Unraveling the Mystery of Erythropoietin-Stimulating Agents in Cancer Promotion

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Abstract

Erythropoietin-stimulating agents (ESA) are approved for use in treating chemotherapy-induced anemia in patients with nonmyeloid malignancies. However, recent clinical trials have shown evidence of inferior overall survival and/or locoregional control of tumors in patients receiving ESAs. Given these concerning data, current studies are focused on elucidating the biological mechanisms by which ESAs may contribute to cancer promotion. Evidence suggests that ESAs activate several signaling pathways that are important in altering tumor behavior and response to treatment. Although further research is needed to more precisely elucidate these mechanisms, caution should be exercised in the use of ESAs beyond their approved indication in cancer patients. [Cancer Res 2008; 68(11):4013–17]

Introduction

Erythropoietin (Epo)-stimulating agents (ESA) are the most commonly used growth factors in clinical practice. ESAs are known to stimulate erythroid cell lines and reverse anemia. In contrast to the initial studies that led to their Food and Drug Administration (FDA) approval for chemotherapy-induced anemia, recent clinical trials targeting restoration of hemoglobin to normal and even supraphysiologic levels have shown worse outcomes for cancer patients receiving ESAs compared with placebo. Given these troubling data, current studies are focused on elucidating the biological mechanisms of action of ESAs that may underlie these differences in study outcomes.

Clinical Data

Epo was approved for use in alleviating chemotherapy-induced anemia in patients with nonmyeloid malignancies in 1993 followed by darbepoetin in 2002. The clinical trials on which ESAs were given FDA approval focused mainly on increased hemoglobin levels, decreased transfusion requirements, and reduction in transfusion-associated risks. Interestingly, improvements in quality of life have never been definitively shown in ESA clinical trials.

These early trials were not designed or powered to detect statistically significant differences in overall survival. However, a meta-analysis suggested a favorable trend in survival outcome (1). This led to trials using ESAs that were aimed at enhancing survival. The first of these, the ENHANCE study (2) published in 2003 by Henke and colleagues, enrolled 351 patients with anemia who had squamous cell cancer of the oral cavity, pharynx, or larynx. Patients, undergoing active treatment with radiotherapy, were randomized in a double-blind manner to receive either epoetin beta or placebo. Study drug was initiated at a hemoglobin concentration of <12 g/dL in women and <13 g/dL in men and continued until target hemoglobins of ≥14 g/dL in women and ≥15 g/dL in men were reached. Patients treated on the epoetin beta arm showed worse locoregional progression, and shorter locoregional progression-free survival and overall survival compared with those receiving radiotherapy and placebo.

The multicenter, double-blind, randomized, placebo-controlled Breast Cancer Erythropoietin Survival Trial by Leyland-Jones and colleagues (3) sought to maintain normal hemoglobin levels in 939 women with metastatic breast cancer undergoing first-line chemotherapy. With a target hemoglobin level of 12 to 14 g/dL, study drug was allowed once hemoglobin reached ≤13 g/dL. Following evidence of higher mortality in the Epo arm, the study drug was discontinued and additional data were collected. Patients treated with Epo had an increased incidence of disease progression and thromboembolic events, and more adverse prognostic factors at baseline. Overall, the authors concluded that these differences could not account for the survival differences seen (4).

Wright and colleagues (5) conducted a trial to measure changes in Functional Assessment of Cancer Therapy-Anemia scores in 300 non–small cell lung cancer patients with disease-related anemia being treated with Epo. Epoetin alfa or placebo was initiated for hemoglobin levels ≤12 g/dL. Adjustments were made to the study drug if the hemoglobin reached >14 g/dL. Given rising concerns for Epo-associated risks during the time of the trial, an unplanned interim safety analysis was conducted. The trial was suspended after 70 patients had been randomized after a significantly improved overall survival was seen in patients receiving placebo.

Additionally, Overgaard and colleagues (6) reported the preliminary results of the DAHANCA 10 study conducted by the Danish Head and Neck Cancer Group. This open-label study randomized 522 patients with locally advanced head and neck cancer undergoing curative radiotherapy to receive darbepoetin alfa or red cell transfusion. Administration of darbepoetin alfa continued unless hemoglobin levels exceeded 15.5 g/dL. Following evidence of poorer locoregional control, shorter disease-free survival, and a trend toward shorter overall survival in the darbepoetin group, the trial was terminated.

Two other studies that have been independently analyzed by the FDA (7) also suggest shorter survival times in patients treated with darbepoetin alfa compared with control. The first multicenter, randomized trial compared the use of darbepoetin alfa with placebo in 989 patients with nonmyeloid malignancies that were not undergoing active anticancer therapy. Study drug was held if the hemoglobin value reached >13 g/dL. The second multicenter, double-blind, placebo-controlled trial randomized 344 patients with lymphoproliferative malignancies undergoing chemotherapy to receive darbepoetin alfa or placebo until hemoglobin levels exceeded 15 g/dL in men or 14 g/dL in women.
More recently, two additional studies have released results indicating adverse outcomes in patients treated with ESAs. Amgen announced preliminary conclusions of the PREPARE (8) trial, which randomized 733 breast cancer patients undergoing neo-adjuvant chemotherapy to darbepoetin alfa or placebo in an open-label fashion. Hemoglobin was to be maintained between 12.5 and 13 g/dL. A planned interim analysis revealed higher progression and death rates on the treatment arm. Lastly, the National Cancer Institute Gynecologic Oncology Group (9) studied patients with cervical carcinoma undergoing concurrent chemoradiotherapy. Patients were randomized to receive either epoetin alfa once their hemoglobin was <12 g/dL or simple blood transfusions for hemoglobin <10 g/dL. The study was discontinued after 109 of a planned 460 patients were enrolled due to concerns of higher thromboembolic rates. The final statistical analysis did not confirm increased thromboembolic rates; however, progression-free and overall survival were found to favor placebo-treated patients.

Together, these eight randomized trials show decreased overall survival or locoregional control of tumors in patients receiving ESAs, in contrast to the initial meta-analysis (see Table 1). An updated Cochrane Review was performed to include data from these recent trials. Bohlius and colleagues (10) reported lower transfusion rates, increased risk of thromboembolic events, and a suggestion of decreased survival in ESA-treated cancer patients.

In response to these data, the American Society of Clinical Oncology and the American Society of Hematology recommended the use of ESAs in patients with nonmyeloid malignancies receiving chemotherapy only after hemoglobin levels fall to 10 g/dL. Both societies challenged the clinical relevance of these studies, pointing out that ESAs were administered in unapproved settings, among patient populations not receiving chemotherapy or by targeting higher than approved hemoglobin levels. It has previously been shown that high target hemoglobin levels and rapid increases in hemoglobin are associated with worse outcomes (11). It remains unproven whether high target hemoglobin concentrations are the only factor contributing to the adverse outcomes seen.

**Possible Mechanisms of Tumor Promotion**

Given these concerns, the mechanisms of action of ESAs, particularly their effects on nonerythroid cells, have recently come under investigation. Many common tumor types, including lung (12), squamous head and neck (13), breast (14), colon (15), gastric (16), and uterine (17) cancers, have been shown to express Epo receptor (EpoR). EpoR is classified as a type I cytokine receptor with a single transmembrane domain with three identifiable forms: full length, truncated, and soluble. Its exact physiologic roles in nonerythroid cell lines remain unclear. It is hypothesized that Epo and EpoR are synthesized by tumor cells and stimulation in an autocrine/paracrine fashion affects cancer cell growth and survival (12, 18–20). With prominent expression of EpoR on tumor cell surfaces, endogenous production of Epo can result in downstream signaling cascades. Signaling pathways activated by EpoR stimulation result in increased migration (20), invasion (13, 18), proliferation (21), angiogenesis (17), and inhibition of apoptosis (22, 23). Activation or up-regulation of EpoR in malignant tissues does not seem to confer these actions because Sinclair and colleagues (24) have shown that gene transcript levels of EpoR are similar among normal and tumor tissues.

Cell surface expression and correct functional folding necessary for EpoR signaling is induced by Janus-activated kinase 2 (JAK2), a known transducer of important cancer cell signaling. Once activated by ligand, EpoR forms a homodimer that activates a multitude of pathways, including activation of JAK2 by phosphorylation (18). JAK2 is mutated in myeloproliferative disorders, particularly polycythemia vera, causing constitutive proliferation of erythroid cells despite the absence of Epo (25). Could a similar mechanism be at play in human cancers?

In fact, in cell lines from squamous cell carcinoma of the head and neck, Epo promotes tumor cell invasion (13, 18). This Epo-stimulated invasion can be blocked by a JAK inhibitor and in a cell line with signal transducer and activator of transcription (STAT) 5A mutation (13). This highlights the importance of the JAK-STAT signaling pathway in cancer progression, transducing the Epo-mediated signal and thereby enhancing invasion.

Increased tumor survival is also modulated in part by suppression of apoptosis despite genetic damage, irradiation, or other stimuli. Epo induces nuclear translocation of nuclear factor-κB (NF-κB) through stimulation of EpoR. This activation of NF-κB has been proposed to induce antiapoptotic gene transcription (26). Protein kinase B (AKT), activated via the phosphoinositide 3-kinase (PI3K) signaling pathway, has also been implicated in increasing tumor cell survival. Epo increased the levels of activated AKT and one of its downstream targets, mammalian target of rapamycin (mTOR), in a dose-dependent manner in human melanoma cells (19). mTOR is known to affect cell proliferation and angiogenesis, whereas AKT can protect cells from death despite the presence of apoptotic stimuli. The increased viability of cells on Epo treatment was diminished by the addition of a PI3K inhibitor (19).

The Epo-EpoR interaction has also been shown to increase migration of a breast cancer cell line through activation of extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase. Higher Epo and ERK levels were induced by hypoxic conditions, which were associated with substantially increased migration. The effect was mitigated by the presence of either soluble EpoR or anti-Epo antibodies (20). Therefore, increased Epo, especially within a hypoxic state, may potentiate the formation of metastases. Ironically, this contradicts the rationale behind several of the recent clinical trials that sought to reverse tissue hypoxia by improving cellular oxygen-carrying capacity by driving hemoglobin to supraphysiologic levels.

Strengthening the plausibility of the *in vivo* relevance of these mechanisms, it has been shown that patients with non–small cell lung cancers that concurrently express Epo and EpoR have worse disease-specific survival than those who do not (12). A retrospective analysis also found worse locoregional progression-free survival of patients with head and neck squamous cell cancers that expressed EpoR when exogenous Epo compared with placebo was administered (27). In addition, patients who continue to smoke during therapy have worse overall outcomes. In this chronic hypoxic state, with dysregulation of hypoxia-inducible factor-1α, elevated systemic levels of Epo can be detected.

Intratumoral hypoxia may play a key regulatory role in the Epo-EpoR autocrine/paracrine loop, as both Epo and EpoR are up-regulated in hypoxic tumor cell lines (19, 20). Investigators may have expected alleviation of anemia by systemic Epo to increase delivery of chemotherapy or reduce global hypoxia, thus improving patient outcomes. However, this has never been shown in clinical studies. Improved systemic perfusion may not translate into improved intratumoral blood flow, as tumors often have aberrant
A major area of concern and limitation of current studies is proliferation or growth of many EpoR-expressing tumor cell lines. This has also been reported that exogenous Epo does not alter transcriptional levels of EpoR (30). It remains the fact that commercially available antibodies used to identify EpoR are nonspecific in their detection, overlapping with heat shock protein-70 (HSP-70) among other proteins. High expression of HSP-70 in tumors has been linked to poor prognosis, aggressive disease, including formation of metastases, and resistance to chemoradiotherapy (34).

Epo functions as a proangiogenic cytokine during its regulation of physiologic angiogenesis, as shown in the uterus (17). The role Epo-EpoR signaling plays in tumor angiogenesis is not fully established. Ribatti and colleagues (16, 28, 29) correlated Epo-EpoR expression with vascular endothelial cells with the extent of tumor neovascularization, which may obey different physiologic principles than normal vasculature.

Epo in tumor and vascular endothelial cells, with the extent of tumor angiogenesis and grade of malignancy for several tumor types. Higher histologic stages and grades of tumors have higher expression levels of Epo and EpoR, which in turn correlates with higher degree of tumor angiogenesis measured by microvessel density.

There have also been conflicting data regarding the chemoradiosensitivity of cancer cells when cultured with exogenous Epo. Cervical carcinoma cells showed a dose-dependent, Epo-mediated improved survival with decreased apoptosis during treatment with cisplatin and Epo (22). Another study of malignant glioma and cervical carcinoma cells revealed increased resistance to cisplatin and radiation in response to Epo (35). Increased survival of melanoma cells when treated with dacarbazine or cisplatin was also shown to be induced by Epo (36). In contrast, renal carcinoma cells cultured with Epo undergo increased apoptosis when exposed to daunorubicin or vinblastine (37). Squamous cell and colorectal carcinoma xenografts show increased sensitivity to 5-fluorouracil.
despite the presence of Epo and do not exhibit increased proliferative effects (38). Cell line studies likely do not adequately represent the complex milieu of Epo biology, resulting in some discrepancies among in vitro and in vivo experiments. Overall, it seems that the specific tumor environment is critical for determining response to chemoradiotherapy. It may well be that we meaningfully alter this microenvironment by the use of exogenous ESAs.

Observed differences in these experiments may be explained in part by examining the tissue-protective effects of Epo seen in normal cerebral and myocardial cells. Joyceu-Faure (39) reported Epo-mediated protection against cellular damage caused by ischemia, free radicals, or cytotoxic agents. The nonhematopoietic signaling pathways involved may be regulated by alternate forms of Epo, such as desialylated or carbamylated forms, which confer little or no erythropoietic response. Alternate regulation of cellular responses driven by Epo also depends on the dimerization partner or subtype of the activated EpoR. Although a homodimerized form of EpoR stimulates erythropoiesis, its heterodimerization with the β-common receptor (shared by other growth factors and interleukins) confers tissue-protective effects (40). The role of the β-common receptor has yet to be studied in nonmyeloid malignancy.

Summary

Taken together, increasing evidence suggests multiple mechanisms of ESA-mediated action, possibly explaining the differences in study outcomes. It remains to be determined whether one mechanism plays a dominant role or how the multiple pathways interact. Intratumoral effects fashioned in an autocrine/paracrine loop may compete with the systemic effects of ESAs. Continued research is therefore crucial. To date, there are no published survival data from patients with nonmyeloid malignancies undergoing chemotherapy concurrently with ESA treatment to maintain hemoglobin in the recommended range of 10 to 12 g/dL. In the interim, physicians will need to be more evidence based in their prescribing practices and should exercise caution in the use of ESAs, taking into account each patient’s case (41). Most would agree that decreasing transfusions by alleviating anemia is the only definitively proven benefit of these agents, which may not outweigh the possible untoward effects. Recently, the American Society of Clinical Oncology and the American Society of Hematology have updated clinical practice guidelines (42) to reflect our current knowledge regarding both the potential beneficial aspects of ESAs and their possible risks.

In conclusion, Epo-EpoR signaling plays an important role in complex cancer biology that is as yet poorly understood. Although not entirely elucidated, our knowledge of the biological mechanisms by which ESAs activate several survival pathways important for tumor behavior and response to treatment is sufficient to mandate caution in their use beyond their strict indication in cancer patients.

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