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Endogenous erythropoietin and erythropoietin receptors in colorectal cancer; can we answer the questions? ☆

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ABSTRACT

Erythropoietin (Epo) is glycoprotein hormone which binds on erythropoietin receptors (EpoR) promoting proliferation and differentiation. Studies have shown that EpoR, apart from erythrocyte precursors, is expressed on no hematopoietic tissue and various tumor cells.

Despite the progress in modern medicine, colorectal carcinoma (CRC) is still the leading cause of increased morbidity and mortality between oncology patients worldwide. Its precursors are benign villous adenomas, which in certain percentage progress to cancer. Anemia of chronic disease is common finding in CRC patients. Some of them are treated with Epo. Epo/EpoR seems to correlate with tumor progression and metastasizing. Therefore, the identification of at-risk group remains a clinical challenge. Vascular endothelial growth factor (VEGF) is a signal protein that stimulates angiogenesis and concentration of VEGF is positive correlated with tumor growth in numerous tumors.

The importance of Epo in tumor pathogenesis has led to a growing interest in the potential prognostic value. By our point of view there are many open questions about role of Epo/EpoR in CRC.

Introduction

Erythropoietin (Epo) is produced mainly by kidneys and by hepatocytes during fetal stage. Minor amounts are secreted by spleen, lung, testis and brain. Erythropoietin receptors (EpoR) belong to family of hematopoietic growth factors and are only partially expressed on cell surface (10%) while the majority of receptors are located in the cytoplasm, endoplasmic reticulum, Golgi apparatus and other endosome-like structures. The main role of Epo is stimulation of erythropoiesis by preventing programmed cell death.

Epo, however, also exhibits an anti-apoptotic action on numerous cells and tissues, including malignant ones. Data from studies researching on animals support the assumption that Epo is stimulating both lymph node angiogenesis and nodal metastasis by increasing migration, capillary-like tube formation, and dose- and time-dependent proliferation of human lymphatic endothelial cells. Epo expression is in positive correlation with tumor metastasis [1].

EpoR exists as a dimeric molecule, which after binding Epo, forms a homodimer. Epo signaling involves tyrosine phosphorylation of the homodimeric EpoR and subsequent activation of intracellular anti-apoptotic proteins, kinases and transcription factors. Main signaling pathways activated by Epo are JAK2/STAT5, PI3K, RAS/MAP kinase pathway and protein kinase C pathway. The JAK2/STAT5 and RAS/MAP kinase pathways are associated with hormone mitogen action, while the PI3K pathway is related with anti-apoptotic activities. Epo-induced proliferation of cancer cells is associated with the activation of JAK2, JAK3, STAT3, and STAT5 (but not JAK1 or STAT1), AKT phosphorylation, ERK phosphorylation (with hTERT gene transcription by JAK2/STAT5/c-MYC), and hTERT protein phosphorylation by PI3K/AKT [2,3].

Abbreviations: CEA, carcinoembrional tumor marker; CRC, colorectal carcinoma; EMT, epithelial-mesenchymal transition; Epo, erythropoietin Epo; EpoR,, erythropoietin recepotors; HCC, hepatocellular carcinoma; HIF-1α, hypoxia inducible factor 1 alpha; MV, mosaic vessels; TNM, tumor, node, metastasis; VEGF, vascular endothelial growth factor.

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Different tumors, including ovary, breast, lungs, thyroid, prostate, endometrial, cervix, head and neck, kidney, glioma and melanoma express EpoR. Breast cancer stem-like cells isolated from HER2-positive tumors express the EpoR and respond to Epo treatment with increased proliferation and self-renewal. Epo also stimulates epithelial-mesenchymal transition (EMT) in renal cell cancer, and pathological EMT has a key role in cancer progression [4].

Research also suggests that Epo plays a crucial role in the process of neo-angiogenesis and promotion of survival of hypoxic cancer cells. Hypoxia is a main driver for tumors leading to more malignant phenotypes, and it is also the main stimulus for Epo production. Hypoxia promotes the availability of heterodimeric α/β subunits of hypoxia-inducible transcription factors (HIF) which stimulate the Epo enhancer. HIF-1α is a major regulator of tumorigenesis in hypoxic conditions and therefore represents a potential therapeutic target in colorectal cancer (CRC) [5]. Significant correlation of HIF-1α to VEGF expression is reported and HIF-1α and VEGF status are found to be significantly associated with tumor stage, lymph nodes and liver metastases [6]. VEGF-C is a crucial regulator of the development of lymphatic vessels and is involved in the lymph node metastasis of cancer [7]. Expression of both HIF-1α and VEGF remained significantly associated with overall survival and HIF-1α also correlates positively to VEGF in CRC [4]. In CRC, besides processing the vascularisation and angiogenesis, VEGF is also promoting chronic inflammation in different stages od CRC tumorigenesis [8]. The strong co-expression of VEGF-A and CD31 suggests that neoangiogenesis in CRC has a prominent role [9]. Microenvironmental selection pressure during carcinogenesis determine phenotypic traits of malignant tumor. Chemokines, cytokines and growth factors released from tumor cells attract in macrophages, which than also release pro-angiogenic cytokines [10,11]. By increasing antigen uptake and cytokine secretion, Epo enhances macrophage function and their maturation [12].

Endothelium-dependent vessels, mosaic vessels (MV) and vasculogenic mimicry (VM) are participating in tumor blood microcirculation supply. VM positive tumors are usually related to more aggressive tumor biology and poor clinical outcomes [13]. Microvessel density and expression of Epo/EpoR are positive correlated, and they are acting together as endogenous stimulant of angiogenesis during the tumor progression (non-small cell lung cancer, gastric cancer) [14,15]. In gastric carcinoma, VEGF-C has a significant role in the carcinogenesis and progression [16]. In HCC, Epo/EpoR expression is significantly correlated with VM formation, thereby facilitating tumor cell migration and metastasis into the blood and lymphatic vessels [7]. Epo/EpoR positive tumors are correlating with stage of HCC and the worst overall survival rate is found in patients with Epo/EpoR positive and VM positive tumors [17]. Epo/EpoR levels strongly correlate with angiogenesis and progression also in neuroblastoma, squamous cell carcinoma of the tongue, melanoma, glioblastoma and some other tumors [18].

Despite the advance of modern medicine and despite the national and global programs of prevention, CRC remain a disease with high incidence and mortality with huge impact on national health budgets. Why is the biology and pathogenesis of CRC still a challenge? Most of CRCs arise from villous adenomas through the process of adenoma-carcinoma sequence. As in big adenomas, significant proportion of malignant alteration can also be found in smaller ones (5–10 mm) [19]. We know that CRC appearance can be attenuated in significant proportion of cases. When CRC is detected in its early stages (I, II) five-year survival is 80–90%, yet if found in stage III survival falls to 40–60% and in stage IV to 10%.

Chemotherapy applied in adjuvant setting rises percentage of survival, so early detection of CRC is of tremendously significance [4,10,20,21].

We are now focusing our attention upon very controversial reports on Epo/EpoR expression in CRC as well as its still uncertain role in tumor angiogenesis as promoter/stimulator of tumor growth which needs to be furtherly validated. The way of expressing Epo/EpoR in CRC could help us to recognize those patients with worse prognosis leading us to different therapy access.

The hypothesis

We hypothesize that Epo and EpoR expression correlate with CRC progression according to Dukes and TNM classification [16]. If our hypothesis is correct, recombinant Epo should not have a place in treating anemia in CRC patients. We also hypothesize that like HIF-1α, Epo/EpoR expression has a potential as a biomarker for poor prognosis in CRC.

Testing the hypothesis

Research will be carried out by using colon cancer samples. The samples will be fixed in 10% formalin paraffin and embedded in hematoxylin and eosin (H&E), and will hence be examined by light microscopy. We plan to apply anti-CD31 antibodies on tissue samples to determine mean vascular density (MVD), by a method used by Wiedner et al. [22]. MVD analysis and counting will be done by two independent researchers (see fig. 1).

To determine whether Epo shows immunohistochemical positivity, we will analyze three areas with invasive tumor growth, in every tissue samples. Semiquantitative analysis will be according to available data [23]. To determine immunoreactivity we will count the percentage of positive tumor cells on 100× magnification. The analysis will be done by two independent researchers. We will categorize and score the data as follows: negative (up to 10%), weakly positive (10–30% of positive tumor cells), moderately positive (31–60%), strongly positive (61%+). Immunoreactivity will finally be displayed as Histo-score which will be obtained by multiplying intensity with percentage of positive cells (EpoR H-score = (%x1) + (%x2) + (%x3). Similar method is used by variety of authors [24] (see fig. 2).

We also plan to determine EpoR positivity by immunohistochemistry in similar manner as described above, using same semiquantitative analysis as in appropriate studies [23]. Stratification of the data will be done by the same method and grades as Epo ranking, and finally, immunoreactivity will be shown as Histo-score (see Fig. 3).

Evaluation of the hypothesis

There are certain limitations that need to be mentioned. First of all, primary antibodies are not specific enough for detecting Epo/EpoR on tumor cells and can give us partly false positive results [8,25]. In study by Gombos et al. increased Epo/EpoR immunostaining was found following the progression of adenoma to adenocarcinoma, but when comparing carcinoma samples (based on their grade and stage) they did not found any difference in Epo/EpoR expression [26,27]. Increase of Epo/EpoR was found in ischemic and necrotic areas of tumor samples indicating that Epo signaling pathway together with HIF-1α and VEGF play important role in colon carcinogenesis [27]. Still, although HIF-1α expression in several studies has been shown to promote tumor progression and resistance to chemotherapy, data for HIF-1α expression and CRC prognosis are inconsistent despite the results of large study (731 CRC specimens). Baba et al. however proved that HIF-1α (but not HIF-2α) overexpression was independently associated with poor prognosis [28].