Mini-Review

The Erythropoietin Receptor: Its Role in Hematopoiesis and Myeloproliferative Diseases

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ERYTHROPOIETIN (EPO)[1] is a serum glycoprotein hormone required for the survival, proliferation, and differentiation of committed erythroid progenitor cells (21), and is the principal hormone regulating the level of circulating red blood cells. The administration of recombinant human EPO to anemic patients suffering from chronic renal failure, AIDS, or bone marrow suppression due to chemotherapy has dramatically alleviated their need for blood transfusions. In this review we focus on several aspects of the cellular receptor for EPO (EPO-R): its expression, its structure and function, the role of receptor mutations in human disease, and two ways in which the receptor can be activated for proliferation independent of hormone. In animal models such growth factor–independent activation of the EPO-R can initiate the development of leukemia.

Erythropoietin Receptor Expression

In contrast to many other hematopoietic growth factors, EPO acts primarily on erythroid progenitors within the fetal liver and adult bone marrow. The earliest erythroid progenitors identified by cell culture are the slowly proliferating burst-forming unit-erythroid (BFU-E) cells, which are not responsive to EPO alone. After growth in the presence of additional growth factors (GM-CSF, IL-3, or SCF), "mature" BFU-E develop which are weakly responsive to EPO. Further proliferation in culture gives rise to colony-forming units-erythroid (CFU-E) which are highly responsive to EPO and generate erythroblast colonies. The sensitivity of these erythroid progenitors to EPO is transient. Beyond the late basophilic erythroblast stage, a nondividing cell, the level of the EPO-R drops and the cells are no longer dependent on EPO for continued maturation. Whether EPO affects differentiation as well as proliferation is not clear.

An EPO-R is also expressed by other nonerythroid hematopoietic cells, including megakaryocytes (14), embryonic stem cells and their differentiated hematopoietic progeny (18, 33), and a number of cell lines arrested at different stages of lineage development (18). The functional relevance of this developmentally diverse EPO-R gene expression is not clear. Also, an EPO-R has been detected in placenta (32), where it may function in transepithelial transport of EPO (20), and in endothelial cells (1). Quite apart from its function in hematopoiesis, EPO-Rs may play other roles in non-hematopoietic cells.

Erythropoietin Receptor Structure

A murine EPO-R cDNA was isolated by expression cloning (8) and encodes a type I membrane-spanning protein of 66 kD which is a member of the cytokine, or hematopoietin, receptor superfamily (Fig. 1). Initial binding studies on transiently transfected COS cells detected two affinities for EPO (k_D = 30 pM and 210 pM) (8); however, subsequent studies have detected a single binding affinity for EPO (k_D = 300–800 pM) in heterologous hematopoietic cells (10, 27), fibroblasts (9), or COS cells transfected with the EPO-R cDNA (D. J. Hilton, S. S. Watowich, and H. F. Lodish, unpublished results). Binding studies on human and murine erythroid progenitor cells have detected either one (4) or two (30) affinities for radioiodinated EPO. Two affinities for EPO may suggest the presence of other components which modulate the binding activity of the cloned EPO-R. However, it is unresolved whether there are indeed EPO-Rs of multiple affinities.

Proteins of ~100 and ~85 kD that cross-link to EPO are detected on the surface of a variety of cell types examined and limited peptide mapping suggests that these two proteins may be related (31). Conflicting studies have suggested that the 100–110- and 75–85-kD proteins are or are not immunologically related to the cloned EPO-R (25, 26). A third component of ~95 kD, specific to hematopoietic cells and immunologically unrelated to the EPO-R, was also detected by EPO cross-linking (26). Clearly, further work remains to delineate the molecular structure of the cell-surface EPO-R complex.

A constitutively active (hormone-independent) form of the

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1. *Abbreviations used in this paper*: BFU-E, burst-forming unit-erythroid; CFU-E, colony-forming units-erythroid; EPO, erythropoietin; EPO-R, EPO receptor; F-MuLV, Friend murine leukemia virus; hGH-R, human growth hormone receptor; PV, polycythemia vera; SFFV, spleen focus-forming virus.

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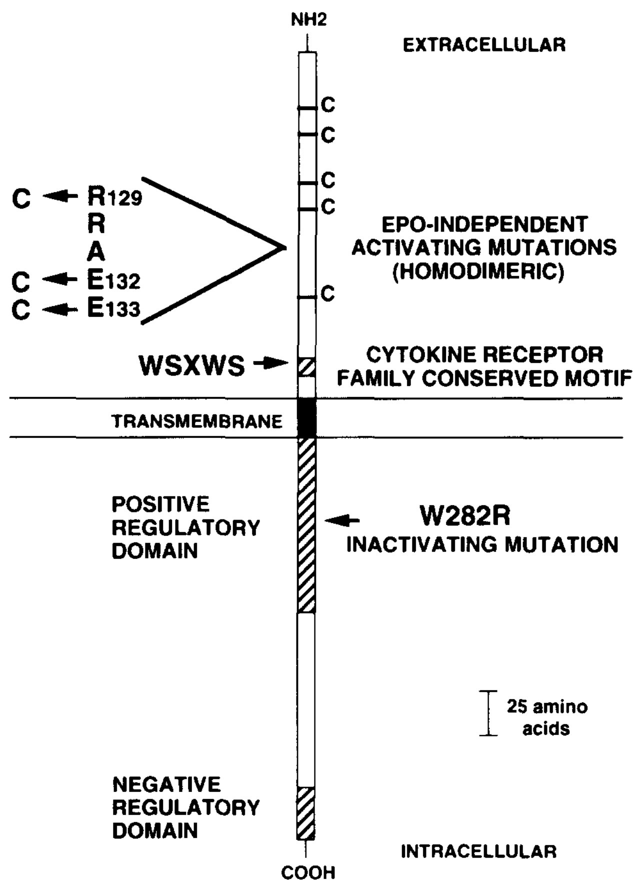

Figure 1. Functional organization of the EPO-R molecule. The schematic diagram shows the relative position of the four conserved Cys residues (initial 4 horizontal black bars from NH2 end; *C*), a fifth Cys (black bar; *C*) unique to the EPO-R, the activating point mutations R129C, E132C, E133C, the conserved WSXWS motif, the inactivating W282R mutation, and cytoplasmic positive and negative regulatory domains of signal transduction.

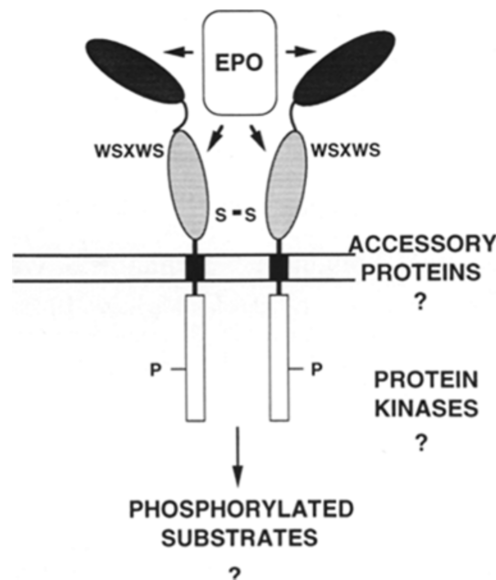

Figure 2. Schematic model of the EPO-R complex. Ligand(s) (*EPO*) induce homodimerization of the EPO-R. By analogy with the structure of the hGH-R, the amino acids that generate the activating mutations R129C, E132C, and E133C, are thought to be at the dimer interface. The WSXWS motif is not at the dimer interface or ligand binding site(s). Phosphorylation (*P*) of both the EPO-R and additional substrates may occur following dimerization initiating the intracellular signaling pathway. The identity and role(s) of accessory proteins in the EPO-R complex is not clear.

EPO-R was isolated from a retroviral transduction system and found to contain a single point mutation, resulting in an Arg to Cys change at residue 129 of the exoplasmic domain (Fig. 1) (37). The R129C receptors form disulfide-linked homodimers independent of hormone, which are assembled in the ER and are transported to the plasma membrane, where they bind EPO with a single affinity (k_D = 700 pM) (34). Since several members of the cytokine receptor family are active as ligand-induced homodimers (12, 15), we hypothesized that the disulfide-linked dimers might mimic the structure of the hormone-bound form of the EPO-R (Fig. 2). Crystal structure analysis of the related human growth hormone receptor (hGH-R) demonstrated that a single nonsymmetrical GH molecule induces homodimerization (12). Residues in the membrane-proximal region of hGH-R stabilize the ligand-induced homodimer and an alignment of the hGH-R and EPO-R sequences shows that Arg129 of the EPO-R maps to this dimer interface region. Mutation of residues flanking Arg129 to cysteine also generates constitutively active EPO-Rs, which form disulfide-linked homodimers in the absence of EPO (Fig. 1; S. S. Watowich, D. J. Hilton, and H. F. Lodish, manuscript submitted for publication), further suggesting that ligand-induced dimerization of the wild-type EPO-R is crucial for transmembrane signaling.

Signaling by the Erythropoietin Receptor

Although the cytosolic domain of the EPO-R does not contain an obvious protein kinase domain, phosphorylation of cytoplasmic proteins appears to be important for EPO-induced proliferation (27). A recent study using chimeric cytokine receptors suggests that the exoplasmic domain is responsible for determining the pattern of receptor-specific cellular protein phosphorylation (7). In contrast, however, other studies implicate the membrane-proximal region, and specifically residue W282, of the EPO-R cytoplasmic domain as crucial for EPO-dependent proliferation (10, 27, 28). The EPO-R is tyrosine phosphorylated upon EPO binding and associates with the cytoplasmic tyrosine kinase JAK2, activating its kinase activity (18a, 27, 35). Whether JAK2 phosphorylates the EPO-R is not known. It is essential to identify proteins interacting with the EPO-R and determine the role they play in EPO-induced proliferation; some of these proteins may be specific for the EPO-R while others, such as JAK2 kinase, may interact with several cytokine receptors.

Deletion of 40 or 91 amino acids from the carboxy terminus of the EPO-R enhances the activity of the receptor without altering its affinity for EPO or cell surface number, suggesting that the COOH-terminal cytoplasmic region of the EPO-R contains a domain that down modulates EPO-R signaling (Fig. 1) (10, 37). Affected members of a Finnish fam-

ily with autosomal dominant benign erythrocytosis were shown to have a mutation in one allele of the EPO-R, which introduces a premature stop codon and predicts the synthesis of an EPO-R lacking the carboxy-terminal 70 amino acids (11). Cultured bone marrow erythroid progenitors from these individuals are hypersensitive to EPO (19). Thus, carboxy-terminal deletions have phenotypically the same effects on murine and human EPO-Rs, and in humans contribute, in a dominant fashion, to a mild pathologic condition. Deletion of the carboxy-terminal 40 amino acids of the murine EPO-R reduces receptor tyrosine phosphorylation (35), suggesting that a phosphorylated tyrosine in this region may serve as a recognition site for a protein which negatively modulates EPO-R signaling. Alternatively, truncated EPO-Rs may preferentially interact with positive signaling molecules. Assuming that the functional EPO-R is a homodimer, it remains to be determined if heterodimers of the wild-type receptor and carboxy-terminal truncated EPO-Rs are functionally distinct from wild-type receptor homodimers.

The Erythropoietin Receptor and Murine Leukemia

The role of growth factors and their receptors in the leukemic process has been the focus of a great deal of investigation. Activating mutations in some hematopoietic growth factor receptors (CSF-1R, EPO-R) have, in experimental murine models, led to the development of leukemia in a multistep process (17, 24). Specifically, the EPO-R has been implicated in two modes of leukemia induction in mice.

Friend virus, an acutely transforming murine erythroleukemia retrovirus has long served as a model for the study of the multistep nature of leukemia (3). A replication-defective, pathogenic, spleen focus-forming virus (SFFV) and a replication-competent, helper, Friend murine leukemia virus (F-MuLV) comprise the Friend virus complex. The SFFV genome lacks any known oncogenic sequences, but the env gene encodes a recombinant/deletion membrane glycoprotein, gp55, that is directly involved in leukemogenesis. Co-expression of the murine EPO-R and the gp55 envelope protein of SFFV in hematopoietic cell lines confers hormone-independent cell proliferation (23, 29). The EPO-R and gp55 interact, as judged by co-immunoprecipitation, in the ER (36) as well as the cell surface (6). The cell surface, disulfide-linked dimeric form of gp55 (16) is the biologically active form (22), and can be cross-linked to radioiodinated EPO (6). Since gp55 itself cannot bind EPO, these results demonstrate that gp55 molecules are associated with cell surface EPO-Rs, and that the gp55 binding site on the EPO-R is distinct from the ligand-binding domain (6).

This association between the EPO-R and gp55 is likely to be the principal cause of the initial stage of Friend disease: EPO-independent polyclonal erythroblastosis. The later evolution to clonal erythroleukemia is thought to result from inactivation of the p53 suppressor oncogene and clonal expansion of cell lines expressing one of the members of the *ets* gene family of transcription factors, PU.1/*spi*-1, or *fli*-1 (3). The target cell for transformation by SFFV is the "mature" BFU-E or CFU-E (2), the erythroid progenitor maximally responsive to EPO. Thus, induction of proliferation by the gp55 gene of SFFV is restricted to those cells expressing a functional EPO-R.

If the *env* gene of SFFV is replaced by a cDNA encoding a constitutively active form of the erythropoietin receptor, EPO-R(R129C), the resultant recombinant virus SFFVcEPO-R also induces erythrocytosis and leukemia in adult mice (24). Analysis of mice infected by the SFFVcEPO-R virus indicates that, in contrast to gp55 of SFFV, EPO-R(R129C) can signal proliferation and induce transformation of cells other than erythroid progenitors (G. D. Longmore, H. F. Lodish, manuscript submitted for publication). Erythrocytosis in SFFVcEPO-R–infected mice is associated with a transient rise in platelet count (24*a*). Clonogenic progenitor cell assays of marrow cells from infected mice suggest that the target cell of SFFVcEPO-R can be a multipotent committed progenitor with predominant erythroid-megakaryocytic features. Several factor-independent leukemic cell lines, derived from the spleens of infected mice, exhibited properties of primitive erythroid, lymphoid, and monocytic cells. Thus, when aberrantly expressed in vivo the EPO-R is capable of transducing functional growth signals in nonerythroid as well as early erythroid progenitor cells.

Similar to those generated by infection of SFFV, all leukemic cell lines generated after SFFVcEPO-R infection contained mutations in the p53 gene (24). However, in contrast to infection by SFFV, activation of PU-1 gene expression by retroviral integration was not observed (24) (G. D. Longmore, and H. F. Lodish, manuscript submitted for publication). Thus, infection by SFFVcEPO-R, in contrast to SFFV, can lead to transformation of nonerythroid as well as erythroid cells, and the sites of proviral integration in clonal leukemic cell lines are distinct from those formed by SFFV.

Patients with polycythemia vera (PV), a clonal myeloproliferative disorder, present primarily with erythrocytosis, although elevations in granulocytes and platelets also occur. Erythroid progenitors from patients with PV can, in culture, be hypersensitive to or independent of EPO for maturation (5, 13). This situation is reminiscent of activation of the murine EPO-R either by COOH-terminal truncation, constitutive point mutation, or by binding of gp55. There are no specific cytogenetic markers or genetic linkage data that implicate mutations in the EPO-R gene in the pathophysiology of PV, but appropriate molecular diagnostic techniques should help resolve the role of EPO-R in this disorder. Alternatively, a human equivalent of the SFFV gp55 protein may activate the EPO-R in PV.

Future Directions

The provocative parallels between mouse and human phenotypes expressing mutant EPO-Rs have shown the utility of in vitro mutational analysis and animal models of disease in understanding human pathophysiology. With the identification of disease processes involving mutations in the EPO-R, work needs to be concentrated upon defining the cell surface organization of the EPO-R complex, the identification of signaling molecules activated by or associated with the EPO-R, and the molecular nature of the EPO-R that contributes to these associations.

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