In the hematopoietic system, the selective action of Epo in cells of the erythroid lineage is mediated through its specific cell surface receptor EpoR to regulate RBC production. Functional EpoR is expressed in many nonerythroid cell types, and an accumulating body of experimental evidence indicates that Epo is a pleiotropic cytokine with significant biological effects in nonhematopoietic tissues (1). The ubiquitous expression of EpoR in nonhematopoietic cells including tumor cells and the broad tissue–protective properties of Epo in diverse organs have not been associated with consistent biological effects of Epo in tumor cells to modulate cell proliferation, apoptosis inhibition, or chemoradiotherapy responses in preclinical experimental models (2). A series of recent randomized, placebo-controlled clinical trials reported detrimental effects including enhanced tumor progression and increased mortality associated with ESA therapy in some cancer patients, the mechanisms of which remain to be characterized (3, 4). Although a direct effect of exogenous Epo on tumor cells to induce signaling through a functional EpoR has been proposed as a mechanism by which Epo may contribute to tumor progression, other potential local and systemic effects of Epo, as illustrated in Fig. 1, deserve consideration in future efforts to elucidate the role of Epo in cancer biology using novel preclinical experimental models and clinical trial designs.

Effects of Exogenous Epo in Preclinical Studies

The expression in cancer cells of EpoR—the mediator of the biological effects of Epo in erythroid cells—has been examined by many laboratories using a variety of techniques including reverse transcription-PCR to show EpoR mRNA transcripts, and immunoblotting, immunoprecipitation, or immunohistochemistry studies to investigate EpoR protein expression (2). In some studies, expression of cell surface EpoR has been examined in cultured cancer cell lines using radiolabeled Epo binding or a novel receptor-mediated endocytosis assay (5), reporting either the absence of specific radioligand binding (6) or the presence of significantly fewer frequency of cell surface receptors compared with erythroid cells that are nevertheless sufficient to mediate proliferative or antiapoptotic effects in some cell lines (5, 7, 8). Thus, a correlation between the level of tumor cell EpoR expression and the responsiveness to exogenous Epo to induce functional intracellular signal transduction in cancer cells has not been established using the available preclinical experimental models, and further investigations are required to characterize the mechanisms and consequences of EpoR expression and activation in tumor cells (5, 9). EpoR protein expression in primary tumor specimens has been examined by immunohistochemistry assays using various antibodies, with many studies using a commercially available polyclonal antibody (C-20) against EpoR (2). This antibody can specifically immunoprecipitate...
EpoR protein from whole cell extracts (10–12), but in direct immunoblotting assays of cell lysates and in immunohistochemical analyses of primary tumors, the C-20 antibody was reported to exhibit binding to other cellular proteins such as heat-shock protein 70—a potential marker of more aggressive tumor behavior (12–14). Batch-dependent differences in C-20 antibody specificity in immunoblotting assays have also been reported (15). Although the potential for nonspecific protein binding in immunohistochemistry assays using C-20 limits the quantitative assessment of EpoR expression in primary tumor specimens, positive C-20 immunostaining in head-neck cancer specimens has nevertheless been associated with the adverse clinical outcomes associated with epoetin-therapy, a finding that will need to be confirmed in prospective studies (16). The optimization of assays to quantify EpoR expression in primary tumor specimens and the characterization of potential differences in the determinants of Epo signaling activation in erythroid versus tumor cells—such as the putative role, if any, of the heteromeric βc receptor subunit—will require further studies (5, 17, 18).

Many preclinical experiments have investigated the effects of recombinant Epo in tumor cells and reported a wide range of findings. In some in vitro studies, EpoR expression in tumor cell lines has been associated with Epo-induced stimulation of proliferation (8, 19–21), increased cellular migration and invasion capacity (6, 22–25), apoptosis inhibition (5, 26–28), and chemoradiation resistance (25, 29–33), whereas other studies have not found significant effects of exogenous Epo on cellular proliferation (31, 34–39) or in vitro chemosensitivity (40). In various xenograft and syngeneic rodent tumor models consisting of anemic or nonanemic animals, exogenous Epo has not promoted tumor growth or modulated chemosensitivity (6, 27, 41–43), and in some preclinical studies, Epo therapy has actually been associated with enhanced chemoradiotherapy efficacy (44–49). The beneficial effect of Epo in enhancing chemosensitivity was attributed in one study to tumor vessel remodeling in colon cancer xenografts (50), whereas enhanced radiotherapy effects are thought to be associated with increased tumor oxygenation (51–53), whereas immunomodulatory effects of Epo have been proposed to play a role in the antitumor effect of Epo in a preclinical multiple myeloma model (54). The variation in the range of in vitro effects of exogenous Epo on cellular proliferation, apoptosis resistance, and chemoradiation sensitivity may be potentially attributable to (a) differences in experimental assays and design, (b) autocrine activation potential of Epo-EpoR signaling as suggested in some studies (26, 32, 39), (c) differences between Epo responses of specific tumor cell types, and (d) variable EpoR expression levels. On the other hand, the in vitro proliferative and cytoprotective properties of Epo reported in some studies have not been associated with a predictable in vivo growth-promoting effect of exogenous Epo in studies involving diverse types of tumors in animal models. It is important, however, to consider the limitations of the commonly used animal tumor models including (a) the relatively short period of time (frequently between 2 and 4 weeks and <8 weeks in the majority of the studies) during which exogenous Epo is administered, (b) the development of marked polycythemia in many of the tumor-harboring animals treated with Epo, and (c) limited feasibility of continued follow-up of tumor progression beyond 1 to 2 months in animals harboring large tumors. In an effort to address these limitations, novel animal
tumor models designed to investigate Epo-EpoR biology in cancer are needed.

In view of the ability of Epo to activate tissue-protective intracellular signal transduction pathways in many diverse nonhematopoietic cell types (1), it is not surprising that several preclinical studies using monolayers of continuous cancer cell lines have reported Epo-induced signaling activation in cancer cells. For instance, Epo-induced phosphorylation of JAK2 and STAT5 in head-neck cancer cells was associated with increased invasion capacity of the cells (23, 24). Epo was also found to induce STAT5 phosphorylation in prostate cancer (21) and neuroblastoma cells (5, 28), but not in breast cancer cells, in which mitogen-activated protein kinase family members including extracellular signal-regulated kinase, p38, and c-Jun-NH2-kinases were phosphorylated by Epo (9, 40). In neuroblastoma cells, STAT5 and AKT activation by EpoR-mediated signaling was required for its antiapoptotic effect (28). In a non–small cell lung carcinoma cell line, impaired down-regulation of EpoR protein was associated with Epo-induced activation of JAK2-STAT5, extracellular signal-regulated kinase, and PI3K-AKT pathways but without a stimulatory effect on cellular proliferation (38, 55). In breast cancer cells, extracellular signal-regulated kinase activation was reported to play a role in increased migration capacity of the cells in an in vitro assay (22). Epo-induced phosphorylation of AKT in melanoma and mammary carcinoma cells was associated with cytotoxic effects in vitro (27, 56). In an in vivo study, systemic Epo administration to rats resulted in increased phosphorylation of AKT in mammary carcinoma cells in flank tumors, but without an effect on the antitumor activity of paclitaxel, suggesting that activation of in vivo signaling may not be sufficient to predictably confer cytoprotection or promote growth (27). In a more recent study, overexpression of a constitutively active EpoR in mammary carcinoma cells promoted in vivo tumor progression and led to increased Epo sensitivity and enhanced extracellular signal-regulated kinase 1/2 and c-Jun-NH2-kinase phosphorylation (9). These findings have suggested that EpoR overexpression and signaling activation in tumor cells have the potential to promote growth and that the development of novel animal models designed to investigate the consequences of Epo-EpoR signaling in tumor cells may be feasible (9).

**Recent Clinical Trials of ESAs—What Can We Learn?**

Despite the variable biological effects of recombinant Epo in the available preclinical experimental tumor models and the absence of a demonstrable in vivo growth-promoting effect, a series of recent large randomized, placebo-controlled clinical trials reported adverse outcomes including increased tumor progression and mortality associated with ESA therapy in some cancer patients, the mechanisms of which are yet to be determined. These clinical trials have several features that contrast with the design of many prior ESA trials including the following: (a) the hemoglobin target of >12 g/dL that is higher than the currently recommended range, (b) the enrollment of relatively homogenous tumor types instead of mixed nonmyeloid malignancies, and (c) adequate power to detect significant differences in progression-free and/or overall survival. A potential direct effect on tumor progression was suggested by the findings of the multicenter, double-blind ENHANCE trial involving 351 head-neck cancer patients treated with radiotherapy and randomized to placebo or epoetin-β, reporting worse locoregional control and survival in the Epo group, particularly in the patient subgroups with manifest cancer (57). More recently, an interim analysis of the presently unpublished DAHANCA 10 study involving 522 patients with locally advanced head-neck cancer treated with radiotherapy also reported an unfavorable locoregional failure rate and a trend toward poorer survival associated with darbepoetin-alfa compared with placebo, leading to the early termination of this trial (58). A third trial involving 939 metastatic breast cancer patients treated with first-line chemotherapy and randomized to epoetin-alfa or placebo was also terminated early due to higher mortality in the epoetin-alfa arm, but the extent to which disease progression may have played a role in the increased death rate was not entirely clear (59, 60). More recently, a quality-of-life study involving advanced non–small cell lung cancer patients not routinely treated with chemotherapy was stopped early when an unplanned safety analysis revealed significantly poorer survival in the epoetin-alfa group compared with placebo (61). In a placebo-controlled, randomized trial involving 989 anemic patients with various types of malignancies targeting a hemoglobin value of >12 g/dL, darbepoetin-alfa was associated with shorter survival (62). It is noteworthy that patients enrolled in this trial were not given antitumor therapy and therefore did not receive darbepoetin-alfa in the context of its indication for the treatment of chemotherapy-induced anemia. Finally, in a 344 patient cohort consisting of lymphoproliferative malignancies treated with chemotherapy, darbepoetin-alfa was associated with significantly shorter overall survival.¹ In contrast to the published and unpublished trials listed above, preliminary results of a phase III study involving 600 patients with extensive stage small cell lung cancer treated with platinum-based chemotherapy reported no significant effect of darbepoetin-alfa on survival or progression.² These unpublished findings parallel the results of a published randomized, placebo-controlled trial involving small cell lung cancer patients treated with chemotherapy that did not find a significant detrimental effect of epoetin-alfa on tumor response or survival, although these conclusions were considered preliminary due to early trial closure as a result of poor patient accrual (63).

The final published results of the terminated trials and other ongoing studies of ESAs in cancer patients are awaited, but they are not anticipated to immediately shed light on the mechanisms of the detrimental effect on progression-free and overall survival. To date, there is no conclusive evidence that ESAs directly promote cancer progression. A retrospective analysis of a subgroup of the primary head-neck tumor

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Table 1. Potential modulatory factors involved in overall tumor response to exogenous Epo

- Concomitant administration of chemoradiotherapy
- The presence and extent of tumor burden in adjuvant versus metastatic setting
- Tumor type-specific differences in tumor biology and Epo response
- Indirect systemic effects of Epo such as immunologic effects, tumor oxygenation, microcirculation
- Variable EpoR expression levels in tumor cells
- Epo effects on cell types in tumor microenvironment such as tumor endothelium and macrophages
- Epo concentration achieved in tumors and required to elicit activation of in vivo signaling

Conclusions and Implications for Future Studies

The absence of an apparent growth-promoting effect of recombinant Epo in commonly used preclinical in vivo tumor models, the variable effect of Epo on chemoradiation sensitivity with some animal studies reporting enhanced antitumor efficacy, the absence of a consistent in vitro proliferative or antipapoptotic effect in different types of cancer cells despite the ability of Epo to activate intracellular signal transduction, the potential contribution of endogenous Epo-EpoR to tumor angiogenesis and progression, and the adverse outcome signals in the recent clinical trials indicate that the role of Epo in cancer biology is clearly not straightforward. The potential mechanisms of the adverse outcomes observed in clinical trials are not completely understood and this issue constituted the focus of the ENHANCE study for EpoR protein expression reported that the significant detrimental effect of epoetin-β on survival was limited to the cohort of patients whose tumor cells expressed EpoR protein (16). EpoR expression was assessed independently in pathology laboratories blinded to clinical outcome data and by immunohistochemical analysis of EpoR expression using an arbitrary cutoff of ≥10% of tumor cells exhibiting positive immunostaining. The findings of this study have suggested that exogenous Epo may exert direct effects on tumor growth and impair progression-free survival, only if EpoR is expressed in the tumors as determined by positive C-20 antibody immunostaining. Despite the issue of C-20 antibody specificity in immunohistochemistry analyses (12–14), these findings have suggested that qualitative and quantitative assessment of EpoR expression in primary tumor samples may be theoretically helpful in the prospective selection of patients for ESA therapy. For instance, the finding of a correlation between the level of EpoR expression and clinical outcome as a function of Epo therapy may be potentially informative (16), with the caveat that detection of EpoR protein and/or mRNA transcripts in tumors would not necessarily indicate in vivo receptor functionality in tumor cells or in other cell types in the tumor microenvironment. Thus, in addition to the need for the development of novel reagents and techniques for quantitative assessment of EpoR expression in primary tumors, carefully designed studies are required for the concomitant investigation of the biological and clinical consequences of ESA therapy in a prospective manner.

Endogenous Epo-EpoR as a Therapeutic Target?

Several preclinical studies have focused on the investigation of the role of endogenous Epo signaling in tumor xenograft or syngeneic models by local intratumor or systemic administration of Epo-EpoR antagonists, leading to tumor regression and apoptotic death of tumor and vascular endothelial cells (64–67). These studies and others demonstrating endogenous and hypoxia-inducible Epo expression in tumor cells have suggested that autocrine or paracrine activation of endogenous Epo signaling may—through either direct or indirect effects—modulate tumor angiogenesis and progression (26, 32, 39). In a more recent study, stable expression of an Epo antagonist in fluorescently labeled tumor cells has been associated with inhibition of tumor cell-induced neovascularization and delayed primary tumor growth visualized directly in dorsal skin-fold window chambers by intravital microscopy (9). Epo antagonist expression in tumor cells resulted in near-complete abrogation of primary tumor formation in an orthotopic xenograft model of breast cancer, suggesting that a functional endogenous Epo-EpoR system may contribute to tumor formation, angiogenesis, and progression (9). The findings of these studies suggest that further investigation of endogenous Epo-EpoR signaling as a potential target of antiangiogenic and antitumor approaches is warranted. The presence of functional endogenous Epo-EpoR signaling that plays a role in tumor progression may modulate the response to exogenous Epo and contribute to the complexity of Epo biology in cancer.
of discussions at the “ESAs and Tumor Growth Workshop” sponsored by the National Cancer Institute in December 2007. Expression of EpoR in diverse tumor types may not be sufficient to affect tumor biology in response to exogenous Epo in a predictable manner and an interplay of complex factors is likely involved to modulate the overall effect in patients (Table 1). Optimization of ESA use as an important supportive therapy modality in cancer patients and further investigation of the role of Epo in cancer biology will require a combination of carefully designed preclinical and clinical studies. Randomized, placebo-controlled, adequately powered clinical trials enrolling patients with chemotherapy-induced anemia being treated for a homogenous tumor type can be designed to investigate not only the effect of Epo therapy on clinical outcomes such as tumor response, progression-free, and overall survival but also the potential effects of Epo on specific factors related to tumor biology and chemoradiation sensitivity such as Epo-induced in vivo phosphorylation and activation of downstream signaling molecules in tumor cells, modulation of tumor angiogenesis, and effects on indices of tumor cell proliferation and apoptosis (Fig. 2). As the safe clinical practice guidelines for ESA use in cancer patients are evolving (68), future scientific challenges include, but are not limited to, (a) the characterization of the structure of the cellular receptor that mediates the nonhematopoietic biological effects of Epo in normal and cancer cells, (b) the development of new reagents, techniques, and clinical investigation models to quantify Epo expression and ascertain its in vivo functionality in primary tumors, (c) the generation and availability of novel animal models for the preclinical study of Epo biology in cancer, (d) the elucidation of the mechanisms of systemic nonhematopoietic tissue effects of Epo including its prothrombotic properties, and (e) the development of selective nonhematopoietic Epo-EpoR antagonists for the characterization of the role of endogenous Epo-EpoR signaling as a therapeutic target in cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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