

To Give or Not to Give Recombinant EPO to Anemia Endangered Cancer Patients

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Abstract: EPO is known as an inducer of maturation and proliferation of erythrocytes. Moreover, it favours angiogenesis. In several studies it was encountered that EPO is a trophic agent that mediates survival and inhibits apoptosis of hypoxia affected cells, particularly those which build masses of irregularly vascularized cancers. The main task concerning EPO for oncologists is the choice to give or not to give recombinant EPO to anemia endangered cancer patients. EPO can do the quality of life better and cause recovery from anemia post chemotherapy and radiation of cancer patients. Nevertheless, EPO therapy shortens survival of patients in some cancers, in which antiapoptotic effect of EPO predominates directly in malignant cells. Thus, separately in every type of cancer, therapeutic use of recombinant EPO calls for prior investigations, if EPO signaling causes proliferation of cancer cells by direct stimulation of EPOR positive malignant cells. Unless the proliferative effect of EPO on cancer cells is excluded, its use in the therapy of anemia in cancer patients is not quite safe.

Characteristics of EPO signalling

Upregulation of EPO

Kidneys and fetal livers synthesize EPO in response to hypoxia [1]. HIF-1 (Hypoxia-Inducible Factor-1) switches on EPO gene that encodes an inducer of erythropoiesis [2]. Traditionally classified as hematopoietic growth factor, EPO is 30.4kD glycoprotein that is intensively glycosylated chain of 193 amino acids [3]. However its mature form that is secreted from the cell consists of 165 amino acids [4]. There are a few evidences for HIF-1 dependant induction of EPO. Namely, hypoxia boosted quantity of hypoxia-dependent subunit of HIF-1, HIF-1 α

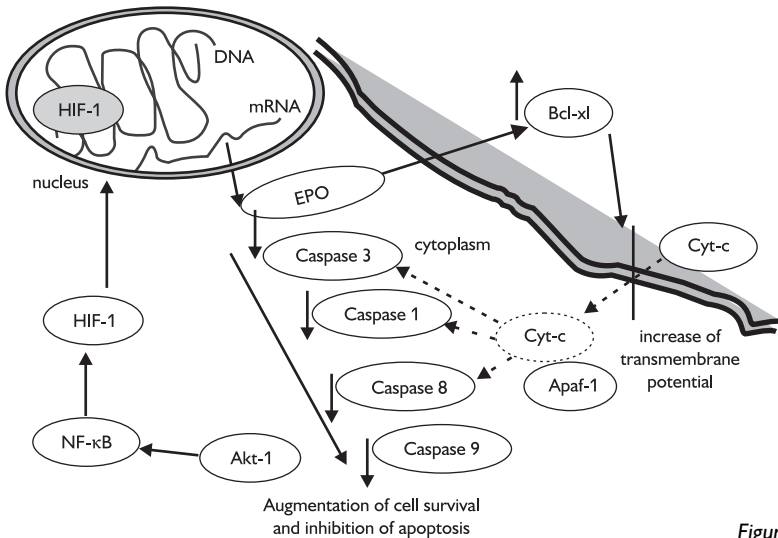


Figure 1 – EPO scheme.

protein in SH-SY5Y and Kelly neuroblastoma cell lines before they started to generate EPO molecules. Several other regulators participate in downstream pathways that lead to biosynthesis of EPO. For instance, – among of them – there is hepatocyte nuclear factor 4 α (HNF4 α) which is indispensably induced prior to biosynthesis of EPO in Hepatoma cell line HepG2 [5]. Regulation of EPO expression also involves Akt-1, which role was elucidated on human hepatocellular cancer cells. Akt-1 is required for action of NF κ B and recruitment of HIF-1 that switches on production of EPO [6]. At last it was reported that EPO production by endometrium is triggered by estrogen on the basis of the homology of 5'flanking region of EPO gene to binding site for estrogen and negative correlation between EPO and estrogen or progesterone receptors (ER or PR) in normal endometrium [7–8]. However this correlation failed to occur in endometrial cancer [8].

Molecular scheme of EPO action

Basic function of EPO is to provide sufficient number of red blood cells by stimulation of their formation. The signaling pathways of EPO were extensively studied in isolated cultures of in brain endothelial cells that were exposed to injury by well-known vasodilator – nitric oxide (NO). EPO was shown to prevent or limit escape of cytochrome c from mitochondria by upregulation of Bcl-xl. EPO blocked cytoplasmic binding cytochrome c to apaf-1 and cyt-c and apaf-1 complex dependent activation of caspase 9 and 3 [9]. Similarly, in vascular endothelial cells, EPO was found to be co-activated by protein kinase B (Akt1) and sustains mitochondrial membrane barrier against release of cytochrome c. As a result, functions of caspase 8, 1 and 3 were silenced [10–11]. These signal pathways are probably common in other types of cells. EPO binds to its receptor EPOR that activates tyrosine phosphorylation of some intracellular mediators such as JAK-2, STAT5, and MAP kinases [12–14]. EPOR belongs to Type I cytokine receptor superfamily but lacks function of intrinsic kinase and as a target protein for EPO mediates signaling through cell membranes [15].

EPO and EPOR related cytoprotection and cell proliferation.

Anti-ischemic effect of EPO

EPO is a proliferative agent for progenitors of blood red cells and cancer cells. EPO binding with EPOR stimulates Bcl-2 and Bcl-xl expression in blood red cells [7]. EPOR mediates signals for proliferation in both non neoplastic and non erytroid cells e.g. endothelial, renal, myoblastic and intestinal cells [16–19].

EPO has overall anti-ischemic and cytoprotective effects in cases of brain hypoxic injury [20–22]. The neurons expose EPOR that is stimulated by astrocyte derived EPO [23]. In addition, EPO stimulation of EPOR caused the angiogenesis in menstruation cycle in uterus [8]. Tumor oxygenation and hemoglobin content increase independently in rodent mammary carcinomas after treatment with

EPO [24]. EPO also favours angiogenesis in wound healing [25]. On the other hand, chronic inflammation is involved in renal cortical atrophy, which is treated with EPO to limit anemia. The specific antibodies are produced directly to extrinsic agent in the form of recombinant EPO. These antibodies can abolish some of EPO activities. Another side effect of EPO administration can be thrombosis. Thrombosis was reported in patients devoid of EPO antibodies that certainly had higher levels of free circulating EPO than patients that developed antibodies against EPO. This implies that recombinant EPO increases the risk of thrombosis that can be due to EPO-boosted cellular density of blood [26]. Thus, main anti-ischemic effects of EPO are enhancement of red cell production, direct modulation of metabolism in EPOR presenting cells (e.g. neurons) and stimulation of angiogenesis.

EPO as direct inducer of cancer cell proliferation

Several cancer types revealed responsiveness to regulation by EPO because they expressed EPOR at surface human tumor cells melanoma and of female genital tract and kidney [27–31]. At transcription level two EPOR isoforms were found in colon, lung, prostate, ovarian and breast cancers [32]. Human prostate cancer cells also contained EPOR and EPO proteins and transcripts [15]. However, prognostic factors as PSA level and Gleason scoring system also remained uncorrelated with EPO and EPOR. Their expression at mRNA and protein levels was uncorrelated with proliferation index as assessed by MIB-1 or with TUNEL evaluation of apoptosis. The comparison between EPO or EPOR and proliferation and apoptosis markers failed to show significance of EPO and EPOR in regulation of cancer growth [15]. In the light of these findings the recombinant EPO seems to be safe agent to administer as anemia prevention without side effect of turning up growth speed of prostate cancer. On the contrary, EPO and the EPO receptor (EPOR) accelerated development of head and neck squamous cell cancers. Particularly, they increase tumor cell invasion, which was indicated by elevated expression of this hormone and its receptors in lymph node metastases [3]. If exogenously supplied, EPO increased circulating levels of different hormones like cortisol and DHEAS and decreases PRL, growth hormone (GH) and somatomedin-C (Insulin Growth Factor-1, IGF-1) [33]. It was produced and entered the circulation in a manner of feedback axis with GH. Inhibition of IGF-1 made administration of erythropoietin not exert its proliferative effects on tumor growth in anemic patients who suffer from malignant disorders [33]. EPO was constitutively overexpressed in malignant cell lines of melanoma both in normoxic conditions and to a greater extent in hypoxia compared with normal melanocytes of the skin “*in vitro*” [34]. Among squamous cell carcinomas and adenocarcinomas of the lung transcripts of mRNA EPO, EPOR, HIF-1 α were detected with reverse transcription-PCR and presence of its protein products was visualized with immunohistochemistry. Non-small cell cancers constitute large sources of the

Epo [35]. Autocrine secretion of EPO contributed to survival of MCF-7 human breast cancer cell line that diminished hypoxia-stimulated apoptosis and increased expression of Bcl-2 and Bcl-XL. That could be implicated in drug resistance of breast tumors [36]. The ominous importance of EPO was confirmed in a study with breast cancer patients. Namely, erythropoietin treated women were characterized by increased death rate compared to placebo group [1]. Consequently, the Z-chamber model that was constructed with breast adenocarcinoma cells R3230Ac showed that EPO antibody or soluble EPOR significantly reduced tumor growth by neutralizing EPO or by competing with membrane EPOR [36]. EPO and EPOR were located in benign glandular cells of endometrium with the peak of immunohistochemical detection in late secretory and gestational phases. It was reported that they were also in endometrial cancers but only EPOR staining varied increasingly with progress of malignant behavior and development of features like invasion of lymph vasculature, involvement of lymph nodes and ER expression decreased to absolute negativity. Accordingly, EPO expression was associated with poor survival likewise EPOR, which was additionally correlated with recurrence free survival of endometrial cancers [37]. EPO and EPOR were variably detected in human papillary thyroid cancers with higher incidence of EPOR that was associated with smaller size of tumor and less frequent recurrence [38]. The expression of EPO and EPOR was also found in pediatric tumors Ewing's sarcoma, neuroblastoma, rhabdomyosarcoma, hepatoblastoma nephroblastoma medulloblastoma, astrocytoma and ependyoma. The presence was accompanied by expression of Bcl-2, Bcl-xl and Mcl-1 and enhanced binding of nuclear factor kappa B39 which suggested augmentation of cancer growth by EPO in these neoplasms.

Therapeutic implications

It has been suggested that EPO sensitized cancer cells via hyperoxygenation to respond better to radiation [40–41] but in many cases this hormone caused an accelerator of malignant proliferation of the cells. Although β -epoetin treatment upregulated hemoglobin concentration, it caused significant local progress of tumors and shorten life spans in head and neck cancer patients [42]. On the other hand, there was a synergy of exogenous erythropoietin, cisplatin and mitomycin C in management of Lewis lung carcinoma (LLC) in a murine model [43]. In human cancer lines EPO and EPOR were abundantly expressed and the xenografts of these neoplasms stopped developing in nude mice after appliance of EPO-R antagonist [44]. In squamous dysplasia and squamous cell carcinoma of the uterine cervix, erythropoietin inhibited proapoptotic activity of cisplatin in HeLa cervical carcinoma cells [28]. Thus, endogenous EPO could protect cancer cells from death and from faster development of cancer [28, 42]. In opposition to this statement, Hardee et al. demonstrated that human recombinant erythropoietin (rEpo) did not influence growth and angiogenesis in rodent tumors like R3230 rat mammary

adenocarcinomas, CT-26 mouse colon carcinomas, HCT-116 human colon carcinomas, and FaDu human head and neck neoplasms [45]. In other study exogenous EPO limited of tumor mass in murine lung cancer model [43]. EPO and HIF-1 α were intensively co-expressed in dysplasias and invasive squamous cell cervical cancers. EPO counteracted hypoxia and prevented from the resistance to chemotherapy [28]. Furthermore, recombinant human erythropoietin (rHuEpo) emerged as a useful anticancer drug that proved its potency to treat multiple myeloma (MM) in monotherapy with previously reported efficiency on murine myeloma models [46–47]. Therefore, function of EPO was far more complex than a simple resuming pool of erythrocytes due to chemotherapeutic cytotoxicity. It probably could recruit CD8+ T cells to struggle with cancer cells [46–47]. Antiapoptotic function of EPO was maintained by induction of Bcl-2 and Bcl-xl that promoted cell survival and limited apoptosis in gastric cancer. EPOR level correlated with degree of microvessel density and advancement of malignant phenotype in IV stage of this neoplasm in immunohistochemical studies with anti-CD31 and anti-EPOR. Therefore, it was presumed that EPO could improve oxygenation of gastrointestinal cells via its trophic properties in vascular network [48].

Conclusions

EPO could act as autocrine and paracrine growth factor, controller of apoptosis and stimulator of differentiation. Recombinant EPO is supplied to rescue cancer patients from post radiation and post chemotherapy anemia. To sum up, advantages of recombinant EPO therapy include improvement of life quality and recovery from anemia post chemotherapy and radiation [49]. Disadvantages are promotion of cancer growth and angiogenesis [9, 50, 51]. Some studies proved EPO supplementation was completely safe and rHuEpo had no side effects as stimulation of cancer development [45]. Astonished by the adverse EPO effects, its real nature in cases of cancer should be analyzed carefully and independently because the predominant EPO action can vary in regard to certain types of neoplasm.

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