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Preoperative administration of erythropoietin stimulates tumor recurrence after surgical excision of colon cancer in mice by a vascular endothelial growth factor–independent mechanism

Marta Pascual, MD,[a,b] *Bernhard Bohle, MD,*[a] *Sandra Alonso, MD,*[a,b] *Xavier Mayol, PhD,*[b] *Silvia Salvans, MD,*[a,b] *Luis Grande, MD,*[a] *and Miguel Pera, MD*[a,b,*]

[a] *Colorectal Surgery Unit, Department of Surgery, Hospital del Mar, Barcelona, Spain*

[b] *Colorectal Cancer Research Group, Cancer Program, IMIM (Institut Hospital del Mar d'Investigacions Mèdiques), Barcelona, Spain*

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ABSTRACT

Background: It has been suggested that preoperative administration of erythropoietin (Epo) in patients with gastrointestinal cancer reduces transfusional needs and is also associated with lower morbidity. On the other hand, experimental and clinical studies show that Epo might enhance tumor growth and angiogenesis. Our aim was to ascertain whether preoperative administration of Epo has any effect on tumor recurrence after curative surgery using an experimental model of colon cancer.

Materials and methods: We induced tumors by injecting B51LiM colon cancer cells into the cecal wall of Balb/c mice. We randomized the animals into three groups of treatment with (1) recombinant human Epo, (2) recombinant mouse Epo, or (3) vehicle alone, for 12 d until cecectomy. On postoperative day 12, we killed mice and analyzed tumor recurrence. We measured serum levels of vascular endothelial growth factor and determined vascular endothelial growth factor expression and tumor microvessel density by immunohistochemistry. We also investigated the *in vitro* effect of Epo on B51LiM cell line proliferation.

Results: All three groups displayed tumor recurrence, but the final tumor load score and total tumoral weight were higher in the two groups that included Epo. The differences were statistically significant when we compared the recombinant mouse Epo group with the control group. We found no evidence of increased angiogenesis or enhanced cell proliferation as possible mechanisms of Epo-induced recurrence.

Conclusions: Preoperative administration of Epo stimulates tumor recurrence in an animal model of colon cancer. Our results point to the need for further research on the mechanisms of tumor growth enhancement by Epo, to better understand the benefits or disadvantages of Epo treatment.

* *Corresponding author*. Colorectal Surgery Unit, Department of Surgery, Hospital del Mar, Passeig Marítim 25-29, 08003 Barcelona, Spain. Tel.: +34 932483207; fax: +34 932483433.
E-mail address: mpera@parcdesalutmar.cat (M. Pera).

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1. Introduction

Erythropoietin (Epo) is the principal hematopoietic growth factor that regulates cellular proliferation and differentiation along the erythroid lineage. The main function of Epo is to maintain hemoglobin levels in peripheral blood under physiologic conditions [1]. Nowadays, exogenous administration of recombinant human Epo (rHuEpo) is accepted for the treatment of anemia in patients with chronic kidney disease, and chemotherapy-induced anemia in cancer patients. Three different forms of rHuEpo have been approved for these indications: epoetin-α, epoetin-β, and darbepoetin-α [2]. Several clinical trials have shown the beneficial impact of these different forms of rHuEpo in patients with cancer, increasing hemoglobin, decreasing transfusional needs, and improving functional outcomes and quality of life [3,4].

Preoperative anemia is not only the main risk factor for transfusion; it is also independently associated with an increased risk of 30-d morbidity and mortality in patients undergoing major surgery [5,6]. For this reason, in the setting of elective surgical procedures with major blood loss, anemia should be corrected preoperatively. In this sense, administration of Epo with iron supplementation has also been proposed in anemic patients undergoing procedures such as valvular heart surgery [7] and total hip and knee arthroplasty [8]. This treatment strategy significantly reduces the perioperative transfusion requirement. Furthermore, a number of clinical trials have suggested that preoperative administration of rHuEpo in patients with gastrointestinal cancer not only reduces postoperative transfusional needs [9–11], but is also associated with lower morbidity [12].

Nevertheless, in contrast to the evidence suggesting all these beneficial effects, three placebo-controlled clinical trials, including patients with head–neck cancer [13], metastatic breast cancer [14], and advanced non-small cell lung cancer [15], and one meta-analysis [16] have found that Epo therapy has a negative effect on survival. The mechanisms by which Epo treatment resulted in poorer oncologic outcomes was a subject of previous research, which was most studies performed with tumor cell lines. Expression of Epo receptor (Epo-R) has been found in renal carcinoma cells [17], melanoma cells [18], and other tumors [19]. Whereas some *in vitro* studies demonstrated that Epo does not stimulate tumor cell proliferation [20,21], others have reported increased cell proliferation after Epo-R activation in human and mouse renal adenocarcinoma [17] and breast cancer cells [22]. Some studies also investigated the effects of Epo on tumor cells *in vivo*, similarly giving rise to conflicting results [23]. The results of all these studies prompt further preclinical research to clarify the real extent of Epo effects on tumors.

Another putative mechanism by which Epo could enhance tumor growth is angiogenesis. Tumor angiogenesis is induced at the level of proliferation and migration of endothelial cells by means of secreted factors produced by tumor cells and other cells present in the tumor microenvironment [24]. Some studies *in vitro* have shown that Epo signaling modulates the regulation of angiogenesis by stimulating endothelial cell proliferation and migration [25,26]. *In vivo*, Epo stimulates the physiological angiogenesis that takes place in the developing mouse and chick embryos [27].

A proposed mechanism for Epo-mediated stimulation of angiogenesis is an increased expression of vascular endothelial growth factor (VEGF), one of the most potent activators of tissue neovascularization [28]. In a model of femoral artery ligation using Epo-R null adult mice that lack non-hematopoietic Epo-R expression, blood flow recovery and activation of VEGF-VEGF receptor system were impaired in Epo-R null mice, compared with wild-type mice [29].

From all these data, the increased levels of Epo and Epo-R found in tumor cells have been interpreted as a putative mechanism that facilitates cell proliferation, prevents apoptosis, and enhances angiogenesis in tumors. It is thus conceivable that the negative oncologic results of the three clinical randomized trials mentioned above resulted from Epo effects on tumor cell growth and survival [13–15]. The purpose of the present study was to investigate whether rHuEpo or recombinant mouse Epo (rMoEpo), stimulates tumor recurrence after surgical resection of a primary tumor in a mouse model of colon cancer. This model was aimed at mimicking the preoperative administration of Epo in the context of curative surgery of colon cancer. We analyzed VEGF expression and microvessel density (MVD), and VEGF serum levels after Epo administration to assess angiogenesis as a putative mechanism of tumor growth enhancement by Epo. We also investigated the effect of Epo on tumor cell proliferation *in vitro*.

2. Materials and methods

2.1. Animals

We used 84 female Balb/c mice (Harlan Interfauna Iberica, Barcelona, Spain), 6–8 wk of age, weighting 19–21 g, for the experiments. The animals were allowed free access to a standard laboratory diet and water. All procedures were reviewed and approved by the IMIM-Hospital del Mar Animal Care and Use Committee in accordance with European guidelines.

2.2. Tumor cell line

We used a colonic adenocarcinoma cell line (B51LiM), obtained from Robert S. Bresalier, MD, at the Henry Ford Health Sciences Center, Detroit, Michigan, in this study. The B51LiM line is syngeneic to the Balb/c mouse strain [30]. Cell cultures were kept in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin, and streptomycin at 37°C in an atmosphere of 5% carbon dioxide. Cell viability, which we determined by the trypan blue exclusion test, always exceeded 95%.

2.3. Induction of a colonic solid tumor

We induced a solid tumor in the cecum, as described previously by Bresalier *et al.* [30] Mice were anesthesized with an intraperitoneal injection of 100/10 mg/kg ketamine-xylazine. We made a 5-mm midline incision and injected 5.0×10^6