International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com

Review Article

# A Review on erythropoietin receptor (EpoR)

***Obeagu, Emmanuel Ifeanyi[1], Obeagu,Getrude Uzoma[2], Dr.Nchuma,Blessing Ogechi.[3] and Amazue, Paul O.[4]**

[1]Diagnostic Laboratory Unit, Department of University Health Services,Michael Okpara University of Agriculture, Umudike,Abia State,Nigeria.
[2]Department of Nursing Science, Ebonyi State University, Abakaliki, Nigeria.
[3]Medical Doctor, Department of Medicine and Surgery, Ebonyi State University, Abakaliki, Nigeria.
[4]Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria.
*Corresponding author

**Abstract**

Erythropietin (Epo) is a glycoprotein hormone that regulates erythropoiesis and performs other nonhaematopoietic roles such as wound healing and protection to memory and improves memory as well. Erythropoietin is present in minute amounts in the circulation under homeostatic conditions, whereas erythropoietin stress, such as hypoxia or anaemia,can stimulate a dramatic increase in erythropoietin production in the kidney, leading to a significant rise in circulating hormone amounts and subsequently increased erythropoiesis. Erythropoietin stimulates red blood cell production by binding and activating a high affinity receptor(EpoR) that is expressed predominantly on the surface of immature erythroid cells. The EpoR is a member of the type 1 cytokine receptor superfamily, sharing specific structural motifs with other members of this receptor family including 2 extracellular immunoglobin-like domains,4 spaced cysteine residues and the sequence SXWS. Signal transduction through the EpoR is initiated by ligand binding,which induces a dimerisation and/or reorientation of EpoR monomers within a dimeric receptor structure. The predominant pathway activated by EpoR and other cytokine receptors is the JAK/STAT signaling cascade.

**Keywords:** Erythropoietin,Erythropoietin receptor(EpoR),Molecular structure of EpoR, Role of dimerisation in EpoR Activation.

## Introduction

Erythropoietin is present in minute amounts in the circulation under homeostatic conditions, whereas erythropoietin stress, such as hypoxia or anaemia,can stimulate a dramatic increase in erythropoietin production in the kidney, leading to a significant rise in circulating hormone amounts and subsequently increased erythropoiesis (Koury et al.,1989;Lacombe et al.,1988;Krantz,1991).Erythropoietin stimulates red blood cell production by binding and activating a high affinity receptor(EpoR) that is expressed predominantly on the surface of immature erythroid cells(Watowich,2011).The EpoR is a member of the type 1 cytokine receptor superfamily, sharing specific structural motifs with other members of this receptor family including 2 extracellular immunoglobin-like domains,4 spaced cysteine residues and the sequence SXWS(Bazan,1990).Signal transduction through the EpoR is initiated by ligand binding, which induces a dimerisation and/or reorientation of EpoR monomers within a dimeric receptor structure(Livnah et al.,1999;Syed et al.,1998;Watowich et al.,1994).The predominant pathway activated by EpoR and other cytokine receptors is the Jak/STAT signaling cascade(Darnell et al.,1994).Jak tyrosine kinases are constitutively associated with the membrane proximal regions of cytokine receptor intracellular domains and are activated upon ligand binding and receptor reorientation. The EpoR associates selectively with the

Jak2 kinase. After EpoR activation,Jak2 phosphorylates tyrosine residues in the intracellular region of the EpoR, providing docking sites for signaling molecules with phosphotyrosine binding motifs, including the signal transducer and activator of trnascription protein STAT5,which mediates the main intracellular signaling pathway elicited by the EpoR(Cui et al.,2004).

**Molecular structure of the Erythropoeitin receptor(EpoR)**

The 3-dimensional structure of the EpoR extracellular region was first determined at 2.8 A resolution in complex with the agonist peptide EMP1 and later in complex with erythropoietin at 1.9 A resolution. Both approaches showed that the erythropoietin extracellular region comprises 2 immunoglobulin-like domains, each formed by a β-sandwich-like structure containing 7- β-strands. The membrane-distal(D1) domain and membrane-proximal(D2) domain are linked by a short hinge and are oriented at approximately 90 degrees to one another. The D1 domain contains the 4 conserved cysteine residues, which form 2 intracellular disulphide bridges that stabilise the EpoR WSXWS motif, this motif seems to stabilise the EpoR teriary structure.

Th EpoR:Epo complex revealed that Epo has 2 discrete binding sites for the EpoR. One binding interface of the ligand governs a high affinity interaction with the receptor, comprising a hydrophobic core surrounded by hydrophylic residues,a motif that has been referenced as a hot spot in terms of directing cytokine:cytokine receptor interactions. The high affinity site ehibits a dissociation constatnt of approximately 1µm and is thought to contribute the majority of the ligand binding energy. A second binding site that uses a distinct set of determinant residue on erythropoietin as well as EpoR has an affinity approximately 1000-fold lower. Thus,a sequential binding model for lignad-mediated activation of EpoR has been proposed, in which ligand interacts first via the high affinity interaction with the second EpoR monomer. Despite the asymmetry of this complex both monomers seem to be functionally similar in terms of activating signal transduction (Zang *et al.*,2009).Moreover, interactions between the transmembrane and membrane proximal cytoplasmic domains of EpoR monomers facilitate dimerisation and/or stabilisattion of the EpoR dimeric complex. The importance of the asymmetric complex and the dimerisation model or receptor activation is supported by observation that an erythropoietin molecule mutated in the site 2 region(R103 A) or EpoRs mutated at the binding region for erythropoietin site 1 or site 2 fail to elicit receptor signaling in haematopoietic cells (Zang *et al.*,2009).The erythropoietin R103A mutant also has provided an opportunity to characterise the EpoR complex on nonhaematopoietic cells. Cytoprotective activities orf erythropoietin on the differentiated neuroblastoma cell line were suppressed considerably with erythropoietin R103A or via RNA-mediated interference of EpoR expression, indicating that survival signaling elicited by erythropoietin on neuronal cells is most likely due to low levels of the EpoR expressed in the configuration of the haematopoietic receptor(Um *et al.*,2007).

The dimeric EpoR structure formed by interaction with erythropoietin or the EMP1 agonist differ in several aspects,likely explaining the fact that EMP1 exhibits reduced potency in terms of EpoR activation, as EMP1 is required at significantly higher concentrations than erythropoietin to elicit erythropoietic responses. The angle between the D1 domains differs in each EpoR:lignad complex. Moreover, the EpoR:Epo complex shows the D2 domain positioned within the same plane while they are twisted at an approximately 45-degree angle in the EpoR:EMP1 structure. This distinction may affect the ability to activate the associated Jak2 kinase, as a particular orientation may be favoured for full Jak2 activation via autophosphorylation. Hence, efficient erythropoietin agonists will likely need to more closely mimic the ligand-occupied receptor orientation(Watowich ,2011).

**Role of dimerisation in EpoR Activation**

The activation mechanism for EpoR was elucidated first through investigation of a constitutively acting form of the EpoR, which was isolated from a retroviral transduction screen in the interleukin 3-dependent murine cell line Ba/F3 by virtue of its ability to support cytokine-independent proliferation. Initially, the biochemical basis for the constitutive activity was unclear; however,a point mutation at residue 129 in the extracellular region that rendered an arginine to cysteine substitution (EpoR R129 C) suggested the

possibility that aberrant disulphide bond formation was involved. The constiutive actiivity of EpoR R 129 C is attributed to acquisition of cysyteine and not loss of arginine.Studies with the constitutive EpoR provided a paradigm for the process of type 1i and ii cytokine receptor activation because it was subsequently recognised that oligomerisation or structural reorientation of receptor subunits was a common activation mechanisms within the cytokine receptor family.

Equilibrium binding experiments withiodinated erythropoietin demonstrated that Epo:receptor complexs containing either wild –type EpoR or EpoR R129 C where governed by a single affinity,indicating that the 3-dimensional structure of EpoR R 129 C is similar to the wild-type receptor, at least within Epo-binding domain. This suggested that covalent dimerisation of EpoR R 129 C mimics the Epo/EpoR confirmation, further supporting the role for receptor dimerisation in the activation process.EpoR R 129 C stimulates Epo-independent colony forming unit-erythroid(CFU-E) development, as judged by in vitro assays,indicating that it supports erythroid proliferation and dimerisation.Mice infected with retrovirus carrying EpoR R129C develop erythropoiesis and splenomegaly and show increased amounts of circulating red blood cells, demonstrating that EpoR R129C stimulates expansion of the erythroid compartment in vivo and indicating deregulation of homeostatic mechanism most likely due to constitutive signaling of EpoR R129C.This,EpoR R129C seems to mimic the biological activity of the Epo:EpoR complex in terms of directing proliferation and differentiation of red blood cell precursors, without perturbing the reythroid developmental programme. Interestingly, overexpression of EpoR R129C in haematopoietic progenitor cells can enhance the generation of other myeloid lineages, early evidence that suggest redundancy in the signal pathways between blood cell growth factors. EpoR dimerisation by bivalent antibodies,analysis of chimeric receptor molecules, or biochemical studies of the purified poR extarcellular region further supported the idea that receptor clustering is an important step in the activation process. The EpoR;Epo complex is a dimeric receptor occupied by a single Epo molecule in a 2:1 EpoR:Epo ratio.EpoRs seem to be transported to the plasma membrane, where ligand activation induces a confirmation change in the orientation of receptor subunits without the dimer.

The quarternary structure of the EpoR has important implications for haematopoiesis in humans as it suggests that EpoR-mediated signal transduction could be altered in individuals with heterozygous mutation of the EpoR gene resulting from inherited or acquired events. Individuals from an extended Finnish family with dominant benign familial erythrocytosis provide such an example; unfortunately, in this instance,the phenotype is mild.Certain individuals within this Finnish family wrer found to have enhanced erythrocytosis, accompained by increased haematocrits and haemoglobin amounts.Erythroid progenitor cells isolated from these individuals demonstarte hypersensitivity to erythropoietin in culture,as judged bt their ability to undergo effective proliferation and differentiation in reduced erythropoietin amounts *in vitro*, compared with progenitor cells from unaffected individuals.The individual aexhibiting erythrocytosis possess a mutation in one copy of the EpoR gene,which generates a premature stop codon and truncated receptor isoform lacking approximately 70 amino acids from the carboxy-terminus. This truncated EpoR is missing an important negativel regulatory region in the cytoplasmic domain that is responsible for recruitment of haematopoietic cell phosphatase 1,which has beeen shown to suppress signaling from the EpoR as well as other cytokine receptors upon its association with activated receptors complexes. Assuming both wild-type and mutant EpoR alleles are coexpressed in individuals may express different EpoR complexes versus those with only wild-type EpoR. The EpoR complexes may include homdimers of the truncated EpoR and heterodimers of wild-type and mutant EpoRs, which may alter receptor signal transduction resulting in hypersensitivity to erythropoietin and mild erythropoiesis. Cytokine-dependent cells that were engineered to express both wild-type and truncated EpoR mimicking the Finnish mutation,or similarly truncated EpoR expressed in affected members of a Sweddish family with dominant erythrocytosis, exhibited enhanced Epo-mediated signal transduction and cellular proliferation compared with cells expressing only wild –type EpoR.In both Finnish and Sweddish families,the EpoR mutation seemsto be inherited with Mendelian frequencies. Moreover, the EpoR mutation functions in a dominant

manner relative to the wild-type allele of the EpoR gene(Watowich,2011).

**Roles for EpoR-mediated Jak2 and STAT5 signaling in erythropoiesis**

One of the aerlist detectable signaling events elicited upon EpoR activation is tyrosine phosphorylation of several intracellular proteins.Because the receptor lacks a kinase domain within its cytoplasmic region,these results indicated that protein tyrosine kinase function is carried out by a distinct factor.Subsequently,the Jak2 protein tyrosine kinase was identified as associating with the EpoR and serving as the principal kinase involved in mediating Epo-responsive signal transduction.Jak2 is constitutively bound to the EpoR intracellular region and seems to provide a chaperone function for newly assembled EpoR molecules,aiding their transit through the secretory pathway from the endoplasmic reticulum to the plasma membrane.

Significantly,Jak2 function is important in human erythropoiesis.A mutation within the Jak2 pseudokinase domain rendering a valine to phenylalanine substitution at residue 617(V617F) and hormone-independent kinase activity of myeloproliferative disorders(MPDs) including polycythaemia vera.In vitro and in vivo approaches to study Jak2V617F show that this mutant protein mediates erythropoietin-independent erythroid progenitor growth and development as well as erythroid cell expansion in vivo(.The EpoR contains 8 tyrosine residues within te membrane –distal portion of cytoplasmic tail,docking sites for intracellular signaling molecules including the transcription factors STAT5A and STAT5B,the p 85 subunit of phodphoinositol 3′ kinase (PI3K),the cytokine suppressor CIS,and the phosphatase SHP-1.Tyrosine phosphorylated Jak2 also seems to interact directly with STAT5A and STAT5B,indicating that it can serve as a scaffold for signal protein activation in addition to its enzymatic role in EpoR signal transduction.Recruitment of signaling molecules in proximity to Jak2 in the EpoR complex enables their tyrosine phosphorylation,which is an important step in subsequent activation of their respective signaling cascades.The STAT5A and STAT5B proteins are predominant signal transducers for EpoR;these proteins are activated within seconds of erythropoietin binding and accumulate in the nucleus to mediate erythropoietin-responsive transcription.

## Conclusion

Erythropietin is a glycoprotein hormone that regualtes erythropoiesis by binding to erythropoietin receptor (EpoR) expressed mainly on the erythroid cells in the bone marrow. It is after the binding that the activation happens by phosphorylation of tyrosine residues.EpoR is vital in the function of erythropoietin. Recruitment of signaling molecules in proximity to Jak2 in the EpoR complex enables their tyrosine phosphorylation, which is an important step in subsequent activation of their respective signaling cascades.

## References

Lacombe, C., Da Silva, J.L. and Bruneval, P.(1988). Peritubular cells are the site of erythropoietin synthesis in the murine hypoxic kidney. *Journal of Clinical Investigation Lancet.365:1054-1061.*

Lawrence,T., Goodnough, B.S. and Carlo,B.(2000). Erythropoietin,Iron, and Erythropoiesis *.Blood.96(3):823-833.*

Livnah, O., Johnson, D.L., Stura, E.A., Farrell, F.X., Barbone, F.P., You, Y., Liu, K.D., Goldsmith, M.A., He, W., Krause, C.D., Pestka, S., Jolliffe, L.K.and Wilson, I.A. ( 1999). "An Antagonist Peptide-EPO Receptor Complex Suggests That Receptor Dimerization Is Not Sufficient For Activation". *Nature Structural & Molecular Biology 5 (11): 993–1004.*

Watowich,S.S.(2011).The Erythropoietin Receptor: Molecualr Structure and Haematopoietic Signaling Pathways.*Journal of Investigative Medicine 59(7):1067-1072.*

Um,M.,Gross,A.W.and Lodish,H.F.(2007). A Classical Homodimeric Erythropoietin Receptor is ssential for the Antiapoptotic effects of Erythropoietin on Differential Neuroblastoma SH-SY5Y and Pheochromocytoma PC-12 Cells.*Cell Signal.19:634*-645.

Cui,Y.,Reidlinger, G. And Tang, W.(2004).Inactivation of Stat5 in mouse Mammary Epithelium During Pregnancy Reveals Distinct Functions in Cell Proliferation, Survival,and Differentiation. *Mol.Cell Biol.24:8037-8047.*

Zhang, Y.L., Radhakrishan, M.L .and Lu,X. (2009).Symmetric Signaling by an Asymmetric 1

Erythropoietin 1 :2 Erythropoietin Receptor Complex.*Mol.Cell.33:266-274.*

Koury,S.T.,Koury,M.J. and Bondurant,M.C. (1989).Quantitation of Erythropoietin-Producing Cells in Kidneys of Mice by in situ hybridisation:Correlation with Haematocrit,Renal Erythropoietin mRNA,and Serum Erythropoietin Concentration.*Blood.74:645-651.*

Krantz,S.B.(1991).Erythropoietin.*Blood.77:419-434.*

Basan,J.F.(1990).Structural Design and Molecular Evolution of a Cytokine Receptor Superfamily.*Pro.Natl.Acad.Sci.U.S.A.87:6934-6938.*

Syed,R.S., Reid,S.W. and Li,C.(1998).Efficiency of Signaling through Cytokine Receptor Depends Critically on Receptor Orientation. *Nature.395:511-516.*

Watowich, S.S., Hilton, D.J. and Lodish, H.F. (1994).Activation and Inhibition of Erythropoietin Receptor Function: role of Reeptor Dimerisation. *Mol.Cell Biol.14:3535-3549.*

Darnell, J.E., Kerr, I.M. and Stark, G.R.(1994).Jak-STAT Pathways and Transcriptional Activation in Response to IFNs and other Extracellular Signaling Proteins.*Science.264:1415-1421*.