Erythropoietin Receptors on Tumor Cells: What Do They Mean?

JOACHIM FANDREY

Institut für Physiologie, Universität Duisburg-Essen, Essen, Germany

Key Words. Erythropoietin • Erythropoietin receptor • Erythropoietin receptor antibody • Heat shock protein (HSP) 70

Disclosure: J.F. has received educational speaker honoraria and travel reimbursements from Janssen Pharmaceutica NV. No other potential conflicts of interest were reported by the author, planners, reviewers, or staff managers of this article.

Abstract

Given the apparent presence of erythropoietin receptors (EPORs) in cancer tissues, questions have been raised about the possible influence of erythropoiesis-stimulating agents (ESAs) on tumor growth and proliferation. Preclinical studies of ESAs have shown no greater tumor proliferation in cell lines and no adverse effect on treatment outcomes in animal models. Furthermore, it appears that the commercially available antibodies that have been used in clinical studies are not specific to EPORs. In particular, they detect isoforms of heat shock protein 70, which is found in tumor cells and is associated with poor prognosis. For this reason, results from clinical studies purporting to relate the administration of ESAs to shorter survival must be considered inconclusive and complicated by methodological and sampling issues. Ongoing studies will help clarify whether the existence of the EPOR has any relevance at all in the cancer setting. The Oncologist 2008;13(suppl 3):16–20

Introduction

In 2003, Henke et al. [1] published the results of a study of patients with head and neck cancer undergoing radiation therapy who were randomized to a control group or additional treatment with epoetin beta (300 U/kg). The purpose of the study was to investigate the potential radiosensitizing effects of raising the level of hemoglobin (Hb) toward a target value of 14 g/dl in women and 15 g/dl in men. Epoetin beta treatment effectively raised the Hb level, but was associated with significantly shorter progression-free and overall survival times.

A subsequent report presented data on the erythropoietin receptor (EPOR) status in tissue samples from 154 patients who were among the 351 patients enrolled in the randomized controlled trial [2]. This retrospective analysis assessed the effect of epoetin beta on progression-free survival in relationship to EPOR expression. Staining studies used C20 antibody (sc-695, Santa Cruz Biotechnology, Santa Cruz, CA). Around two thirds of samples stained positive and, among this EPOR-positive group of patients, the local disease-free-survival rate was significantly lower in patients treated with epoetin than among controls (Fig. 1A). Among the smaller number of patients whose tumor tissue did not stain for the EPOR, there was no significant difference in outcome between the placebo and treated groups (Fig. 1B).

These findings raised the question of whether the presence of EPORs might stimulate tumor cell growth when bound by EPO or an EPO-like ligand [3]. However, the basis for such a study is the presumption that the EPOR antibody detects what it is meant to detect, and not other proteins; and it is by no means clear that this is the case [4–6]. Data from several groups suggest that the antibodies currently available are not valid tools for determining the EPOR status of tissue sections obtained from cancer patients.

What Might Be the Relevance of EPORs?

RBC, or erythrocyte, production is mainly regulated by the hormone EPO [7, 8]. Hypoxic conditions lead to increased synthesis of EPO, which is regulated by the transcription...
factor complex hypoxia-inducible factor 1 [9–11]. To maintain daily renewal of RBCs, a steady-state production of EPO is required [7]. The EPOR is a member of the cytokine family of receptors and is expressed as a preformed dimer on the surface of erythroid progenitor cells [12]. EPO binds to the EPOR on the extracellular domain of bone marrow erythroid precursors [12]. Binding of EPO to the EPOR causes a conformational change in the EPOR and activates the phosphorylation of its intracellular domain, triggering a cascade of intracellular events (including the Janus kinase 2–signal transducer and activator of transcription 5 pathway) and stimulating erythroid progenitors to proliferate and differentiate, resulting in the rapid formation of functional RBCs (Fig. 2) [12–15]. Changes in the EPO level or mutations to the EPOR are associated with changes in hematocrit.

Whereas EPO’s principal role is in the regulation of RBC formation, both EPO and the EPOR have been found in nonhematopoietic tissues and cells, including solid tumors, endothelial cells, the central nervous system, etc. [16].

Indirect evidence for the existence of EPORs on human tumors is derived in part from studies showing that prior absorption of EPOR proteins eliminates tumor EPOR staining on immunohistochemistry [17, 18]. The second source of indirect evidence is from trials demonstrating shorter locoregional progression-free survival or overall survival times among EPOR-positive patients treated with erythropoiesis-stimulating agents (ESAs) rather than placebo [1, 2, 19].

**Preclinical Studies**

Direct evidence of EPORs on human tumors might be derived from the demonstration by immunohistochemistry of EPORs on tumor cell lines or tumor tissue samples using polyclonal rabbit antisera. The vast majority of in vitro studies, however, showed no promotion of tumor cell growth, decreased apoptosis and/or resistance to treatment, and the existence of EPOR-related signaling [20]. In contrast, from a few cell culture studies, increased proliferation of tumor cells has been reported [20]. Those studies, however, are limited by the absence of appropriate vehicle controls and the general use of suprapharmacologic doses of ESAs that would not be found in vivo in poorly perfused tumors [17]. Cells were studied under artificial circumstances, were deprived of serum, and were synchronized. None of these reflects the state of a heterogeneous population of tumor cells in natural circumstances. In addition, the very moderate proliferative effect of EPO on tumor cells does not compare with the response of an erythroid cell line, in which pharmacologically relevant doses (0.01–0.4 U/ml EPO) cause a several hundredfold increase in proliferation [20].

More generally, there are no reasons to suppose that EPORs should play a role in tumor progression. The EPOR gene is not an oncogene, and there is no selective advantage...
for tumors to overexpress it. EPO mRNA is detectable in tumor cell lines [16, 20], but is not elevated compared with nontumor tissues. Tumor cell lines show no or only weak binding to EPO. Surface expression of EPORs on tumors has not been unambiguously demonstrated [20]. In fact, most of the staining is in the cytoplasm, a site in which the receptor cannot, of course, bind its ligand [6].

In more than 25 malignant and benign human cell lines, EPO did not increase the proliferation rate of EPOR-positive tumor cell lines, nor did it affect c-fos mRNA expression in these cell lines [16, 21]. The EPOR did not seem essential for tumor cell growth in these cell lines [21]. Collectively, the majority of in vitro studies have shown that ESAs are likely to have a neutral effect on human cancers [17, 20].

More indicative of a (patho)physiological role of ESAs on tumor growth are in vivo animal models. So far, the in vivo tumor model studies with ESA treatment may be generally grouped into one of three categories: (a) regression of tumor mass, (b) enhancement of tumor- ablative therapies, and (c) no enhancement of tumor-ablative therapies. As recently reviewed by Sinclair et al. [20], all 23 in vivo studies revealed no tumor-promoting effect of ESA administration alone. As expected, ESAs increased hematocrit in anemic animals that had tumors and improved tissue oxygenation, which may have contributed to restore the effectiveness of radiation and photodynamic therapy as well as chemotherapy in some (but not all) studies [20].

**Commercially Available Polyclonal Anti-EPOR Antibodies Detect Heat Shock Proteins**

One relevant consideration is that Henke et al. [1, 2] in their study of patients with head and neck cancer could evaluate receptor expression, but not receptor activity, in what were formalin-fixed tissues. The specificity of the antibodies used in their study in Western blot analyses has been called into question [22]. One cause for concern is that a mouse knockout model in which the EPOR gene was deleted, so that there was no expression of EPOR protein, produced tissue that showed the same staining to “EPOR” antibody as tissue from wild-type mice that did not have the gene deleted (Fig. 3) [22]. The only possible conclusion is that the EPOR antibody is detecting some other protein.

Examination of the amino acid sequence of the purported EPOR demonstrated by Western blotting revealed that it was not that protein at all but one of several isoforms of heat shock protein (HSP)70 [22]. C20 recognized HSP70-2 almost exclusively, which may be a result of a high degree of sequence similarity between the EPOR peptide used for immunization and HSP70-2 [22]. Furthermore, when the HSP70-2 isoform was added in excess, binding to what was supposed to be the EPOR was completely eliminated (Fig. 4) [22].

This fact is not without interest to oncologists because HSP70 is a highly conserved family of chaperone proteins that are induced in normal cells by stress and that have important functions in promoting cell survival and resistance to apoptosis [23]. They are found in tumors, particularly those of an aggressive phenotype. Expression correlates with shorter survival and resistance to treatment in many tumors [24, 25], including those of the
lung, breast, head and neck, prostate, and bladder. Expression increases under conditions of hypoxia. It appears that there is sufficient sequence homology between the antigen used to generate C20 polyclonal antibodies and HSP for most of the “EPOR” detected to be HSP70 isoforms 2 or 5 [22, 23]. Unfortunately, similar controls for HSP70 were not presented in the study by Henke et al. [2], and it remains possible that HSP70-2 was detected by the antiserum. Whether HSP70 detection could be in any way used to predict EPO response currently appears highly speculative and requires further studies.

**Conclusion**

Certain clinical studies using commercially available antibodies of doubtful specificity have suggested a relationship between the presence of EPORs and adverse clinical outcome following treatment with ESAs. However, the limitations of the reagents available to detect EPORs are such that we must question these results. It would not necessarily be reasonable to use the conclusions they came to as the basis for deciding on treatment.

Ongoing studies will help clarify the role of EPORs in the cancer setting, and crucial to this will be work with more specific antibodies. Meanwhile, studies using antibodies of dubious specificity should be treated with caution.

**Acknowledgment**

The author acknowledges the assistance of medical writer Julia O’Regan, Bingham Mayne and Smith, Medical Communication.

**References**


15 Wittuhrn BA, Quelle FW, Silvennoinen O et al. JAK2 associates with the erythropoietin receptor and is tyrosine phosphorylated and activated following stimulation with erythropoietin. Cell 1993;74:227–236.


