes ventricular remodeling [97, 98]. These protective effects may be due to a reduction of apoptosis in cardiac myocytes [99–101] and fibroblasts [99]. Other studies stated that in patients with congestive heart failure EPO improves ventricular performance and exercise capacity and reduces hospitalization rates [102–104].

EPO may reduce renal injury due to ischemia/reperfusion and it may accelerate renal tubular regeneration, reducing the severity of renal failure. It appears that EPO exerts its renal protective effect by inducing renal HIF-1 production [105]. It is also known that EPO maintains the integrity of podocytes by preserving their cytoskeleton [106].

It has also been demonstrated that EPO protects against intestinal ischemia/reperfusion injury in rats by

reducing oxidative stress, apoptosis, and leukocyte infiltration [107, 108].

In summary, EPO interferes with apoptosis, inhibits the spread of infection by restricting injury to adjacent tissues, and minimizes the effects of ischemia, trauma [90], or toxins [75]. It also interferes with the activities of proinflammatory cytokines and stimulates healing following injury [35].

Recent studies delineate cellular pathways controlled by EPO and have focused on new therapies averting deleterious effects. These once unknown strategies were reviewed by Maiese et al. [109]. Several nonhemopoietic effects of EPO on the nervous and cardiovascular systems and its modulation of immunological and inflammatory mechanisms in wound healing were extensively reviewed by Arcasoy [110].

Other Actions of EPO

Blood vessel development consists of 2 distinct phases: vasculogenesis and angiogenesis. In the former, endothelial progenitor cells form *de novo* vessels. In contrast, angiogenesis is the formation of new vessels from existing vessels through endothelial proliferation, migration, sprouting, and anastomosis.

Physiological angiogenesis is a tightly regulated process. Insufficient angiogenesis induces ischemia and organ failure. As shown in chick [111] and mouse embryos [112], EPO and EPO-R signaling are important in physiological angiogenesis. Interestingly, EPO and EPO-R-null embryos exhibit defective angiogenesis but relatively normal vasculogenesis, suggesting that earlier vasculogenesis is EPO and EPO-R independent [112]. Endogenous EPO/EPO-R are also implicated in the regulation of cyclic uterine angiogenesis [64] and are involved in the wound healing cascade, promoting angiogenesis and granulation tissue formation [113].

Endothelial proliferation is an area of major scientific interest due to the pivotal role of angiogenesis in neoplasia. Human endothelial cells express EPO-R [50]. Synthetic EPO increases endothelial cell proliferation in human umbilical vein and bovine capillary endothelial cell cultures [13]. Due to its chemotactic effects, EPO also increases endothelial cell migration [51]. In primary endothelial cell cultures, it also induces EPO-R, eNOS expression, and NO production, especially in hypoxia [51].

EPO also acts on blood vessels to mobilize bone marrow-derived endothelial progenitor cells and to shift them into circulation [114, 115]. This process both pro-

motes endothelial cell proliferation [116, 117] and stimulates neovascularization [111]. EPO affects the apoptosis and function of immature endothelial cells [118]. The influence of EPO is also manifest in embryonic vasculogenesis persisting into adulthood, suggesting a continuous interrelation between hemopoiesis, vasculogenesis, and angiogenesis [118]. Further investigation is necessary to clarify the precise role of EPO in blood vessel formation.

Vascular smooth muscle cells possess EPO-R, leading to their contraction, in primary smooth muscle cell cultures [53]. EPO also causes vasoconstriction in isolated vessels [52], an effect enhanced in genetically hypertensive rats [119]. EPO/EPO-R also stimulates angiogenesis in diabetic retinopathy [120, 121].

Endogenous EPO signaling is essential in early embryonic neural development and contributes to both neurogenesis and neuron survival in adults. The temporal and spatial regulation of EPO and EPO-R expression in the developing embryo suggests a role of EPO signaling in early neurogenesis [122]. Both EPO and EPO-R-null embryos have no absent major nervous structures although their brains are smaller and less developed than their littermate controls. This underdevelopment also affects their choroid plexus [122]. EPO has also been reported to induce the proliferation of neural progenitor cells and to prompt their proliferation and maintenance; thus, EPO-R expression levels are higher in such cells than in mature neurons [123]. The specific role of EPO and EPO-R in neural development remains to be determined.

McPherson and Juul [60] reported that EPO affects the development of rat intestine wherein it (a) promotes the division of intestinal smooth muscle cells and enterocytes and (b) stimulates the migration of intestinal epithelial cells and prevents their apoptosis.

EPO has the ability to affect immunological mechanisms. It enhances the immune response in murine experimental models [124] and acts on B lymphocyte responses, thus suggesting a role in immunomodulation. Dendritic cells, which initiate the immune response, are targets of the immunomodulatory function of EPO [125] and macrophages are also influenced by EPO [126]. Indeed, an excessive availability of EPO resulted in the enhancement of the proinflammatory phenotype and function of both splenic steady-state and inflammatory-induced peritoneal macrophages [126]. Interestingly, EPO has no effect on neutrophil function [127].

Lastly, it was also reported that EPO possesses anti-inflammatory effects in different animal models, indicating that it decreases apoptosis and reduces the expression of proinflammatory cytokines [124, 128]. The mech-

Fig. 2. Nonerythropoietic actions of EPO in nontumorous tissues. Contributions of EPO activity to tissue protection and other actions.

anisms of the immunological and anti-inflammatory effects of EPO require further investigation. A summary of the nonerythropoietic actions of EPO in nontumorous tissues is outlined in figure 2.

EPO and EPO-R Expression in Tumors

Tumoral hypoxia is known to be associated with poor patient survival. Various factors contribute to it. For example, the abnormal structure and function of tumor microvasculature decreases perfusion, enhances the extravasation of fluid leaking into the extravascular space, and increases the viscous resistance to flow. As a result, a reduced blood flow leads to hypoxia, especially at lower hemoglobin concentrations. Thus, anemia arising as a result of either the malignant process per se or anticancer treatment plays a significant role in reducing tumor oxygenation [129].

Tumor hypoxia has '2 sides' and can be regarded as a prognostic factor. Although tissue oxygen concentrations

below 1% ($pO_2 < 7$ mm Hg) can exert antiproliferative effects, restrict cell proliferation, stimulate differentiation, and induce apoptosis as well as necrosis, some cell clones adapt to hypoxic stress through the modification of gene expression resulting in the emergence of an aggressive phenotype and the promotion of local and distant spreading [129]. Hypoxia also induces angiogenesis and may promote metastasis via the downregulation of adhesion molecules. Affected tumor cells rapidly become adapted and acquire resistance to chemo- or radiotherapy [129].

Opposing mechanisms have been proposed to explain the effects of EPO on tumor growth. According to Jelkmann et al. [130], tumors are often hypoxic and increasing hemoglobin levels with erythropoiesis-stimulating agents may enhance tumor oxygenation with a resultant increased effectiveness of radiation and chemotherapy and, thus, increased patient survival. Alternatively, it has been suggested that EPO directly promotes tumor cell growth. Yasuda et al. [131] reported EPO and EPO-R expression in 24 malignant human tumor cell lines, regardless of histological type, genetic characteristics, and bio-

logical properties. In addition, several individual cell lines secreted small amounts of EPO and responded to hypoxic stimuli by an increased secretion of EPO functioning in an autocrine manner [131]. Udupa [132], who studied 12 tumors and 2 cancer cell lines, detected EPO-R in all tumor types as well as both cell lines. In contrast, only 9 of the 12 tumors expressed EPO. Induction of cell proliferation in tumor cell lines exposed to EPO has been reported [132]. Thus, it is well established that EPO-R is present in most cancer cells and that EPO exerts significant effects on them. What is then the function of EPO-R in cancer cells? Some studies have found induction of proliferation in tumor cell lines exposed to EPO. Therefore, either endogenously produced or exogenously administered, EPO can lead to cell proliferation and improve the survival of EPO-R-expressing cancer cells.

Malignant neoplasms consist not only of tumor cells but also of a capillary network and other supporting cells. Most such tumors contain EPO-responsive elements in the form of both neoplastic cells and vascular endothelium. According to Yasuda et al. [131], EPO contributes to growth, angiogenesis, and viability in almost all malignant tumors, suggesting that they possess EPO-responsive sites. Although autocrine EPO levels are much lower than ambient physiological levels, they may nonetheless be sufficient to preserve the tumor microenvironment and lead to the stepwise acquisition of self-sufficient growth independent of the normal environment of surrounding tissues. As has been demonstrated, administration of EPO-R antagonists inhibits angiogenesis and decreases tumor cell survival. Aside from local EPO concentrations in tumors and/or their environment, the length of exposure to EPO may also be of importance [133]. Hardee et al. [134] found EPO to be an important angiogenic factor regulating tumor cell-induced neovascularization as well as tumor growth during the early stages of tumorigenesis. They also noted a suppression of tumor angiogenesis and progression by EPO blockade, suggesting that EPO is a potential target in the therapeutic modulation of angiogenesis.

If EPO has a direct effect on tumor cell growth and survival, then the neoplastic cells must have a functional EPO-R, i.e. a cell surface protein specifically binding EPO to elicit a response through the activation of signaling pathways. Recent studies have demonstrated the presence of EPO-R in various primary tumors and cancer cell lines. Clinical trials reported adverse outcomes after EPO treatment in anemic patients undergoing cancer chemotherapy [135]. Such results are likely due to tumor progression [136]. Shorter patient survival might be related to the pres-

ence of EPO-R on tumor cell surfaces. Thus, administration of recombinant EPO might increase the growth of a given tumor [137, 138]. On the other hand, Sinclair et al. [139] found no evidence that EPO-R is overexpressed in tumors or present on tumor cell surfaces, thus suggesting that overexpression of EPO-R yields no selective advantage to tumors. Similarly, Fandrey [140] concluded that EPO-R plays no role in tumor progression. These results question the very functional relevance of EPO-R gene transcription in tumors. Given that the EPO-R gene is not an oncogene, there appears to be no selective advantage for tumors overexpressing it. Although EPO mRNA is detectable in tumor cell lines, it is not significantly increased when compared with nontumoral tissues. Lastly, observing a diminution of tumor mass in murine models of T lymphocytic neoplasms, Katz et al. [141] suggested that EPO may act as an antitumor immunotherapeutic agent. Further studies are needed to determine the precise role of EPO-R in tumor progression.

It is of note that commercially available anti-EPO-R antibodies do not accurately detect EPO-R expression since (a) they lack specificity and (b) they cannot distinguish between its cell surface and intracellular expression [136, 142, 143]. It is also known that Western blot cannot discriminate between EPO-R at the 2 locations [144]. Directed against EPO-R, the antibody C20 is of limited utility for detecting EPO-R expression (by using reverse transcriptase polymerase chain reaction, immunofluorescence, and Western blot analysis [145]) since it also recognizes other, non-EPO-R proteins. The latter include several isoforms of heat shock protein (HSP) 70, a highly conserved family of chaperone proteins induced by stress that promote cell survival and resistance to apoptosis. HSPs are highly expressed in tumors, particularly ones with an aggressive phenotype. Their presence correlates with resistance to treatment, formation of metastasis, and, in many instances, a poor prognosis.

EPO-R expression was reported in a variety of tumors and cell lines but a lack of specificity of some anti-EPO-R antibodies has led several authors to question the significance of these immunohistochemical studies in human tumors, concluding that further studies are needed to determine whether EPO-R are in fact expressed.

Potential Risks of EPO Therapy

Adverse effects of treatment are associated with erythropoiesis-stimulating agents, particularly EPO. Jelkmann et al. [130] reported that in patients with chronic

kidney disease EPO administration increased mortality due to cardiovascular and thromboembolic events and possibly tumor growth promotion. Corwin et al. [146] also reported an increased incidence of thrombosis and nonfatal myocardial infarction. The most common adverse effects in cancer patients undergoing combined chemotherapy and EPO treatment include pyrexia, vomiting, shortness of breath, paresthesia, upper respiratory tract infection, diarrhea, and edema [147]. EPO should be used with caution in patients with hypertension since both short- and long-term administration of EPO may significantly elevate blood pressure [148]. Clearly, EPO anti-anemic treatment needs to be a focus to minimize its side effects.

In vivo studies in animal tumors with erythropoiesis-stimulating agents may be grouped into 3 categories: regression of tumor mass and enhancement and no enhancement of tumor-ablative therapies [140]. The majority of in vitro studies showed that erythropoiesis-stimulating agents are likely to have a neutral effect on human cancers [140]. The study of Sinclair et al. [143] confirmed the lack of a tumor-promoting effect. Erythropoiesis-stimulating agents increase the hematocrit of anemic, tumor-bearing animals and improve tissue oxygenation; the extent to which they contribute to restore the effectiveness of radiation and chemotherapy is unsettled.

Future of EPO

The introduction of exogenous EPO in the form of rHuEPO led to significant progress in the management of anemia due to a variety of conditions, including chronic renal failure and chemotherapy. In recent years, research has focused on the use of rHuEPO in patients with cancer. It is available in 5 forms: epoietin- α , epoietin- β , epoietin- ω , epoietin- δ , and darbepoietin- α . All have the same amino acid sequence, but their glycosylation varies as a result of type- and host cell-specific differences reflected in the manufacturing process [83]. For example, the α and β forms are similar in terms of molecular characteristics and pharmacokinetics, but the β form has a higher molecular weight. In contrast, darbepoietin has 2 additional glycosylation sites, hence its increased biological activity and longer half-life [36].

Cytoprotective agents that are effective are precisely targeted and safe. They are used primarily in nervous system and cardiovascular diseases [3]. EPO has been shown to be safe and well tolerated in patients with anemia and

chronic kidney diseases, as well as in acute ischemic stroke and cardiac infarction. EPO may be an ideal cytoprotective agent, but toxicity may occur. In patients with congestive heart failure, elevated EPO plasma levels may increase the severity of the disease and negatively affect the prognosis [98]. This is, in part, due to its hypertensiogenic effect [149]. Thus, the use of EPO is contraindicated in patients with uncontrolled hypertension since both short- and long-term administration can precipitate hypertensive crises. EPO treatment has been associated with other alterations like thrombosis, pyrexia, vomiting, and paresthesia [146].

Apart from erythropoiesis, EPO has roles in hemopoietic and non-hematopoietic organs. In patients with malignancies, an unknown mechanism activates EPO and EPO-R transcription [131]. According to these authors, EPO signal transduction is involved in angiogenesis and therefore in the growth of tumors, and it is a putative mechanism contributing to the development of almost all malignancies. Consequently, factors that generate disordered EPO signaling in tissues appear to contribute to malignant transformations [131, 150]. The nature and effect of such factors remain to be elucidated.

Several authors have shown that EPO is indispensable to the growth and viability of malignant tumors. Thus, disruption of EPO signaling may be a promising treatment. Many studies have focused on reducing the harmful side effects of EPO. For example, it has been found that extended and once weekly doses of EPO provide comparable safety and efficacy in the treatment of chemotherapy-induced anemia [151]. It is of note that prolonged administration of EPO may not be as beneficial since it can induce the formation of anti-EPO antibodies, occasional red blood cell aplasia, and reduced EPO-R expression on cell surfaces. The latter results in loss of EPO function at any concentration [3]. Further studies are needed to separate the deleterious and beneficial effects of EPO.

At present, according to the US National Institutes of Health website (clinicaltrials.gov) a total of 457 trials are under study using 'erythropoietin' as the keyword. Collectively, these trials seek to analyze the clinical effects of EPO in patients with a variety of disorders, including anemia, kidney disease and renal failure, myocardial infarction, cancer, and brain and spinal cord injury. Of these, 103 are still recruiting volunteers or have not yet been started. The other 347 trials are open studies; some are ongoing, others have been completed, and 14 terminated with results. Six were withdrawn prior to recruitment and 1 study was suspended.

Conclusions

EPO increases the red cell mass resulting in an elevated oxygen delivery. This may act as a survival factor for cells that express EPO-R. We have reviewed EPO and EPO-R expression in various tissues in its disease states and have discussed the clinical uses and side effects. EPO offers protection to the neurological and cardiovascular systems and has been shown to influence the immune response. We have also discussed the potential dangers of EPO and their role in decreased patient survival. Further

studies are underway to explore the full potential of EPO and its benefits as well as the elimination of side effects. We are confident that the future of EPO therapy is promising. Obviously, further experimental studies and clinical trials are needed in order to fully understand the mechanisms underlying the effects of EPO.

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