Putative Oncogenic Role of the Erythropoietin Receptor in Murine and Human Erythroleukemia Cells

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To determine whether the erythropoietin receptor (Epo-R) plays a role in the course of malignant erythropoietic disorders, this gene was studied in murine and human erythroleukemia cells. An altered Epo-R gene was found in a murine Friend erythroleukemia cell line, FCL1, due to a spleen focus-forming virus (SFFV) long terminal repeat insertion within the noncoding region of the first exon, leading to Epo-R mRNA overexpression. A similar mechanism of Epo-R activation has previously been described in the T3CL-2 Friend erythroleukemia cell line. An elevated number of Epo-binding sites has been observed in two human erythroleukemia cell lines, TF-1 and UT7. In UT7 cells, homogeneously staining region of the short arm of chromosome 19 [hsr (19)] was evidenced, which contained an amplification of the Epo-R gene. This Epo-R gene amplification was confirmed by the quantification of Southern blots in which the intensity of the Epo-R signal was compared in UT7 DNA and in DNA from normal cells. The Epo-R gene was present in UT7 at a mean number of seven to eight copies per cell. Interestingly, the Epo-R gene was rearranged; the breakpoint region was located near the 3’ end of the gene, 3 kb downstream from the end of the last exon. Taken together, these results suggest that, in both murine and human systems, genetic alterations of the Epo-R gene are not rare events and could be involved in the occurrence of the erythroleukemic process.

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ERYTHROPOIETIN (Epo) stimulates proliferation and/or differentiation of erythroid progenitor cells by binding to specific membrane receptors. The erythropoietin receptor (Epo-R) is a member of the cytokine receptor superfamily that includes receptors for such cytokines as interleukin (IL)-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), leukemia inhibitory factor (LIF), and oncostatin, as well as for growth hormone, prolactin, and the ciliary neurotrophic factor. The cloned Epo-R chain possesses the common structure described for this receptor superfamily, characterized by a cytoplasmic domain without kinase catalytic activity, a single membrane-spanning domain, and an extracellular domain containing a set of four spatially conserved cysteine residues and a membrane-proximal Trp-Ser-X-Trp-Ser motif. Different modifications in the activation of some cytokine receptors, such as constitutive activation by interaction with retroviral glycoproteins or mutation in the extracellular domain, have been shown to be important steps in leukemic processes. Thus, the interaction between the envelope glycoprotein of Mink cell focus-forming viruses and the IL-2 receptor induces a growth factor–independent proliferation of lymphoid cells. Similarly, the glycoprotein gp55 encoded by the env gene of the polycythemia-inducing Friend spleen focus-forming virus (SFFVp) can interact with the Epo-R. Binding of gp55 to Epo-R appears to mediate the Epo-R complex expressed at the cell surface and the SFFVp-mediated proerythropoietic effect during the preleukemic stage of the Friend disease. Recent data, in accordance with those previously published, showed a correlation between the occurrence of the SFFVp–Epo-R complexes expressed at the cell surface and the SFFV pathogenic potential. Constitutive activation of the Epo-R in the absence of viral products has also been documented, since a single mutation located at codon 129 in the Epo-R extracellular domain (replacement of an Arg by a Cys residue) constitutively triggered the activation of this receptor in the absence of Epo addition. It is noteworthy that a retrovirus carrying this mutated form of Epo-R gained oncogenic properties and induced erythroleukemia with polycythemia in susceptible mice.

More recently, a direct relationship between an abnormality of the Epo-R and pathogenicity has been demonstrated. Indeed, a highly significant linkage between a familial erythrocytosis and a polymorphism of the Epo-R gene was established. In human myeloid cell lines with erythroid potential, the exact role of Epo and its receptor in the occurrence and development of erythropoietin remains to be elucidated. K562, HEL, and OCI16 cell lines harbor variable numbers of Epo-binding sites, but do not respond to Epo. KU812 and KJ18 cells are sensitive to Epo. Epo increases hemoglobinization in KU812 cells, whereas it enhances the plating efficiency of KJ1 cells, which spontaneously differentiate into red blood cells. Two more recently established human leukemia cell lines, TF-1 and UT7, are strictly growth factor–dependent for their proliferation, and Epo is required for the appearance of erythroid differentiation features in UT7 cells. The heterogeneity in the response to Epo of these different leukemia cell lines raises the question of the structure and functionality of the Epo-R in these cells.

We previously described a DNA rearrangement of the Epo-R gene in the T3CL-2 Friend-induced erythroleukemia cell line related to a retroviral insertion and leading to Epo-R transcriptional activation.

In this report, we analyzed the Epo-R gene structure and

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expression in other murine and human erythroleukemia cells. We found that in another Friend erythroleukemia cell line, FCL1, the Epo-R gene is altered by a mechanism similar to that described for T3CL-2. Moreover, we found a complex alteration of the Epo-R gene in the human UT7 cell line, characterized by an amplification of the Epo-R locus and a genomic rearrangement located outside the coding sequence, 3 kb 3′ from the end of the last exon of the Epo-R gene.

**MATERIALS AND METHODS**

**Erythroleukemia cell lines and tumors.** Murine erythroleukemia cell lines were cultured in alpha medium plus 5% heat inactivated fetal calf serum (FCS) in a humidified incubator (5% CO₂, 95% air) at 37°C. Different cell lines were studied, including polycythemic Friend-induced erythroleukemia cells T3CL-2, FCL1, TFP10, F4N, F4+, FLc, 707, and 745; anemic Friend-induced erythroleukemia cells CTF 23-30; Rauscher erythroleukemia cells Red 5-1.5; and helper F-MuLV–induced cells IJW32, IW35, 201 IW32, NN10.24

The two factor-dependent erythroleukemia cell lines UT7 and TF-1 were grown in alpha medium containing 10% FCS and 2.5 ng/mL recombinant human GM-CSF, whereas KU812 and JK1 cell lines were grown in RPMI 1640 containing 10% FCS.

Most primary Friend tumors were isolated from spleen and liver of ICFW mice infected with the polycythemic Friend viral complex (Lilly and Steeves) or Ikawa. Friend-induced erythroleukemia cells T3CL-2, FCL1, TFP10, F4N, F4+, FLc, 707, and 745; anemic Friend-induced erythroleukemia cells CTF 23-30; Rauscher erythroleukemia cells Red 5-1.5; and helper F-MuLV–induced cells IJW32, IW35, 201 IW32, NN10.24

**Polymerase chain reaction amplification of murine erythroleukemia genomic DNA.** Oligonucleotides were synthesized on a Milli-Biosearch 8700 DNA synthesizer (Millipore, Bedford, MA) and purified on a 20% acrylamide urea gel. The oligonucleotide position and sequence used for polymerase chain reaction (PCR) amplification and sequencing have been described previously.25 Briefly, INTS-310 5′-(AACTCTGCTGTCTGCCCCA)-3′ is an oligonucleotide located at the Epo-R promoter region, and INTS-190 primer 5′-(CCA-GGCTGCCAGGAAGTGA)-3′ corresponds to the Epo-R exon 1.

Genomic FCL1 DNA (1 μg) was added to 100 μL of a PCR mixture containing buffer, dNTPs (10 mmol/L), 2.5 U Taq polymerase (Perkin Elmer Cetus, Norwalk, CT), and 1 mmol/L of each oligonucleotide primer. The amplified fragments were electroeluted and cloned in pUC18 as described previously.25 Inserts from recombinant pUC18 vectors were sequenced on both strands by the dideoxy chain termination method (Sanger) with a Pharmacia T7 sequencing kit (Pharmacia, Uppsala, Sweden), using universal primers or specific oligonucleotides as internal primers.

**Western blot analysis.** Sample preparation, electrophoresis, and transfer were performed as previously described.26 A goat antiserum to purified Rauscher Envelope (Env) protein was kindly provided by Dr J. Gruber, National Cancer Institute, Bethesda, MD. This antiserum recognizes the F-MuLV and the SFV Ef Env proteins. It was used at 1/250 dilution for Western blots. An alkaline phosphatase–labeled rabbit anti-goat IgG antiserum was purchased from Sigma (St Louis, MO). The revelation was performed using the nitroblue tetrazolium (NBT)/5-bromo-4-chloro-3 indolyl phosphate (BCIP) method.

**Northern blot analysis.** Total cellular RNA from different cell lines was extracted according to the method of Chirgwin et al21; after glyoxal denaturation, 20 μg of total RNA was electrophoresed and transferred to GeneScreen membranes (Dupont NEN, France).

**Southern blot analysis.** High–molecular weight DNA were digested with restriction endonucleases; 10 μg was blotted to nylon membranes after electrophoresis and hybridized to the β- labeled probes.

cDNA and genomic probes. Genomic and cDNA murine Epo-R probes have been described previously.22 Human Epo-R probes have been isolated from cDNA and genomic clones.22 For fluorescence in situ hybridization (FISH) analysis, the Epo-R probes (two genomic insert DNAs encompassing 15 kb of the Epo-R gene) and the low-density lipoprotein receptor (LDL-R) probe (genomic insert DNA of 5.3 kb in pcdV1 vector, ATCC 57005; ATCC, Rockville, MD) were digoxigenin-labeled by nick translation according to the Boehringer protocol (Boehringer Mannheim, Mannheim, Germany). The chromosome 19–specific painting probe was used according to the Oncor protocol (Gaithersburg, MD).

A 3-kb genomic fragment corresponding to the minor cluster region of Bcl2 gene, provided by C.J. Larsen, INSERM U301, Paris, was used as a standard for quantification.

**Chromosome preparation and FISH.** Slides containing metaphase spreads of the UT7 cell line were prepared by standard procedures and treated with 5-bromodeoxyuridine (BrdU) to induce R-banding.23 Hybridization was performed using the Epo-R and LDL-R probes at a concentration of 10 ng/μL with 50-fold excess human total DNA as competitor. For double-color FISH, chromosome 19 biotinylated painting probe and Epo-R probe nick translated with digoxigenin were pooled after denaturation (10 μL of the painting probe and 10 μL of the Epo-R probe at the concentration of 15 ng/μL with 50-fold excess human total DNA). After overnight hybridization at 37°C, the slides were rinsed, then incubated sequentially with antibiotin (goat) antibody (Vector Laboratories, Burlingame, CA), antidigoxigenin (mouse) antibody (Boehringer), fluororescin isothiocyanate (FITC)-conjugated anti-goat antibody (Biosys, Compiegne, France), and finally with sheep rhodamine-conjugated anti-mouse antibody (Boehringer). Slides were stained with propidium iodide (0.1 μg/mL), briefly rinsed, and mounted in a solution of antifade p-phenylenediamine (PPD). Green and red fluorescence were viewed through a double-band pass filter (Omega Optical, Brattleboro, VT), on a conventional epifluorescence microscope without any image processing technique. Metaphases were photographed using Ektachrome ASA 400 film (Kodak, Rochester, NY).

**RESULTS**

**Study of Epo-R gene structure in murine erythroleukemia cell lines and tumors.** Several murine erythroleukemia cell lines were tested for the presence of Epo-R genomic rearrangements. Southern blot analyses were performed using a probe containing sequences located 5′ to the transcription initiation site and encoding the first exon of the Epo-R gene.22 Among 13 cell lines tested, one cell line, FCL1, had a rearranged Epo-R allele. The size of the rearranged fragment appeared identical to that previously described for the T3CL-2 cell line (Fig 1A), which was shown to contain a viral LTR insertion.22 Two oligonucleotides, INTS 310 and INTS 190, located 5′ and 3′, respectively, of the T3CL-2 DNA rearrangement, were used to perform a PCR amplification on FCL1 DNA. In addition to the normal fragment (354 bp), a 0.5-kb longer fragment (869 bp) was obtained, which corresponded to the rearranged allele (data not shown). After cloning and sequencing of this fragment, it appeared that a viral long terminal repeat (LTR) sequence was inserted in the first exon of the Epo-R gene, 5′ of the coding sequence. Thus, the nature of the rearrangement in FCL1 was similar to that described for T3CL-2. However, the viral insertion in the FCL1 and T3CL-2 Epo-R genes exhibited differences: (1) the LTR insertion in FCL1 was localized 8 bp upstream from the T3CL-2 insertion and the duplication of five nucleotides flanking the LTR insertion was GGAGC in FCL1 and CAGGG in T3CL-2 (Fig 1B); and (2) there were 10 nucleo-
Fig 1. Epo-R gene rearrangement in Friend erythroleukemia cell lines. (A) Southern analysis of the Epo-R gene in Friend erythroleukemia cell lines. DNA (10 μg) was digested with EcoRV and hybridized with the 1.3-kb murine Epo-R 5' genomic probe. Lines 1 to 14 correspond to FCL1, 745, CTF 23-30, 707, Red 5-1.5, TFP10, F4N, F4+, IW32, IW35, FLC, T3CL-2, 2011W32, and NN10. The germ-line allele is indicated as A (9 kb), and B (6.5 kb) is the size of the rearranged alleles in FCL1 and T3CL-2. (B) Schematic comparison between LTR insertions in T3CL-2 and FCL1 cell lines. On the middle line, the 5' sequence of exon 1 of the normal Epo-R allele is depicted. The position 0 corresponds to the normal cap site. The ATG initiation codon of the Epo-R is at the position +147. The LTR insertion in the T3CL-2 cell line is represented at the top. The 5-bp repeats GAGGG flanking the LTR insertion on each side are boxed; this sequence is found at position +103 of the normal allelic sequence of the Epo-R. The LTR insertion in the FCL1 cell line is represented at the bottom. Duplicated repeats GGACG are boxed and the corresponding normal sequence is located at position +95 of the normal allele. In these two cases, a new LTR transcriptional initiation site (triple arrow) located downstream of the normal Epo-R promoter cap site (single arrow), was used.

We further assessed the independent origin of the two cell lines by hybridizing genomic DNAs with Spi-1 and p53 probes, two genes known to be frequently altered in Friend erythroleukemias.30,31 After hybridization with Spi-1 and p53 probes, an additional band was seen in FCL1 and T3CL-2 DNAs when compared with normal DNA from DDD mice, and it is noteworthy that these two cell lines harbored different hybridization patterns (Fig 2). These results definitely exclude cell line contamination and indicate that these two cell lines have a different origin. The viral LTR insertion led to Epo-R mRNA overexpression in FCL1 cells (Fig 3A). However, the number of Epo-binding sites determined by 125I-Epo fixation to the cell surface was not increased (1,000 Epo-binding sites per cell). It has been shown that the gp55 Env protein of the Friend SFFV could retain the Epo-R intracellularly; as a consequence, the presence of the gp55 Env was verified by Western blot analysis: both cell lines expressed high levels of gp55 Env protein (Fig 3B). Since FCL1 and T3CL-2 cell lines were established in culture a long time ago, we were unable to determine whether the alteration of the Epo-R gene had occurred in vivo or during the in vitro expansion of these cells. We therefore screened 23 Friend primary tumors isolated from Friend virus-infected mice for Epo-R rearrangement. Two probes were used for the analysis of the Epo-R gene: the previously described 5' genomic probe, and a 1.7-kb cDNA fragment corresponding to the whole coding sequence. No rearrangement could be detected in these tumors.

Epo-R abnormalities in human erythroleukemia cell lines. The number of Epo-binding sites on the cell surface of the human erythroleukemia cell lines is quite heterogeneous. Some, such as K562, HEL, JK1, and KU812, harbor a low number (<300/cell), while others, such as OCI1M1, UT7, and TF-1, exhibit a high number of Epo-binding sites (from 3,000 to 11,000/cell).

The TF-1 erythroleukemia cell line is dependent on various hematopoietic growth factors for its proliferation and harbors 1,700 to 3,000 Epo-binding sites per cell. High levels of Epo-R mRNA transcripts were recently described in this cell line, associated with a structurally abnormal Epo-R gene.32,34 The TF-7 cell line, established from a patient with a megakaryoblastic leukemia, is also dependent on growth
factors, including Epo, GM-CSF, IL-3, or IL-6. It expresses a high number of Epo-binding sites, between 7,000 and 11,000/cell. This prompted us to look for genomic alterations of the Epo-R gene in the UT7 cell line. Two cDNA probes were used: a 5' probe containing human exon 1 and a 3' probe (probe A) containing exons 7 and 8 of the human Epo-R gene (see Fig 5).

By Southern blot analysis, no abnormality was observed at the 5' end of the Epo-R gene for the four cell lines tested, KU812, JK1, TF-I, and UT7, when the 5' probe was used; in contrast, Southern blot analysis with probe A, located at the 3' end of the gene, demonstrated the presence of abnormal Epo-R gene fragments in both TF-1 and UT7 DNAs (Fig 4A). In TF-1 DNA, in addition to germ-line fragments (SacI, 9.2 kb; BglII, 25 kb; HindIII, 5 kb; BamHI, 4.3 kb), new fragments (SacI, 4 kb; BglII, 5 kb; HindIII, 3.5 kb; BamHI, 6 kb) were observed, showing that these cells had one rearranged Epo-R allele as previously described by Ward et al. In UT7 DNA, these results showed that the Epo-R gene was rearranged in all four digests tested, allowing us to exclude a polymorphism of the Epo-R alleles. From these data, a map could be established, indicating that the Epo-R rearrangement in UT7 DNA occurred within a 3.2-kb HindIII-SacI fragment localized outside the Epo-R gene coding sequence, 3 kb downstream from the end of the last exon (Fig 5). Similar to the TF-1 cell line, UT7 overexpressed Epo-R mRNA, which appeared to be structurally normal (data not shown).

Karyotype of UT7 cell line and in situ hybridization studies. The karyotype of UT7 cell line (chromosome number, 82 ± 4; range, 79 to 91) exhibited multiple rearranged chromosomes (>30/metaphase). The representative formula was as follows: 79-91, XXXY, +add(X)(p22),add(1)(pl1),add(1)(q31), +add(1)(q12), der(2)t(2;5)(p24; q14)x2,-3, add(3)(p12), add(3)(q11), -5, -5, -5, der(5)t(5;7)(p14; q12), add(6)(p21), add(7)(q11), add(8)(p12)x2,-9,-9, dic(9;?) (p12; ?), dic(9;?) (p13; ?), -10,-10,-11, add(11)(q12), -12, del(12)(q24), -13,-13,add(13)(p12), -14,-15,-16,-17,add(17)(p1), +hsr(19)(p1), -21, i(21q), -22,-22,+der(?) (?); 19)(q11)x2+10mar [cp 13] (Fig 6).

One of the rearranged chromosomes was homogeneously stained after R-banding. Its short arm was similar to the long arm of chromosome 19 and its long arm suggested a possible amplification [homogeneously staining region = hsr (19)]. Chromosome painting using a chromosome 19-specific painting probe exhibited a positive staining on five chromo-
Fig 4. Southern analysis of the Epo-R gene in four human erythroleukemia cell lines. (A) DNAs (10 μg) from KU812 (lane 1), JK1 (lane 2), TF-1 (lane 3), and UT7 cell lines (lane 4) were digested with SacI, BglII, HindIII, and BamHI, and hybridized with a Epo-R cDNA probe encompassing exons 7 and 8 (probe A, see Fig 5). A lambda HindIII scale is positioned on the left. (B) DNA (10 μg) from UT7 (lane 1), TF-1 (lane 2), and human placenta (lane 3) were digested with BamHI, HindIII, and SacI, and hybridized with a 3′ genomic probe of the Epo-R gene (probe B, see Fig 5).

Fig 5. Restriction map of the human Epo-R gene. The eight coding exons are depicted as black boxes; the positions of translational initiation ATG and termination TAG codons are indicated on exons 1 and 8, respectively. Probe A is a cDNA probe encompassing exons 7 and 8. Probe B is a genomic fragment localized 3.5 kb downstream of the TAG termination codon. Positions of UT7 and TF-1 Epo-R gene rearrangement regions are indicated by arrows.

Stained, except for the distal segment of the long arm, which was lightly stained (Fig 7B).

FISH of Epo-R probe, localized on band 19p13.2 by Burd et al., exhibited recurrent FISH spots on the two normal chromosomes 19, in a position corresponding to band 19p13.1 or 19p13.2. In addition, a strong signal, corresponding to multiple FISH spots, was observed on one submetacentric, corresponding to the presumed hsr (19), demonstrating the presence of an amplified sequence. The quantification of the FISH spots was difficult, but up to 12 spots per chromosome (six per chromatid) could be identified. These spots were distributed in a segment corresponding to approximately 30% of the length of the presumed hsr (19) chromosome, localized in the median position of the long arm (Fig 7A). Chromosome painting and FISH of EpoR probe performed on the same preparations conclusively demonstrated that the submetacentric stained by chromosome painting also hybridized the EpoR probe, and was thus an hsr (19)(p1) (Fig 7C).

In situ hybridization with a LDL-R probe, localized in 19p13.3, gave similar results to those of the Epo-R probe on the hsr (19), suggesting that a large segment of the 19p arm was involved in the amplification (data not shown).
Fig 7. FISH exhibiting signals on chromosome 19 and its derivatives. (A) Hybridization of the Epo-R probe on the hsr (19)(p1) chromosome. (B) Painting of chromosome 19. D, normal chromosomes 19; E, +der(7)(7;19) (7; q11); F, hsr (19)(p1). (C) Double-color FISH of the Epo-R probe (orange) and chromosome painting (green) of the hsr (19)(p1).

Fig 6. Representative R-banded karyotype of UT7 line. Normal chromosomes are at the top and bottom (rows A and E). Derivative chromosomes of known origin are shown in rows B and D [notice the three der(19)]. Marker chromosomes (unknown origin) are shown in row C.
Finally, these data suggest that in UT7 cells, the chromosome hsr (19), despite its fairly homogeneous staining (Fig 6), is composed as follows: (1) the 19q arm; (2) the centromere of chromosome 19; (3) the proximal segment of the 19p arm; (4) an amplified segment (19p13.1 or 19p13.2 → 19p13.3); (5) the distal segment of the 19p arm; and (6) a segment of unidentified origin.

Quantification of the Epo-R amplification on Southern blots. To confirm the noticeable amplification of the Epo-R gene evidenced by FISH analysis, we quantified the Epo-R signal detected on Southern blots. UT7 DNA and DNA from human placenta were digested by five different restriction enzymes; the resulting blots were hybridized simultaneously with the Epo-R probe A and with a Bcl2 probe used as an internal control. Bcl2 gene is located on chromosome 18, two copies of which are present in UT7 cells (see Fig 6). The Epo-R and Bcl2 signals were scanned with a PhosphorImager 400 (Molecular Dynamics, Sunnyvale, CA), and the ratio of Epo-R to Bcl2 in UT7 and normal DNAs was calculated in the five digests, allowing us to determine that the Epo-R gene was amplified 3.7 ± 0.5-fold in UT7 cells when compared with normal placental DNA (Fig 8). This amplification corresponded to 7.4 ± 1 copies of one Epo-R allele, a result fully consistent with the data obtained by FISH.

DISCUSSION

These results clearly show that Epo-R genomic alterations are not rare events during erythroleukemic processes. Indeed, for virally induced murine erythroleukemia cell lines, two (T3CL-2, FCLI) of 14 tested exhibited Epo-R gene alteration. In addition, Epo-R gene abnormality in the Friend F5-5 cell line has been described elsewhere. In human erythroleukemia cell lines, the Epo-R gene is amplified in UT7 cells. By FISH analysis, at least six spots of Epo-R could be identified per chromatid hsr (19), plus two normal chromosomes 19, giving a total of at least eight Epo-R copies for 82 chromosomes. The quantification of the Epo-R amplification by Southern blot analysis in comparison with Bcl2 signal gave a mean number of 7.4 ± 1 copies of the Epo-R gene, which is in total agreement with the data given by the karyotype and FISH analysis. In addition, the Epo-R gene appeared rearranged in two (UT7 and TF-1) of four cell lines tested. It remains to be determined whether the actual frequency of this event is higher by analyzing the Epo-R genomic locus with 5' and 3' probes more distantly located. Interestingly, the Epo-R gene alteration in UT7 and TF-1 cell lines occurred in a common genomic region localized to the 3' part of the gene. In TF-1 cells, the Epo-R gene breakpoint has been mapped to exon 8, 5' to the translation termination codon, whereas the breakpoint in UT7 cells is located further 3', outside the coding sequence. In both murine and human systems, genetic defects of the Epo-R gene are associated with overexpression of Epo-R mRNA; the resulting excessive activity of the Epo-R is probably involved in the occurrence of leukemogenesis. In human erythroid progenitors, the half-life of the Epo-R mRNA has been determined to be approximately 90 minutes; an identical half-life for the Epo-R mRNA in UT7 cells was reported by Komatsu and Fujita, indicating that there is no change in mRNA stability. In UT7 cells, the breakpoint is located outside the coding sequence, which implies that the transcriptional unit is unchanged; as a result, it is impossible to differentiate normal from rearranged Epo-R transcripts. Moreover, the mechanism leading to an increase of transcriptional activity in UT7 cells could be due either to the Epo-R gene amplification or to the presence of an exogenous enhancer brought at the 3' end of the gene, or both. Only

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Fig 8. Quantification of the Epo-R and Bcl2 signals. DNAs from normal human placenta (N) and UT7 cells (U) were digested by five different restriction enzymes (EcoRV [1], SacI [2], XbaI [3], HindIII [4], SphI [5]). The Southern blots were hybridized with both Epo-R (E) and Bcl2 (B) probes and were scanned with a PhosphorImager 400 after 3 days of exposure.
the clonage and expression study of the Epo-R rearranged allele will allow clarification of this point.

UT7 and TF-1 human erythroleukemia cell lines with abnormal Epo-R genes express a high number of Epo-binding sites in comparison to their normal counterparts, i.e., human erythroid progenitors, and in comparison to KU812 and JK1 cells, which exhibit 200 and 300 Epo-binding sites, respectively. It is striking that UT7 and TF-1 cell lines are both highly responsive to Epo for proliferation and/or differentiation. Similarly, two clones of Ba/F3 cells expressing variable murine Epo-R levels were generated, and the clone expressing the higher Epo-R level was more sensitive to Epo. Analogous results were observed in M1 cells transfected with the IL-6-R, which were found to be hypersensitive to IL-6 (by 36-fold) when they display a high number of IL-6-binding sites per cell.

In contrast, for T3CL-2 and FCL1 murine erythroleukemia cell lines, there was no correlation between the overexpression of mRNA and the number of Epo-binding sites displayed at the cell surface. The most likely explanation is an intracellular retention of the complex formed by the Epo-R and the gp55 Env protein, as previously described for these Friend erythroleukemia cell lines. Neither of these SFFVp-infected cell lines shows any response to Epo for proliferation or differentiation. However, in these established erythroleukemia cell lines, it is now accepted that gp55 protein is responsible for the constitutive activation of the Epo-R due to the interaction between Epo-R and viral gp55 glycoprotein. In T3CL-2 and FCL1 cell lines, interaction between gp55 and the overexpressed Epo-R may provide a positive pressure in the development of erythroleukemia.

The role of Epo-R abnormalities and overexpression in the genesis of human erythroid disorders is not yet clearly established. The fact that Epo-R gene rearrangements were associated with overexpression of the Epo-R mRNA and protein argues for a role of this genetic alteration in the erythroid cell line phenotype. Recently, a highly significant link between a dominant Epo-R gene mutation and a familial erythrocytosis has been described. This Epo-R defect gave rise to a protein truncated for 70 amino acids downstream of the mutation, leading to clinical erythrocytosis and to hypersensitivity to Epo of erythroid precursor cells in vitro. Until now, the onset of human erythrocytosis has not been shown to be related to alterations of chromosome 19, where the human Epo-R gene is located, but several abnormalities involving this chromosome, among many others, were described in UT7 and TF-1 erythroleukemia cell lines. In UT7, two distinct rearrangements involve chromosome 19. One is an unbalanced translocation of the long arm of chromosome 19 with another chromosome of unknown origin. This rearrangement occurred before the tetraploidyization, as suggested by the presence of two identical derivative chromosomes. The other abnormality was a hsr (19) on which the Epo-R and LDL-R genes were found to be coamplified. In the hypothesis of a simple amplification process, this suggests that the amplicon was large, since the two genes involved are located at bands 19p13.2 or 19p13.1 and 19p13.3, respectively. It is usually difficult to detect, in a large amplicon, which genes are of importance; that Epo-R gene is highly transcribed suggests that this gene might be the target of the amplification and thus important in the leukemogenic process.

Finally, it remains to be determined whether Epo-R genetic defects could not be related to a selective pressure in vitro cultures, and if they can be detected in de novo erythroleukemias. The screening of 23 Friend tumors in vivo failed to find any Epo-R abnormality with the probes mentioned earlier. A study of human erythroleukemias is currently in progress to determine whether Epo-R gene abnormalities are associated with these disorders.

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Oncogenic Role of the Erythropoietin Receptor

Putative oncogenic role of the erythropoietin receptor in murine and human erythroleukemia cells

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