RAPID COMMUNICATION

The Gene for the Human Erythropoietin Receptor: Analysis of the Coding Sequence and Assignment to Chromosome 19p

By John C. Winkelmann, Laura A. Penny, Larry L. Deaven, Bernard G. Forget, and Robert B. Jenkins

The full-length coding sequence of the human erythropoietin receptor has been assembled from cDNA and genomic DNA. The derived 508 amino acid sequence is 82% identical to the murine erythropoietin (Epo) receptor with one single residue gap in alignment. There is no major structural difference between the human and murine receptor molecules. Nucleotide sequence homology is, as expected, very high within the coding domain. Unexpectedly, there are two distinct, short stretches of 3' untranslated sequence homology between human and murine cDNAs. The functional significance of this sequence conservation is unknown. The human Epo receptor gene is localized to human chromosome 19p by in situ hybridization. This chromosome assignment is confirmed by hybridization to a panel of sorted human chromosomes.

© 1990 by The American Society of Hematology.

ERYTHROPOIETIN (Epo) is a glycoprotein hormone that regulates erythropoiesis. Its synthesis by the kidney is stimulated by decreased oxygen delivery. Epo exerts its effect on early erythroid progenitors, resulting in cell proliferation and erythroid differentiation. The molecular mechanism of Epo action is largely unknown. In recent years it has become apparent that, like other glycoprotein hormones, the biologic effects of Epo are initiated by its association with a specific cell-surface receptor.

The recent expression cloning of the murine Epo receptor cDNA has made direct examination of the Epo receptor molecule possible. The reported cDNA clone encodes a 55-Kd peptide with a single putative transmembrane domain approximately in the middle of the molecule. The Epo receptor belongs to a previously unrecognized family of growth factor receptors; it is structurally related to the interleukin-2 (IL-2) receptor β subunit and the receptors for IL-3, IL-4, IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF). Many important questions remain regarding subunit composition, hormone binding characteristics, and signal transduction properties of the Epo receptor.

The human Epo receptor is of interest to investigators studying erythropoiesis in humans and the biology of clinical disorders such as pure red blood cell (RBC) aplasia and polycythemia vera. To determine the structure of the human receptor, we have isolated and characterized a cDNA clone encoding 75% of the human fetal liver Epo receptor using synthetic oligonucleotides derived from the reported murine Epo receptor. Using the nucleotide sequence of this cDNA and exon sequence derived from 5' genomic DNA, we have derived the primary structure of the human Epo receptor. Human chromosome assignment and sublocalization have been performed.

MATERIALS AND METHODS

Molecular cloning. Two synthetic 36-base oligonucleotides, based on the 3' coding sequence of the reported murine Epo receptor, were used as hybridization probes. These oligonucleotides (A: 5'-GATGGCCCTCTATGCAAGACAGCCTT-3', a sense probe; and B: 5'-CACCATTGGGATGCAGAGGCTCT-3', a antisense probe) were labeled by reacting 1 µg of DNA with α-32P-deoxycytidine triphosphate (Amersham, Arlington Heights, IL) in the presence of terminal deoxynucleotidyl transferase (Pharmacia, Piscataway, NJ). Oligonucleotide A was hybridized to one million bacteriophage plaques plated from a human fetal liver cDNA library constructed in λgt11 and transferred to nitrocellulose membranes (Schleicher and Schuell, Keene, NH). Hybridization conditions were 70 mmol/L Tris pH 7.4, 6X SSC (1X SSC; 0.15 mol/L NaCl, 0.015 mol/L Na citrate), 5X Denhardt's (2X Denhardt's: 0.2% sodium each of Ficoll, polyvinylpyrrolidone, and fraction V bovine serum albumin), 100 µg/mL denatured DNA, and hybridization was performed at 68°C for 16 hours. After washing the filters with 2X SSC at 68°C, the filters were exposed to Kodak XAR-5 film for 2-4 days at -80°C.

From the Department of Medicine and Institute of Human Genetics, University of Minnesota, Minneapolis; Departments of Laboratory Medicine, Mayo Clinic, Rochester MN; Departments of Medicine and Human Genetics, Yale University, New Haven CT; and Life Sciences Division, Los Alamos National Laboratory, Los Alamos NM.

Submitted March 9, 1990; accepted April 20, 1990.

Supported by Grant No. HL-39834 from the National Institutes of Health, and a Basil O'Connor Starter Scholar Research Award from the March of Dimes.

Address reprint requests to John C. Winkelmann, MD, Division of Hematology, Department of Medicine, and Institute of Human Genetics, University of Minnesota Medical School, Minneapolis, MN 55455.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1990 by The American Society of Hematology.

0006-4971/90/7601-0034$3.00/0
The human Erythropoietin receptor 25

analyzing the 5' exons is shown.

tion codon (TAG) is underlined.

otide coding sequence of the

otides A and B (see above) to a cDNA library constructed in hgtll

sequence determination. The subcloning strategy used for sequence

the 3' end of ER2.

The position of each intron sep-

phage promoters that flank the pGEM7Z polylinker were annealed

et all5 was used. Primers homologous to the SP6 and T7 bacterio-

3').4

liver. Its identification as an authentic

H. Erythropoietin Receptor

(underlined) at nucleotide 28.

Fig 2. Assembled nucleo-

herring sperm DNA, 100 µg/mL polydeoxycytidine, 0.01 mol/L

sodium pyrophosphate, and 0.1% sodium dodecyl sulfate (SDS) at

in situ hybridization method of Harper and Saunders,” as modified

Computer analysis of nucleotide sequence data was performed on

microcomputer using The DNA Inspector IIe (TEXTCO, West

Lebanon, NH) programs. Nucleotide and amino acid databank

searches and alignments were accomplished on minicomputer using the Intelligenetics programs and GenBank database.

In situ hybridization to human metaphase chromosomes. The

in situ hybridization method of Harper and Saunders,13 as modified by Donlan,14 was used. Fluorescent R-bands,19 as well as routine Wright staining, were used to identify chromosomes.

Hybridization to sorted human chromosomes. Human

metaphase chromosomes were isolated by flow-sorting.10 They were

spotted onto nitrocellulose discs, denatured, hybridized to 32P-

labeled ER2 cDNA as and autoradiographed. The

mRNA from a mouse erythroleukemia cell line.

Nucleotide sequence analysis. The ER2 cDNA and genomic DNA were subcloned into the plasmid pGEM7Z (Promega Biotec, Madison, WI) for restriction enzyme mapping and nucleotide sequence determination. The subcloning strategy used for sequence analysis is shown in Fig 1. The dideoxynucleotide method of Sanger et al15 was used. Primers homologous to the SP6 and T7 bacterio-

phase promoters that flank the pGEM7Z polylinker were annealed to double-stranded plasmid templates. Sequenase (United States Biochemical, Cleveland, OH) was used for all sequence reactions.16

The labeling isotope was α-35 S-thio-deoxyadenosine triphosphate. Deoxyinosine triphosphate was substituted for deoxyguanosine triphosphate in sequencing reactions to resolve sequence ambiguities.

RESULTS

ER2 is a 1,347 base pair cDNA clone from human fetal liver. Its identification as an authentic Epo receptor clone

1  TGGGCTCCCC CTTGCCGCTGG GTCTATCATG GACCACTGAG GGGCCTCCTT

51  CTGGCCCCAG GTCGGTCCTCC TTTGTCTCTC GCTCGTCTGG GCGCGCTTGG

101  CGCCTCCGCC TAACCTCCCC GACGCGAGCT TCGAGAGCGA AGCCGCTTGG

151  CTGGGGCGCC GGGGCGCGGA AGACCTCTTG TCCTTCACCA GACGGGCTGA

201  GGACTGTTGG TGGTCTGTTG TCGCTGGCGT ACTGACATCC GGTGCCTTGA

251  GCAACTCAGT CTTCTCTTAC CAGCTGAGG ATGAGCCATG GAAGCTGTGT

301  CGTCTCCAGG CTGCTGGCGG TCCACGCTTT CTCCGCTTGG AGGGGCTTGG

351  GCTGCCTACA GCCGACACGT CGAGCTTCGT GCCCCTAGAG TTGCGCGTCA

401  CACGCACTCC GTGCGCTTGC GTATACCACT GTGTCATACC CATCAATGAA

451  GCTAGTGCTCC TAGACGCCCC CGTGGGGCTG GTGGCGCGGT TGGCTGACGA

501  GAGCCCAAGG GGGCTTATCC GATGGGCCCT ACTCCAACCC TTATGAGAAC

551  AGTACACTAT CCTGGACCCC AGCTCCCAGC TCTTGCGTCC ATGGACACTG

601  TGCTTTGGCC TCGAAGCCCA GCCCAGAGGG AGCCTCTGCT GCCAGCTTTG

651  TGCTGCCCCG GAACCCGCCC AGTGAGGACC TCCCAGGGCC TGGTGGCAGT

701  AGTGGGCAGT GAGCATGCCC AGGATACCTA TCTGGTGCTG GACAAATGGT

751  AGGACCCACC TGCTTCCCTG GAAGTCCTCT CAGAGCGCTG CTGGGGGACG

801  GAGCGGCCAC GTAGTGTTGC GCTGGCTCCC GCCGCCTGAG ACACCCATGA

851  GCCGGGCTCT GAAGCAGAAG ATCTGGCCTG GCATCCCGAG CCCAGAGAGC

901  GAGCCCAAGG GGGCTTATCC GATGGGCCCT ACTCCAACCC TTATGAGAAC

951  GTACCAGAAT GATGGCTGCC TGTGGTGGAG CCCCTGCACC CCCTTCACGG

1001  AGGCCCAACCC TGGATGCACG GCCCAGAAGT TCTCGCTGCC GGGCGTCCCT

1051  ATGCAAGGGC TGGACGGGCC TCCCTGCTCC CTCCCCAGC CCCGATTGTG

1101  ATGAGCCGAG CTTCGGCGGC TTCTGGAGCG CCTGGTCGGA GCCTGTGTCG

1151  TGCTGCCCCG GAACCCGCCC AGTGAGGACC TCCCAGGGCC TGGTGGCAGT

1201  TGTCAGTCTG AGCTGGTGGG GCCTGCAGA TGATCAGGGA TCCAATATGA CTCAGAGAAC

1251  TGCTTGGGCC TGCAACGCTCC GCCGAACGGC AGCTCTCGCT GCCACGTTCG

1301  AGTACATCTA CGTGGACCCC AGCTCCCAGC TCTTGCGTCC ATGGACACTG

1351  TGCGCTTGAAG TCGCCCTTAC CCCACCCACC CAATAGATCC TGATCCCTGT

1401  GGTATCTGAC TCTGGCATCT CAACTGACTA CAGCTCAGGG GACTCCCAGG

1451  GACGGCAAGG GGGCTTATCC GATGGGCCCT ACTCCAACCC TTATGAGAAC

1501  AGCTTATCC CAGCGGCTGA GCCCTGCTCC CCCAGATGTG TGCTTGTGTC

1551  TGTCAGGACG AGCTGGTGGG GCCTGCAGA TGATCAGGGA TCCAATATGA CTCAGAGAAC

1601  TGTCAGGACG TCAAGCTTA TGGGACGGC ATGGACGGGC TTCTCGCTGG

1651  AGCAAGGGCA TGGCTGATTG TTGCTGCTCC ATCCATCTTG TCTCGAGAAC

1701  CACACCTTGT CATATTTTTT AATAATGATG AGTATTTTTT TTGTATCTTA

1751  TATATATATAT CACATAAAAA AAAAAAAA

Fig 2. Assembled nucleo-

dotide coding sequence of the

human Epo receptor. The cod-
ing sequence begins with ATG (underlined) at nucleotide 28. The position of each intron sepa-

rating the 5' exons is shown

by an arrowhead. The termina-

tion oodon (TAG) is underlined.

The 22 nucleotides of the 3'

end of ER2.

The murine cDNA was isolated by hybridization of oligonucle-

otides A and B (see above) to a cDNA library constructed in hgtll

sequence (5'-ATGGACAAACTCAGGGTGCCCCTCTGGCCT-

3').4

The ER2 cDNA and genomic DNA were subcloned into the plasmid pGEM7Z (Promega Biotec, Madison, WI) for restriction enzyme mapping and nucleotide sequence determination. The subcloning strategy used for sequence analysis is shown in Fig 1. The dideoxynucleotide method of Sanger et al15 was used. Primers homologous to the SP6 and T7 bacterio-

phase promoters that flank the pGEM7Z polylinker were annealed to double-stranded plasmid templates. Sequenase (United States Biochemical, Cleveland, OH) was used for all sequence reactions.16

The labeling isotope was α-35 S-thio-deoxyadenosine triphosphate. Deoxyinosine triphosphate was substituted for deoxyguanosine triphosphate in sequencing reactions to resolve sequence ambiguities.

Computer analysis of nucleotide sequence data was performed on microcomputer using The DNA Inspector Ile (TEXTCO, West Lebanon, NH) programs. Nucleotide and amino acid databank

searches and alignments were accomplished on minicomputer using the Intelligenetics programs and GenBank database.

In situ hybridization to human metaphase chromosomes. The

in situ hybridization method of Harper and Saunders,13 as modified by Donlan,14 was used. Fluorescent R-bands,19 as well as routine Wright staining, were used to identify chromosomes.

Hybridization to sorted human chromosomes. Human

metaphase chromosomes were isolated by flow-sorting.10 They were

spotted onto nitrocellulose discs, denatured, hybridized to 32P-

labeled ER2 cDNA as and autoradiographed. The

position of each sorted chromosome was marked on the filter discs. The authenticity of hybridization signals was established by superim-

posing the autoradiograph on the marked filters.

RESULTS

ER2 is a 1,347 base pair cDNA clone from human fetal liver. Its identification as an authentic Epo receptor clone
was accomplished by nucleotide sequence analysis. Because ER2 does not include the 5' end of the coding sequence, the remainder has been derived from genomic DNA. Exons were identified by hybridization to 5' murine Epo receptor sequences. Figure 1 depicts the molecular clones and the nucleotide sequencing strategy used to analyze ER2 and exons I through III. The assembled coding sequence of the human Epo receptor is shown in Fig 2. The reading frame begins at the underlined ATG. The positions of the 5' introns are designated by arrowheads. The 22 nucleotides at the 3' end of exon 11 are identical to the 5' end of ER2. The cDNA extends through the translation termination codon (underlined) and the 3' untranslated region. There are 14 adenine nucleotides at the 3' end, implying that this is the site of poly(A) addition. The genomic DNA sequence diverges from the cDNA at this point (data not shown), also suggesting that this is the authentic site of polyadenylation. Surprisingly, there is no consensus poly(A) addition signal (AATAAA) upstream of the 3' end.

Figure 3 displays the alignment of the reported murine receptor with the derived 508 residue human Epo receptor amino acid sequence. The predicted peptide has a calculated molecular weight of 55.24 Kd. There is an 82% amino acid sequence identity between murine and human receptors, with a single amino acid gap in alignment. The putative hydrophobic leader and transmembrane domains of the murine receptor align exactly with that of the human sequence, as determined by hydrophilicity plots (data not shown). The degree of homology is similar throughout the length of the molecule.

As expected, there is a high degree of nucleotide sequence conservation between human and murine Epo receptor coding sequences. Interestingly, there is sequence homology between the 3' untranslated regions of the murine and human cDNAs. Figure 4A shows a homology matrix plot of the 3' untranslated sequences. With the parameters used (a search segment of 25 nucleotides, allowing eight mismatches), sequence homology is detected in two discrete segments, designated 1 and 2. Figure 4B shows the sequence alignment of the conserved regions. Segment 1 is 25 bases in length and 76% identical between mice and humans, with no gaps in alignment. A search of nucleotide sequence databanks showed no other genes with close homology to segment 1. Segment 2 is 35 bases long and is 71% identical, with one single nucleotide gap. Segment 2 is just 5' to the apparent poly(A) tail and includes a stretch of simple (TA) repeats. On
THE HUMAN ERYTHROPOIETIN RECEPTOR

**DISCUSSION**

We describe the coding sequence of the gene for the human Epo receptor. As expected, there is a high degree of nucleotide and derived amino acid sequence conservation between the murine and human receptors. If one presumes the murine receptor molecule to be of adult derivation, it is notable that there appears to be no major structural dissimilarity between this molecule and the human fetal liver receptor (encoded by ER2). This would suggest that the relative GM-CSF or IL-3 dependence of Epo action in adult versus fetal RBC precursors is determined by other molecules, perhaps interacting with the Epo receptor.

We observe two distinct, short 3' untranslated region homologies between human and murine Epo receptors (Fig 4). Usually there is no significant sequence conservation between noncoding sequences of mouse and human cDNAs. Several examples exist of such sequence homologies that have important functional implications. Conserved untranslated region sequences are involved in posttranscriptional regulation of gene expression and determination of mRNA stability. There is no evidence that the conserved 3' sequence of the Epo receptor is functionally important. The possibility that these sequences may be involved in Epo receptor gene expression remains to be tested experimentally.

In situ hybridization data sublocalize the gene encoding the ER2 cDNA to human chromosome 19p. This assignment is confirmed by the independent technique of hybridization to a panel of sorted human chromosomes. No inherited disorders of human erythropoiesis have been mapped to 19p. It has two extra chromosomes 19 that are designated 19p+ (ie, 19p has extra genetic material). TF-1 cells are known to express high levels of Epo receptor. Also, this chromosomal region exhibits no extended locus homology with a corresponding mouse chromosome. Interestingly, a human erythroleukemia cell line (TF-1 cells) has several karyotype abnormalities. It has two extra chromosomes 19 that are designated 19p+ (ie, 19p has extra genetic material). TF-1 cells are known to express high levels of Epo receptors. One might speculate that abnormalities in structure or expression in the Epo receptor gene may have a role in the pathogenesis of this erythroleukemia.

The availability of molecular clones for the Epo receptor will allow detailed molecular analysis of normal and pathologic erythropoiesis. Several important questions concerning the receptor itself remain unanswered. First, there are inconsistencies between data obtained from Epo receptor crosslinking experiments and the peptide structure derived from cDNA. Second, the determinants of receptor binding affinity are unknown. The putative...
interactions of the Epo receptor with other membrane proteins are undefined. The solution of these problems will be an important step in understanding erythropoiesis. In addition, studies to determine the precise regions of the receptor molecule involved in ligand binding and signal transduction are possible. Expression of the Epo receptor gene is likely to be one of the earliest events in hematopoietic commitment to the RBC lineage. Therefore, the regulation of this gene is of keen interest.

ACKNOWLEDGMENT

The authors acknowledge Dana M. Carlson, Mary Campbell, Tho T. Dang, Kelly Munson, and Robert Mitchell for excellent technical assistance.
REFERENCES

38. Tojo A, Kukamachi H, Kasuga M, Urabe A, Takaku F:


The gene for the human erythropoietin receptor: analysis of the coding sequence and assignment to chromosome 19p

JC Winkelmann, LA Penny, LL Deaven, BG Forget and RB Jenkins