Human Erythropoietin Receptor: Cloning, Expression, and Biologic Characterization

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We have isolated the human homologue of the murine erythropoietin receptor (mEPO-R) from an erythroleukemia line, OCIM1, and from fetal liver. Both the cDNA and protein sequence of the human receptor were 82% homologous to the mEPO-R. Heterologous expression of the human cDNA in COS cells yielded a protein of about 66 Kd. The protein could be specifically immunoprecipitated with either an antibody raised against the amino terminus of mEPO-R or by a monoclonal antibody that bound EPO bound to its receptor. Cross-linking of radioiodinated EPO to COS cells expressing the human EPO-R gave apparent molecular weights of 68 and 100 Kd for the receptor. The murine interleukin-3–dependent pre-B lymphocyte cell line, Ba/F3, was made EPO-dependent by transfection of the human cDNA into the cells and selecting for growth in EPO-containing media.

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Fig 1. Analysis of the sequence of clone no. 18. (A) hEPO-R nucleotide and predicted amino acid sequences. The putative amino terminus at residue 25 is indicated by an arrow. The sites of potential N-linked glycosylation are boxed. The nucleotide difference with one PCR clone is indicated by an arrowed A. The putative transmembrane domain is underlined. The position of the intron region of the single amino acid insertion in hEPO-R is overlined. Conserved sequences with other hematopoietic growth factor receptors are boxed.

Development of an EPO-dependent cell line. The murine interleukin-3 (mIL-3)-dependent cell line, Ba/F3, was transfected (1 x 10⁵ cells in 0.8 mL of ice-cold PBS) with linearized (EcoRI) pIL-R by electroporation,¹⁹ using a Gene Pulse Electroporator (BioRad). Transfected cells were grown for 2 days in RPMI, 10% FCS, and 10% WEHI conditioned media before selection in media containing RPMI, 10% FCS, and 0.5 U/mL recombinant human EPO (hEPO).

Proliferation assay. EPO-dependent cells expressing the hEPO-R (Ba/F3-EPO-R) were washed three times with RPMI and seeded in a 96-well microtiter plate at 1 x 10⁴ cells/mL (200 µL/well). EPO dose response was measured by growing the cells in various concentrations of hEPO. After 48 hours, cell number and viability were determined.²⁰

RESULTS

Isolation and identification of a cDNA encoding the hEPO-R. The human erythroleukemia cell line, OCI1,¹ expresses a 2-kb RNA transcript that hybridizes to the
full-length mEPO-R cDNA (data not shown). The cDNA to this transcript was isolated by screening a cDNA library prepared from poly-A selected OCIM1 mRNA, with the mEPO-R gene. Two positive clones, nos. 18 and 27, containing inserts of 1.5 and 1.2 kb, respectively, were isolated and sequenced.

Sequence analysis of clones of hEPO-R. Clone no. 27 was incomplete at the 5′- and 3′- ends of the gene when compared with mEPO-R cDNA (data not shown). Clone no. 18 was full-length and identical to clone no. 27 except for a putative intron of 95 nucleotides at nucleotides 914/5 (Fig 1A). The nucleotide sequence of clone no. 18 contains a single open reading frame of 1,524 nucleotides encoding a 508 amino acid protein with a calculated molecular mass of 55-Kd (Fig 1A). The cDNA and protein sequence of the putative human receptor were both 82% similar to the corresponding mEPO-R sequences. The main structural features of the human and murine receptors are conserved between species, except for an amino acid insertion between amino acids 71 through 76 and the absence of an N-linked glycosylation site, in the cytoplasmic domain, for hEPO-R (Fig 1B).

To confirm the functional structure of the hEPO-R gene, two oligonucleotides were synthesized based on the sequence of the open reading frame of clone no. 18 and used to amplify the putative hEPO-R gene from human fetal liver cDNA. The sequence of the PCR clone was identical to the coding sequence of clone no. 18, except for a single T→A difference at nucleotide 235, and it did not contain the putative intron. Two other PCR clones were sequenced in this region, and both did not contain the T→A base change. Subsequently, the structure of the intron was confirmed by sequencing genomic clones isolated from a human lymphocyte library (data not shown).

Expression of hEPO-R gene in COS cells. The DNA sequence containing the intron in clone no. 18 was exchanged for the corresponding region in clone no. 27 to obtain p18-R with a contiguous in-frame coding sequence. Transient transfection of COS cells with p18-R, followed by metabolic labeling, yielded a protein of about 66-Kd. This protein was specifically immunoprecipitated with either a rabbit polyclonal antibody raised against the amino terminus of mEPO-R (Fig 2A, lane 4) or by an immune complex of EPO and the MoAb 1.4.2 (Fig 2A, lane 2).

Chemical cross-linking of [125I]EPO (molecular weight 40-Kd) to COS cells transfected with p18-R gave two bands of apparent molecular weights of 100 and 140-Kd (Fig 2B, lane 2).

EPO-dependent cell-growth in Ba/F3 cells transfected with p18-R. The putative hEPO-R was shown to be biologically active by preparing an EPO-dependent cell line. The mIL-3-dependent pre-B-lymphocyte cell line, Ba/F3, was transfected with p18-R, linearized with EcoRI. The resulting transfecants expressed the 66-Kd hEPO-R by immunoprecipitation of cell lysates from [35S]cysteine-labeled cells (data not shown) and grew in either mIL-3 or EPO-
expressed in COS cells compared with the murine homo-


cross-species reactivity of human EPO, the hEPO-R displays

receptors for erythropoietin on human bone marrow cells and on the

Ba/F3 hEPO-R cells, attempts to cross-link [125I]EPO to the

surface hEPO-R were not successful.

The presence of two cross-linked EPO-R complexes on

COS cells expressing the hEPO-R raises several intriguing

possibilities. First, the results may be an artifact of expression

in COS cells (eg, glycosylation or other protein modific-

ation). Second, the receptor may interact with an endogenous

COS protein. Third, the receptor may dimerize in the

membrane. Fourth, two molecules of EPO bind one molecule

of receptor based on the size of the higher molecule weight

cross-linked product. Most likely the hEPO-R polypeptide

interacts with a second subunit or accessory protein. For

instance, it has been recently shown18 that the gp55 glycopro-

tein of the Friend virus binds to and stimulates the mEPO-R.

This viral glycoprotein may be mimicking a normal second

subunit of the EPO-R.

hEPO-R, like mEPO-R, belongs to a novel and growing

family of hematopoietic growth factors outlined by other.21,22

For instance, the receptor has the conserved “WS-WS”

sequence and a cysteine tetrad motif in the extracellular

domain (Fig 1B, boxed sequences). In the cytoplasmic

domain the receptor shares the proline, serine, and acidic

amino acid-rich regions seen with some,23 but not all,26,27

members of this growth factor receptor family.

The gene for the hEPO-R will provide a valuable tool to

address several issues; the role of these conserved sequences in

signal transduction, and the structure and physiologic

relevance of the different classes of receptors on erythroid

progenitors and leukemic cell lines.

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SS Jones, AD D'Andrea, LL Haines and GG Wong