Editorial Comments

Erythropoietin receptors: their role beyond erythropoiesis

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Introduction

It has been known for ~40 years that erythropoietin, which is mainly produced by the kidney in response to hypoxia, is the primary regulator of red blood cell production and is indispensable for terminal differentiation of erythroid progenitors. It controls proliferation, maturation and also survival of erythroid progenitor cells. The binding of erythropoietin to its receptor, which exists as a preformed dimer, induces a conformational change that brings constitutively associated Janus family tyrosine protein kinase 2 (JAK2) molecules in close proximity and stimulates their activation by transphosphorylation. In turn, JAK2 molecules phosphorylate tyrosine residues in the cytoplasmic domain of the erythropoietin receptor, which then serve as docking sites for various intracellular signalling proteins that contain Src homology 2 (SH2) domains (Figure 1). These proteins can then be activated through JAK2-mediated tyrosine phosphorylation. For example, the transcription factor STAT5 (for ‘signal transducer and activator of transcription 5’) can bind to phosphorylated erythropoietin receptors, become phosphorylated, homodimerize, translocate into the nucleus and activate target genes. Other pathways activated by the erythropoietin receptor through protein phosphorylation include the Ras/MAP kinase and phosphatidylinositol 3-kinase (PI3-kinase) pathways (reviewed in [1]).

Initial northern blot analyses showed that the erythropoietin receptor was selectively expressed in cells of the erythroid lineage, which was consistent with an effect of erythropoietin restricted to red blood cell production. However, subsequent studies using more sensitive methods disclosed a more widespread expression of this receptor, raising the possibility of extra-erythropoietic effects of erythropoietin. For example, the presence of mRNA encoding the erythropoietin receptor has been detected in brain, retina, heart, skeletal muscle, kidney and endothelial cells [2,3]. In addition, low-level expression of erythropoietin mRNA also has been demonstrated in tissues other than kidneys and liver, in particular the brain, suggesting paracrine extraerythropoietic actions of erythropoietin [3]. Recently, the importance of erythropoietin signalling outside the bone marrow has been demonstrated during embryonic development. Moreover, studies in various experimental models have shown that injection of high doses of erythropoietin can protect against acute organ injuries.

Erythropoietin and embryonic development

Analyses of mice harboring a null mutation of the erythropoietin or the erythropoietin receptor gene have shown that erythropoietin is indispensable not only for red blood cell production, but also for normal development of brain, heart and blood vessels. In mice with a disrupted erythropoietin system, the developing heart displays ventricular hypoplasia, defects in the interventricular septum and abnormalities of the vascular network [4]. Similarly, in mice with a targeted deletion of the erythropoietin receptor gene, brain development is normal until 10.5 days post conception, but at subsequent stages it displays a reduction in the number of neural progenitor cells and increased apoptosis of neuronal cells [5]. Analysis of blood vessel formation in erythropoietin and erythropoietin receptor null embryos also shows that vasculogenesis occurs normally, but that angiogenesis is impaired severely [6].
Erythropoietin and experimental acute organ injuries

Nervous system

In 1998, Sasaki’s group [7] reported that intraventricular infusion of erythropoietin can protect neurons against ischaemic injury. Following this pioneer work, experimental data, published mostly by Cerami’s group, have shown that erythropoietin can cross the blood–brain barrier and that systemic injections of large doses of erythropoietin can decrease brain or spinal cord damage in various experimental models. For example, they protect against the consequences of a blunt trauma of the head [8] or spinal cord [9] or of an acute ischaemic injury of the brain, spinal cord or retina [8,10–12]. Erythropoietin also has protective effects in experimental models of multiple sclerosis or status epilepticus [8,13] and it can prevent or partially reverse experimental diabetic neuropathy [14]. In vivo and in vitro analyses have shown that these neuroprotective effects are associated with a strong inhibition of apoptosis [10–12] and with neurotrophic activity of erythropoietin [12]. Such experiments have also shown that erythropoietin can inhibit the release of proinflammatory cytokines and the infiltration by inflammatory cells [13].

Heart

Consistent with data from knockout mice, which show that the heart expresses functional erythropoietin receptors, injections of large doses of erythropoietin can protect against the consequences of transient or permanent coronary artery occlusion [15–18]. After the initial phase, animals treated with erythropoietin exhibited a reduction in myocardial damage, as assessed by histological analyses and by measurement of haemodynamic parameters. These improvements were associated with decreased apoptotic cell death [15–17].

Kidney

Since 2003, different groups have shown that pretreatment of animals with erythropoietin can protect the kidney against ischaemia–reperfusion injury [19–23]. In these studies, injection of erythropoietin at a dose ranging from 300 to 5000 IU/kg provided effective protection against renal dysfunction and reduced morphological damage. These protective effects were associated with a decrease in apoptotic cell death and in caspase activity, but not with increased cell proliferation [19,21].

Mode of action of erythropoietin

Inhibition of apoptosis appears to be essential for the tissue-protective effects of erythropoietin. Different pathways responsible for these antiapoptotic effects have been identified (Figure 2). First, binding of erythropoietin to its receptor provokes the phosphorylation and homodimerization of STAT5, which can then enter the nucleus, bind to cis-acting elements and enhance the transcription of various genes, including Bcl-XL, a gene encoding an antiapoptotic molecule of the Bcl-2 family [24]. Second, binding of erythropoietin to its receptor can lead to the phosphorylation of PI3-kinase, which in turn activates protein kinase B/Akt by phosphorylation [25]. Akt will then phosphorylate and inactivate proapoptotic molecules, such as caspase 9, Bad or glycogen synthase kinase-3β. It also phosphorylates I-κB, which activates the transcription factor NF-κB, and induces cytoplasmic retention of FOXO transcription factors through their phosphorylation. Genes encoding proapoptotic molecules, such as Fas ligand or Bim, are activated by FOXO proteins. Third, analysis of the effects of erythropoietin on neuronal cells has shown that erythropoietin can induce the phosphorylation of I-κB (inhibitor of NF-κB) and, thus, activate the transcription factor NF-κB, which in turn enhances the

Fig. 1. Schematic representation of the consequences of the binding of erythropoietin (EPO) to its receptor. Conformational changes of the receptor (A, B), transphosphorylation of associated JAK2 molecules (B) and phosphorylation of tyrosine residues in the cytoplasmic tail of the receptor (C) allow binding and phosphorylation of signalling molecules (D). See text for details.
transcriptional activity of target genes encoding anti-apoptotic molecules, such as XIAP and c-IAP2 [26].

Fourth, in kidney, Yang et al. [19] have shown that erythropoietin induces heat shock protein 70 (Hsp70) and that inhibition of Hsp70 expression eliminates the protective effects of erythropoietin. The antiapoptotic effects of Hsp70 include inhibition of apoptosis protease-activating factor-1 and of apoptosis-inducing factor.

Recently, Cerami’s group has shown that some of the cytoprotective effects of erythropoietin are mediated through its binding to heterodimers containing the erythropoietin receptor and the common beta receptor [27]. Interestingly, carbamylated erythropoietin binds to these heteroreceptors and exerts tissue protective effects, while it does not bind to the classical erythropoietin receptor and does not stimulate erythropoiesis [27–29]. This is the first evidence suggesting that erythropoietin receptors expressed in different tissues are not identical. Further analysis of such differences will probably be essential for understanding different effects of erythropoietin in different tissues.

Clinical implications of the tissue-protective effects of erythropoietin

The above-mentioned data raise the question as to whether extraerythropoietic effects of erythropoietin are relevant in patients treated with recombinant erythropoietins for the correction of anaemia. However, it is important to emphasize that, so far, extraerythropoietic effects have usually been demonstrated only with concentrations of erythropoietin that are much higher than those usually achieved during anaemia management with erythropoietin. In addition, there is some evidence that the expression of both erythropoietin and its receptor in various tissues is stimulated by hypoxia, so that anaemia correction might even down-regulate local paracrine erythropoietin signalling.

On the other hand, the experimental data suggest that patients with acute organ ischaemia could benefit from (pre-)treatment with high doses of erythropoiesis-stimulating agents. In 2002, Ehrenreich et al. [30] reported the results of a pilot trial that included 40 patients who received either relatively large doses of
Erythropoietin treatment was associated with better clinical outcomes and with a trend towards reduction in infarct size. With respect to other types of organ damage, such as acute renal failure, there are situations in which early administration of high doses of epoetin could theoretically have beneficial effects, such as those associated with a high risk of acute tubular necrosis. Similarly, injection of a high dose of epoetin to kidney donors could possibly decrease the risk of graft failure. However, although the experimental data reported are impressive, comparison of these publications also suggests a significant variability in the results, which raises some concern about their application to the clinical situation. In any case, robust clinical trials proving such potential benefits will be required before the use of erythropoietin for indications other than anaemia correction can be recommended.

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References