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TOPIC HIGHLIGHT

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Functional significance of erythropoietin receptor on tumor cells

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Abstract

Erythropoietin (Epo) is the regulator of red blood cell formation. Its receptor (EpoR) is now found in many cells and tissues of the body. EpoR is also shown to occur in tumor cells and Epo enhances the proliferation of these cells through cell signaling. EpoR antagonist can reduce the growth of the tumor *in vivo*. In view of our current knowledge of Epo, its recombinant forms and receptor, use of Epo in cancer patients to enhance the recovery of hematocrit after chemotherapy treatment has to be carefully evaluated.

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Key words: Erythropoietin; Tumor cell; Receptor; Cell signaling; Proliferation; Erythroid cell

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INTRODUCTION

Erythropoietin (Epo) is a hormone which regulates the red blood cell formation. Epo is produced in the kidney and delivered to the target organ, bone marrow, *via* circulation[1]. The circulatory levels of Epo is usually at a very low level but in response to the loss of red blood cells or during hypoxic exposure its level is increased within a few hours due to the enhanced production in the kidney. The elevated Epo level acts on the bone marrow erythroid cell precursor population to augment the erythroid cell production resulting in the increase of red blood cell mass. Thus EPO acts in a tightly regulated system for the maintenance of red blood cell volume. Action of Epo on the responding cells is mediated through its receptor, EpoR.

The signal provided by Epo and its receptor, EpoR are essential for erythroid cell proliferation and differentiation[2]. Studies have revealed that there is an unique mechanism of Epo binding and activation of EpoR-Jak2 kinase complexes. Erythroid cell populations collected from humans or mice and enriched by various techniques have been used for these studies[3]. Purified human blood erythroid burst forming units, mice fetal liver cells or spleen cells from mice made anemic by two consecutive injections of phenyhydrazine have been used for such investigations[3]. Studies indicated that Epo binds to EpoR and induces a signal for proliferation and survival similar to other cells.

STRUCTURE AND CHARACTERISTICS OF Epo

Epo is a glycoprotein with a molecular weight of approximately 34 000 daltons[4]. The protein backbone of the mature hormone consists of 165 amino acids. The alpha form of the hormone consists of 31% carbohydrate while the beta form consists of 24% carbohydrates. These two forms of Epo have similar biologic and antigenic properties. The carbohydrate moiety of Epo plays an important role in the mediation of its overall effect *in vivo*. Sialic acid, which comprises 30% of carbohydrates, represents the critical component of the carbohydrate moiety for *in vivo* biological activity. The desialation of Epo, while fully functional *in vitro*, results in significant loss of *in vivo* activity due to the rapid clearance by the liver of disialated Epo[5]. The importance of glycosylation of the Epo molecule in the mediation of its *in vivo* effect also became evident when it was found that the recombinant Epo produced in *E. coli* was not effective *in vivo*. Expression of the Epo gene in mammalian cells that provided sugars was needed to attain its full biological effect *in vivo*. Epo is a single chain polypeptide and is resistant to denaturation by heat, alkali or reducing agents. The Epo gene is located on chromosome 7 and unlike other hormones like insulin or ACTH, it is fully synthesized in its active form prior to secretion into circulation.

Epo AND EpoR FUNCTION

Effect of Epo on erythroid cells is mediated through the EpoR similar to other hormones. One single class of EpoR with an apparent molecular weight of about 100 000 daltons occurs and it binds to the hormone with a dissociation constant of about 300 pM[6]. Erythroid cells display between 1000 and 3000 EpoR sites per cell. Only a small percentage of these receptor sites have to be populated with Epo to initiate cell division and this process is dependent largely on Ca^{2+}.

Previously it was thought that EpoR and Epo are required for the survival and proliferation of erythroid cells alone. However, recent investigations have indicated the presence of EpoR in normal cells of other tissues as well. Epo is now known to be important in function and development of the brain. Furthermore the brain makes its own Epo which is slightly smaller in size than Epo produced by the kidney[7]. Epo is needed for the development of new blood vessels in the muscles of athletes training at high altitude. A greater oxygen demand by the exercised muscle tissue is supplemented by the presence of Epo. Requirement of Epo is also shown in ovary, oviduct, uterus, and testis and in all of these organs the presence of EpoR has been shown[4].

PRESENCE OF Epo AND EpoR ON TUMOR CELLS

Presence of EpoR is shown in the breast cancer cells while adjacent normal cells have no such receptors[8]. Authentic EpoR transcripts and protein have been detected in human renal-cell carcinoma and the cell lines derived from these are shown to enhance proliferation by the presence of Epo[9]. Presence of Epo and EpoR has also been shown in breast carcinoma but not in normal breast, benign papilloma or fibrocystic tissues[10]. EpoR is also found in epithelial ovarian carcinoma as detected by Western blot analysis[11]. Using the immunoblot technique, Belenkov *et al* have confirmed the presence of EpoR in the human malignant glioma cell line, U87 and the primary cervical cancer cell line, HT100[12]. Examination of breast cancer biopsies has revealed a high level of EpoR expression in cancer cells in 90% of tumors while Epo expression has been found in 60% of tumors[13]. Wollman *et al* have demonstrated the presence of EpoR in the human neuroblastoma cell line using the RT-PCR technique[14]. Occurrence of EpoR in human prostatic epithelial cells and prostate cancer cells has been demonstrated and in this instance Epo serves as a growth factor for these cells[15]. Yasuda *et al* have reported that signaling pathways of Epo and EpoR are involved in the tumorigenesis of ovarian and uterine cancers[16]. Hepatic tumors, chemically induced in rats, showed the presence of Epo while in normal cirrhotic liver tissues Epo was not detected[17]. EpoR was present in such tumors. Table 1 summarizes the publications which have looked into the occurrence of EpoR and Epo in various tissues. Our own observation on rat pancreatic tumor cell line, AR42J cell, has indicated the presence of EpoR on the surface of these cells and Epo induces proliferation of these cells (unpublished observation). Hence it is now well established that EpoR is present in most of the cancer cells and Epo has a significant effect upon these cells.

Table 1 Presence of Epo and EpoR in the tumors of different tissues of the body. Symbol + in the columns indicate the presence of particular item in the tumor and - indicate either it is not tested or it is absent

Tissue of origin	EpoR	Epo	References
Breast cancer	+	+	[8, 10, 13]
Glioma cell line, U87	+	-	[12]
Cervical cancer cell line, HT100	+	-	[12]
Epithelial ovarian carcinoma	+	-	[11]
Renal carcinoma	+	+	[9]
Nuroblastoma	+	-	[14]
Prostate cancer cells	+	-	[15]
Glioblastoma	+	+	[16]
Gastric cancer	+	+	[16]
Stomach choriocarcinoma	+	+	[16]
Lung small cell carcinoma	+	+	[16]
Pancreatic cancer	+	+	[16]
Head & neck squamous cell carcinoma	+	+	[18]
Hepatic tumor	+	+	[17]

FUNCTIONAL SIGNIFICANCE OF EpoR ON TUMOR CELLS

Question naturally arises what is the function of the EpoR present in cancer cells? Attempts have been made in many investigations to find the role of EpoR in light of induction of signal transduction and cell growth. Some studies, as described earlier, have noted the induction of proliferation of tumor cell lines in the presence of Epo. Transplanting several tumor cell lines into nude mice Yasuda *et al* have confirmed the presence of Epo-responsive sites in xenografts in which phosporylation of the STAT5 (signal transducer and activator of transcription) is detected[16]. In these nude mice when Epo signaling is blocked by EpoR antagonist, angiogenesis and tumor cell survival are inhibited leading to the destruction of tumor mass and the disturbance of phosphorylation of STAT5. Epo mimetic peptide, on the other hand, has promoted angiogenesis and tumor cell survival. Hence Epo is indispensable for the growth and viability of malignant tumor[16].

EpoR on prostate cells is functional and exhibited a dose-dependent proliferative response to Epo and triggered STAT5 phosphorylation[15]. Epo mediated invasion of head and neck squamous cell carcinoma cells in Epo-treated head and neck cancer patients has been shown to be due to induction of limited proliferation effect by the pharmacological dose of Epo[18]. Epo activates the mitogen activated protein kinase, extracellular signal regulated kinase (ERK), and promotes migration in breast cancer cells. This migration can be inhibited by the inhibitor MEK[19]. Further, hypoxia induced Epo mRNA and EpoR expression in breast cancer cells is followed by the activation of ERK and cell migration[19]. When rat mammary adenocarcinoma cells were implanted into rats

in a tumor Z-chamber model and administered with a neutralizing anti-EPO antibody or soluble Epo receptor or an inhibitor of Jak2, all resulted in a delay in tumor growth with 45% reduction in tumor depth in a dose dependent manner[13]. Renal carcinoma cells have been shown to have increased proliferation in the presence of Epo[9]. Several effects of Epo described above with various tumors and carcinoma indicates a compelling proof that cancer cells have EpoR and Epo has an effect on these cells despite the fact that a recent study has indicated that all anti-EpoR antibodies do not always predict the EpoR expression[20]. An exception to this is an early study by Wollman *et al*[14] who have found no proliferative effect of Epo on nerve tumor cells in spite of the presence of EpoR on these cells.

CONCLUSIONS

The reports on studies of Epo and EpoR indicate that they are not specific to erythroid cell proliferation and differentiation alone. They have an active role in other cells as well, normal as well as tumor cells. Use of Epo in cancer patients has to be monitored carefully for its side effects related to its proliferation potential of the tumor for which chemotherapy is given. It is important as more and more patients are being treated with Epo for a rapid hematocrit recovery after chemotherapy treatment. Knowledge is emerging in this regard and further studies are needed to sort out the beneficial effect of Epo from its harmful effect.

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