

EDUCATION SESSION 4: HEMATOPOIETIC GROWTH FACTORS



Erythropoietin

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Erythropoietin (EPO) is a glycoprotein hormone of 30.4 kDa that is produced by the kidney in response to hypoxia¹. The carbohydrate residues make up 39% of its mass and are essential for the synthesis, secretion and the *in vivo* activity of the hormone²⁻⁴.

EPO is synthesized by specialized, peritubular, interstitial cells in the kidney, the exact nature of which is not yet known^{5,6}. These cells, under normal conditions, produce EPO at a constant rate that maintains normal erythropoiesis. When anemia develops the amount of synthesized erythropoietin is increased not by an increase in the rate of EPO synthesis per cell but by recruitment of more of these EPO producing interstitial cells⁷. The hormone is not stored intracellularly but is secreted into the circulation where under normal conditions it is found in the serum at a concentration of 5-30 mU/ml^{7,8}. The concentration of the hormone in the serum is inversely related to the concentration of the hemoglobin. The majority of the circulating hormone is taken up by the erythroid marrow and is catabolized; a small portion of degraded EPO is taken up and metabolized by the liver and a small proportion is excreted in the urine⁸.

The human EPO gene spans an area of 3.6 Kbp in the human genome and comprises five exons and four introns⁹. Only a single copy of the human EPO gene has been mapped to chromosome 7pter-q22¹⁰. Extensive homology exists among the EPO genes of various species. The regulation of EPO gene expression is not yet fully understood. Under conditions of hypoxia the expression of EPO gene is activated via binding of hypoxia-inducing factor 1 to the 5' end of the hypoxia-inducible enhancer located at the 3'-flanking region of the EPO gene. Additional factors, such as hepatic nuclear factor 4, may bind to the EPO gene promoter, which acts synergistically with the enhancer to promote transcription of the EPO gene under conditions of hypoxia. The mechanism by which interstitial EPO-producing renal cells sense hypoxia is not well understood. There is evidence to suggest that oxygen tension may be sensed by a hemoprotein whose conformational change may trigger the signal for gene activation. It has also been suggested that this hemoprotein may signal by converting molecular oxygen to hydrogen peroxide^{11,12}.

Epo acts on erythroid cells through its receptor, a transmembrane protein of 66kDa¹³. EPO receptors are found on erythroid cells, primarily on CFU-E (about 1000 receptors per cell), which can be considered as the target cell for its action. EPO receptors decline in number as erythroid cells mature and they disappear at the stage of orthochromatic erythroblast¹⁴. Following binding of EPO to its receptor, the receptor forms homodimers and undergoes phosphorylation by physically associating and interacting with the tyrosine kinase Jak2. Activation of Jak2 kinase leads to association and phosphorylation of STAT5, which as a transcription factor binds to nuclear DNA carrying the signal from the membrane to the nucleus¹⁵. Using a variety of erythroid cell lines and erythroid cell populations, a number of investigators have reported that EPO leads to activation of multiple other signal transduction pathways including MAP kinase, protein kinase B, phosphatidylinositol 3-kinase, ras, phospholipase C and others¹⁶. None of these signaling pathways is unique to EPO as they have been shown to be activated in non-erythroid cells stimulated with a variety of hemopoietic growth factors and cytokines. It is not yet clear to which extent the activation of each signaling pathway contributes to the action of the hormone on erythroid cells.

EPO is the major humoral regulator of erythropoiesis. It promotes survival, proliferation and differentiation of late erythroid progenitor cells. Since proliferation requires the presence of live cells, prevention of programmed cell death/apoptosis seems to be the most important and crucial effect of this hormone on normal erythroid cells¹⁷. EPO is not required for the commitment of multilineage progenitors to erythroid differentiation, as shown in EPO and EPO-R knockout mice¹⁸. However, once the erythroid cells reach the stage of CFU-E, they become dependent on EPO for their survival. Absence of EPO leads to the activation of events leading to programmed cell death^{17,19}. The prevention of apoptosis in normal erythroid cells is mediated through induction of Bcl-X_L¹⁹, an antiapoptotic protein that belongs to the Bcl-2 family²⁰. The expression of Bcl-X depends on the presence of EPO and factors in the serum other than EPO cannot induce it and rescue the normal erythroid cells from apoptosis¹⁹. Bcl2 does not seem to play any sig-

nificant role in normal erythroid cells but it has been detected in erythroleukemia cell lines that depend on EPO.

The cloning of EPO gene allowed the introduction of EPO into clinical practice. During the last 15 years, the hormone has been used for the treatment of the anemia of renal failure, anemia of chronic disease associated with rheumatoid arthritis, inflammatory bowel disease, AIDS, and cancer, as well as for the treatment of anemia in hematopoietic malignancies, post-bone marrow transplantation, and autologous blood donation.

The use of EPO for the treatment of the anemia of chronic renal failure constitutes a replacement therapy. More than 96% of patients respond to this treatment by reaching a target hematocrit of 32-38%. Improvement of the hematocrit is seen after the first two weeks of treatment and the target hematocrit is achieved within the first couple of months. After achieving the target hematocrit, the dose of EPO can be decreased to a lower maintenance dose. Major side effects include worsening of preexisting hypertension (30%), seizures (4%), iron deficiency, and an increase in the predialysis serum potassium and phosphorus^{20,21}. Careful correction of hypertension can prevent the occurrence of seizures. Arthralgias, myalgias or skeletal pain may occur at the beginning of treatment with EPO, but these symptoms subside with time and they are rarely severe enough to necessitate discontinuation of therapy. Due to the high frequency of development of iron deficiency in previously non-transfused patients, evaluation of the patient's iron status is common practice and oral iron supplementation is frequently given. Lack of response to EPO or development of resistance after initial response is associated with infection, chronic inflammatory disease, iron deficiency, aluminum toxicity, and hyperparathyroidism with marrow fibrosis, trauma, or surgery²²⁻²⁵.

The rationale for using EPO for the correction of the anemia of chronic diseases is that in such conditions (rheumatoid arthritis, AIDS, cancer) a blunted EPO response to anemia has been demonstrated²⁶⁻²⁸. In addition, in vitro studies have shown that the inhibitory effect of IL-1 on erythropoiesis that is exerted through the interferon- γ can be reversed by high concentrations of erythropoietin^{29,30}. Clinical trials of EPO for the correction of anemia of these diseases have shown response (increase of Hct by 6 points or of Hg by 2 g/dl without transfusions) rates of 88% in rheumatoid arthritis³¹, 44% in AIDS³² and 40-60% in cancer therapy receiving or not receiving chemotherapy³³. It should be noticed that patients with AIDS and high (>500 mU/ml) endogenous EPO serum levels had a very low response to EPO. Clinical trials of EPO for the correction of anemia associated with multiple myeloma, non-Hodgkin's lymphoma and chronic lymphocytic leukemia have shown response rates of 40-60%. Responses were primarily seen in patients having pre-treatment serum EPO levels lower than 100 mU/ml³⁴⁻³⁸. In myelodysplastic syndromes the use of EPO is based on the fact that these are clonal diseases and expansion of a normal clone may result in correction of the anemia. Clinical trials have shown a response rate of 28-33%

(RA 39%, RARS 17.5%, RAEB 12.5%) with the pretreatment serum EPO level lower than 100 mU/ml being the best predictor of response. No responses were seen in patients with EPO levels greater than 200 mU/ml^{39,40}. Favorable results with EPO have also been reported for the anemia associated with radiation therapy⁴¹, bone marrow transplantation⁴² and prematurity. Considering the powerful biologic properties of the hormone, it is reasonable to expect a possible therapeutic effect in any type of underproduction anemia in which residual normal erythroid progenitors exist in the marrow, the EPO-response to anemia is suboptimal, and there is no coexisting nutritional deficiency.

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