The t(6;8)(q27;p11) Translocation in a Stem Cell Myeloproliferative Disorder Fuses a Novel Gene, *FOP,* to *Fibroblast Growth Factor Receptor 1*

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In patients with an atypical stem-cell myeloproliferative disorder with lymphoma (B or T cell), myeloid hyperplasia, and eosinophilia, the chromosome 8p11-12 region is the site of a recurrent breakpoint that can be associated with three different partners, 6q27, 9q32-34, and 13q12. Rearrangements are supposed to affect a pluripotent stem cell capable of myeloid and lymphoid differentiation and to involve the same 8p11-12 gene. The t(8;13) translocation has recently been shown to result in a fusion between the *FGFR1* gene that encodes a tyrosine kinase receptor for fibroblast growth factors and a novel gene, *FIM* (also called *RAMP* or *ZNF198*), belonging to a novel family of zinc finger genes. In the present study, we have cloned the t(6;8)(q27;p11) translocation in two patients and found a fusion between *FGFR1* and a novel gene, *FOP* (*FGFR1* Oncogene Partner), located on chromosome band 6q27. This gene is alternatively spliced and ubiquitously expressed. It encodes a protein containing two regions of putative leucine-rich repeats putatively folding in α-helices and separated by a hydrophobic spacer. The two reciprocal fusion transcripts were evidenced by reverse transcription-polymerase chain reaction in the tumoral cells of the patients. The predicted chimeric FOP-FGFR1 protein contains the FOP N-terminus leucine-rich region fused to the catalytic domain of FGFR1. It may promote hematopoietic stem cell proliferation and leukemogenesis through a constitutive phosphorylation and activation of the downstream pathway of FGFR1.

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SPECIFIC RECURRENT chromosomal translocations have been described for many malignant tumors, including leukemias, lymphomas, and sarcomas.[1] Characterization of translocation breakpoints has shown fusion of genes frequently encoding potential transcription factors and nucleic acid-binding proteins or, in some instances, tyrosine kinases,[2] which lead to the generation of chimeric products and/or alteration of gene expression, thereby providing a putative oncogenic stimulus.[3-5]

A recently described distinct myeloproliferative disorder (MPD) is associated with recurrent translocations in which the 8p11-12 chromosomal region is rearranged with three different partners, 6q27, 9q32-34, and 13q12.[6] This hematological malignancy is characterized by myeloid hyperplasia, eosinophilia, and T- or B-cell lymphoblastic lymphoma and generally progresses to acute myeloid leukemia (AML). The biphenotypic feature of this MPD strongly suggests that the genetic abnormality occurs in the hematopoietic stem cell. The 8p11-12 breakpoint has been recently characterized.[7,8] It is distinct from the breakpoints in other translocations evidenced in AML, ie, t(8;16)(p11;p13)[9] and its t(8;14)(p11;q11.1),[10] and t(8;19)(p11;q13)[11] variants and t(8;22)(p11;q13).[12,13] The *MOZ* gene is involved in the t(8;16) translocation[14] and in AML with inv(8)(p11;q13).[15] We have demonstrated that the three different translocations associated with MPD have the same localization of the 8p11-12 breakpoint and that cosmids specific of the fibroblast growth factor receptor 1 (*FGFR1*) gene spanned the breakpoint.[16] *FGFR1* encodes a receptor for members of the fibroblast growth factor family.[17] Four groups at least have cloned the t(8;13) breakpoint. All studies showed that this translocation results in the fusion of the *FGFR1* gene to a previously unidentified 13q12 gene named *FIM*,[18] *RAMP*,[19] or *ZNF198*.[20,21] The putative oncogenic fusion protein contains zinc finger motifs, contributed by the product of the 13q12 gene, and the catalytic domain of FGFR1.[18-22] The chimeric protein is relocalized to the cytoplasm[20] and has a constitutive tyrosine kinase activity.[18] These findings strongly suggest that the genesis of the 8p11-12 MPD syndrome is due to altered FGFR1 signal transduction pathways.

Among the rearrangements specific of the 8p11-12 MPD, the t(6;8)(q27;p11) is a rare recurrent translocation and, to date, only four cases have been described.[16,23,24] In the present study, we report that *FGFR1* is fused to a novel gene we named *FOP* (*FGFR1* Oncogene Partner) in two patients. In both cases, the translocation results in the fusion of a putative leucine-rich domain of FOP to the protein kinase domain of FGFR1. The two reciprocal fusion transcripts are expressed. The t(6;8) breakpoint occurs within *FGFR1* intron 8, as is the case for the t(8;13) translocation.[18]

MATERIALS AND METHODS

Patients. The two patients with a t(6;8)(q27;p11) studied have been partially described in a previous work.[16] The clinical phenotype in each case was consistent with the 8p11-12 MPD.[6]

Patient no. 1 was a 27-year-old man with a white blood cell count (WBC) of 62×10^9/L with 48% myeloid cells. The bone marrow aspirate was extremely hypercellular, with, respectively, 10% and 28% of eosinophilic and lymphoid cell population. A 46,XY,t(6;8)(q27;p11) karyotype was found in the 49 bone marrow-derived metaphases studied. A 7-month treatment with hydroxyurea reduced the WBC. Two years later, a polycythemia vera was diagnosed. Five years later, the

disease progressed to acute myeloid leukemia, and the patient died 6 months later after incomplete remission.

Patient no. 2 was a 19-year-old man. When the patient was 12 years of age, a polycythemia vera associated with a cutaneo-mucous lichen was diagnosed. Complete hematological remission was obtained after treatment with hydroxyurea, but the skin disorder was unresponsive to the immunosuppressive treatment. When the patient was 19 years of age, the number of polymorphonuclear neutrophils increased to 20×10^9/L. Bone marrow examination evidenced hyperplasia in the three myeloid lineages without fibrosis or eosinophilia. At that time, chest x-rays showed enlargement of thymus and histological examination after exeresis showed lymphoid hyperplasia. One year later, cervical and axillary lymphadenopathies appeared and histological examination displayed infiltration by myeloid blast cells. Acute transformation into AML4 with 40% blasts in bone marrow was diagnosed. Bone marrow karyotype showed a t(6;8)(q27;p11) in the 32 mitoses analyzed. After high-dose chemotherapy, a return to chronic phase was obtained. Nine months later, a Lyell syndrome was diagnosed and the patient died from septic shock.

Human cell lines. The following cell lines were cultured as recommended by the supplier: IE8, pre-B/B stage acute lymphoblastic leukemia (ALL); JY, B-ALL; U937, histiocytic lymphoma; KG1, myeloblastic/promyelocytic AML; SU-DHL-1, Ki-1 lymphoma cells bearing a t(2;5)(p23;q35); Daudi, Burkitt's lymphoma; HSB-2, MO, and MOLT-4, T-ALL; HEL, erythroleukemia AML; HL60, promyelocytic AML treated or not with phorbol esters; MIA PaCa-2, pancreas carcinoma; IMR-90, lung fibroblast; A549, lung carcinoma.

All cell lines were purchased from the American Type Culture Collection (ATCC; Rockville, MD), except for IE8 (gift from T. LeBien, Medical School, University of Minnesota, Minneapolis, MN) and SU-DHL-1 (gift from R. Rimokh, Hôpital E. Herriot, Lyon, France).

Fractionation of peripheral blood cells. Peripheral blood cells were isolated from blood samples obtained from healthy donors (Centre Régional de Transfusion Sanguine, Marseille, France) and fractionated in the four major hematopoietic nucleated populations (B and T lymphocytes, granulocytes, and monocytes) as previously described.[25] The percentage of purified normal cells was more than 90%, as assessed by immunophenotype analysis.

cDNA cloning and sequencing. The fusion transcript was analyzed with a Marathon cDNA amplification kit (Clontech, Palo Alto, CA). First-strand cDNA was synthesized from 2 µg of t(6;8) total RNA (case no. 1) by using an *FGFR1*-specific antisense primer (F5R, 5′-ATGGACAGGTCCAGGTACTCC-3′). Second-strand cDNA was synthesized and adapter ligated according to the manufacturer's protocol. The fusion transcript was amplified with an *FGFR1*-specific antisense primer (F-4R, 5′-ACTCTGGTGGGTGTAGATCCG-3′) and adapter primer AP1 from the Marathon kit. Polymerase chain reaction (PCR) conditions were as follows: initial denaturation at 95°C for 5 minutes, then 95°C for 1 minute, 60°C for 1 minute, and 68°C for 4 minutes for 30 cycles, with a final elongation step at 68°C for 10 minutes. One tenth of the first-round PCR product was reamplified with a nested-specific *FGFR1*-specific antisense primer (F-3R, 5′-CTTGGAGGCATACTC-CACGAT-3′) and adapter primer AP2. PCR conditions were the same as for the first-round amplification. The second-round PCR product was precipitated in ethanol and subcloned in the pUC18 vector by using the Sure Clone Ligation kit (Pharmacia, Uppsala, Sweden). Nucleotide sequences were obtained using the T7Sequencing kit (Pharmacia) and the forward and reverse sequencing primers for pUC18. Products were analyzed on 5% polyacrylamide/urea sequencing gels.

The full-length *FOP* cDNA was obtained by screening the SCID t(8;13) library we previously constructed.[18] Approximately 10^6 plaque-forming units were screened with a 1.5-kb *Not* I-*Eco*RI fragment from cDNA clone zs55g02. Clone sequencing was performed as described above or at Génome Express (Grenoble, France) on an automated

sequencer (Applied Biosystems 373; Applied Biosystems, Foster City, CA). Comparisons with GenBank and dbEST entries were made using BLASTN and TBLASTN.[26] Comparisons with protein databases (EMBL; Swissprot, Geneva, Switzerland) used BLASTX, TBLASTX.[27] Protein alignment was performed using multiple sequence alignment software[28] (http://www.toulouse.inra.fr/multalin.html).

Northern blot analyses. The multiple tissue Northern blots (Clontech; no. 7759-1, 7760-1, and 7767-1) were hybridized according to the manufacturer's instructions by using, as a probe, the 1.5-kb *Not* I-*Eco*RI fragment from cDNA clone zs55g02 labeled with [^{32}P]dCTP in random priming reaction.

Determination of the genomic structure of FOP. The genomic structure of the gene was deduced based on the cDNA sequences of *FOP*, ESTs retrieved from dbEST database (http://www.ncbi.nlm.nih-.gov/irx/dbST/dbest_query.html), and the genomic sequence from Pl artificial chromosome (PAC) 167A14 (accession no. Z94721). Sequence comparisons were performed using TBLASTN.[26] Intron position was deduced from the determination of both 5′ and 3′ limits of the exons.[29]

For alternative splicing analysis, reverse transcription-PCR (RT-PCR) experiments were performed. Three primer sets from the 5′ end (sense, 5′-GGAGCGTACCCTGCTGCG-3′; antisense, 5′-CCTTC-GAGACCTTGCAGC-3′), middle part (sense, 5′-TTAGTGGC-TAGTCTTGTTGC-3′; antisense, 5′-TAAAGAAGGGGCTCCCGC-3′), and 3′ end (sense, 5′-GAAGCAAGCAGGAAGTCTGG-3′; antisense, 5′-GCAGTGTTTTTGAAATGCAAGG-3′) of *FOP* sequence were used to amplify *FOP* transcripts. PCR reactions were performed in a Perkin Elmer-Cetus (Norwalk, CT) apparatus using an equivalent of 500 ng of reversed transcribed RNA and 50 pmol of each oligonucleotide as primers. The first cycle was run as follows: denaturation at 95°C for 5 minutes, annealing at 60°C for 30 seconds, and synthesis at 72°C for 1 minute. The next 30 cycles were run using the same conditions except that the denaturation step was only 30 seconds. For the last cycle, the synthesis time was 7 minutes. PCR products were gel-purified in agarose 1%, subcloned and sequenced as described above.

Analysis of gene expression by RT-PCR. RT-PCR reactions were performed from 2 µg of total RNAs as previously described.[18] Fusion transcripts were analyzed for the two t(6;8) patients. *FOP-FGFR1* fusion transcript was amplified with the *FOP*-specific sense (FOP-1F, 5′-CATTCTCCACCAAAGTCACCA-3′) and *FGFR1*-specific antisense (F-9.2, 5′-CATACTCAGAGACCCCTGCTAGC-3′) primers, giving a 162-bp product. *FGFR1-FOP* fusion transcript was amplified with *FGFR1*-specific sense (FA, 5′-ATCATCTATTGCACAGGGGCC-3′) and *FOP*-specific antisense (FOP-1R, 5′-CCCGCTTGTCTTCTTCT-TACC-3′) primers that generated a 197-bp product. PCR conditions were as described above.

The wild-type *FOP* and *FGFR1* transcripts were amplified using FOP-1F/FOP-1R (PCR product of 101 bp) and FA/F-9.2 (PCR product of 258 bp) primer pairs, respectively. PCR conditions were as described above.

The human β2 microglobulin primer pair (sense, 5′-CCAG-CAGAGAATGGAAAGTC-3′; antisense, 5′-GATGCTGCTTACAT-GTCTCG-3′; PCR product of 268 bp) was used as a control for RT and PCR efficiency.

RESULTS

Cloning of the MPD t(6;8) breakpoint. To clone the putative fusion gene, we did PCR amplification of cDNA 5′ ends (5′-RACE) from t(6;8) malignant cell total RNA (case 1) by using *FGFR1*-specific oligonucleotides. The resulting PCR products were cloned. Two chimeric clones of 400 and 800 bp were sequenced, showing that a sequence not previously characterized was fused in a continuous reading frame to

FGFR1 at nucleotide 1272 from ATG (accession no. M34185). Database searches with the novel sequence showed identity with human DNA sequences from one PAC, PAC 167A14 (EMBL Z94721) localized on chromosome 6q27, but did not show significant similarity or identity to any known gene or protein. We named this novel gene *FOP*. ESTs were shown to correspond to PAC 167A14 and we studied some of these cDNA clones. Sequencing of cDNA clone zs55g02 from which two ESTs (AA286845 and AA286846) are available in dbEST showed that it contained the sequences surrounding the breakpoint identified in PCR products from patient RNA.

Cloning of FOP *gene.* A cDNA library we previously constructed[18] was screened with the insert from cDNA clone zs55g02 as a probe. Two of the largest clones isolated were sequenced. They all shared identical sequences with ESTs AA286845 and AA286846, one of which had a 5′ extended end of 150 bp and was approximately 1.7 kb in length. The cDNA and deduced aminoacid sequences of *FOP* are shown in Fig 1. Within the full-length cDNA of 1,630 bp (EMBL: Y18046), a single open reading frame of 1,197 nucleotides starts with the putative ATG at nt 85. The sequence surrounding this codon matches the consensus sequence for optimal translation initiation, as determined by NetStart 1.0, a program for translation start prediction (http://www.cbs.dtu.dk/services/NetStart/). A stop codon at nt 1,282 is followed by a 338-bp long 3′ untranslated sequence, including a polyadenylation signal upstream to a poly(A) tail. It encodes a putative protein of 399 amino acid residues (aa).

The genomic structure of *FOP* was deduced based on the cDNA sequences, ESTs retrieved from dbEST, and the genomic sequence from PAC 167A14. It is composed of 13 exons spanning more than 40 kb of genomic DNA. The size of the exons is highly homogeneous, whereas the size of introns is heterogeneous, ranging from 0.5 to 8.8 kb (Fig 2A). All of the intron/exon boundaries agree with the canonic acceptor and donor splice sites.[29]

Multiple *FOP* cDNA variants were identified as the result of alternative splicing. In some cDNA clones an additional exon, exon 11 (Fig 2A), was identified. Variant transcripts resulting from splices of either exon 7 or exon 11 were cloned by RT-PCR and also found in several human and murine ESTs (including AA919880) and a human EST (N46560), respectively. It must be pointed out that, when exon 11 is not spliced out, a shorter protein of 331 aa can be deduced as the result of the presence of an in-frame stop codon. Moreover, an alternative splice is suspected in the *FOP* 3′ noncoding part.

FOP *sequence motifs.* Analysis of the transcript containing 1,197-bp *FOP* coding sequence suggests that *FOP* encodes a protein of 399 amino acid residues with a predicted molecular mass of 44.3 kD. FOP is a protein largely hydrophilic and contains, in its N- and C-termini, several regions folding in α-helices, as determined using GOR secondary structure prediction program.[30] These two regions are interrupted by a hydrophobic spacer region (Fig 1). The α-helix regions contain repeats with the consensus sequence $L-X_2-L-X_{3-5}-L-X_{3-5}-L$, in one-third of which the leucine is substituted by either a valine or an isoleucine.

Evidence for a novel gene family. The EST database (NCBI, Bethesda, MD) was screened for sequences similar to the *FOP* gene. Several human ESTs, whose deduced amino acid sequence (after translation in the 6 reading frames) has similarities to portions of the FOP protein, were found. These results are evidence for a multigenic family. EST similarities were found not only in vertebrates including human, mouse (AA103209, AA919880), and zebrafish (AA497344), but also in more distant phyla such as monocellular eucaryotes (leishmania, AI034687) and plants (rice, RICS1091A). The alignment of the deduced N-terminus of the FOP protein with translated nonhuman ESTs displaying sequence similarities and encoding putative orthologs is shown in Fig 2B.

Gene expression. Expression of the wild-type *FOP* gene was investigated by Northern blot analysis of poly(A)+ RNAs of various human tissues. Two major transcripts of approximately 1.2 and 1.7 kb were detected in all samples (Fig 3). A high level of expression and an additional transcript of approximately 3 kb were detected in the heart. Using RT-PCR analysis, *FOP* expression was found in normal hematopoietic cells as well as in different malignant cell lines (Fig 4). Similarly, *FGFR1* expression was found in normal and tumoral cells (Fig 4), in agreement with previous studies.[18,31,32] *FOP* and *FGFR1* were expressed in the two t(6;8) patients' samples (Fig 5, rows 1 and 4 in the middle and right panels, respectively).

Predicting the transcription of chimeric messages over the breakpoint, we designed primers from *FOP* and *FGFR1* to do RT-PCR on RNA from the two t(6;8) patients. Both *FGFR1-FOP* and *FOP-FGFR1* reciprocal transcripts were detected in patients (Fig 5, rows 2 and 3 in the middle and right panels, respectively) but not in normal and other tumoral samples (Fig 5, left panel, rows 2 and 3, respectively). They proved to encode in-frame fusion junctions on sequence analysis (Fig 6).

Characterization of the fusion junctions. We next determined the nucleotide sequence spanning the breakpoints on both parental and translocated chromosomes. On chromosome 8, the break occurred in *FGFR1* intron 8, 139 bp upstream of exon 9, which encodes the intracellular juxtamembrane domain. Two variants of the *FGFR1-FOP* fusion transcribed from chromosome der(8) were obtained. They differ by the use of alternative *FGFR1* 5′ splice sites located six nucleotides apart at the 3′ end of exon 8. They lead to inclusion or not of two amino acids, VT (Fig 3), in the juxtamembrane region, a feature described for wild-type FGFR1,[33,34] and in the fusion product resulting from the t(8;13) translocation.[18] On chromosome 6, the break occurred in *FOP* intron 6, 424 bp downstream of exon 6. *FOP* intron 6 is 2.6-kb long and contains two AT rich regions, a CA(n) repeat region and a subtype of long terminal repeat (LTR/MaLR), located downstream of the breakpoint. The chimeric sequence 5′-ATGGTCTTT-3′ of the rearranged intron from the *FOP-FGFR1* fusion gene results from the sequences 5′-ATGGTTTTT-3′ and 5′-ATGGTCTTT-3′ of *FOP* intron 6 and *FGFR1* intron 8, respectively. It is noteworthy that the breakpoint occurs in an identical stretch of nucleotides (but for one) in both *FOP* and *FGFR1* wild-type introns. The characteristics of the wild-type FGFR1 and FOP proteins, the fusion proteins FOP-FGFR1 and FGFR1-FOP, are depicted in Fig 6.

DISCUSSION

In this study, we describe the fusion of the *FGFR1* gene with a previously uncharacterized gene, *FOP*, in the t(6;8) transloca-

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cggccgcgtagcccgcgcgcggagcgtaccctgctgcggccgttggccgttagcgcggcttcggcggttgtcttggagaagcaagATGGC
                                                                                      M  A
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GGCGACGGCGGCCGCAGTGGTGGCCGAGGAGGACACGGAGCTGCGGGACCTGCTGGTGCAGACGCTGGAGAACAGCGGGGTCCTGAACCG
 A  T  A  A  A  V  V  A  E  E  D  T  E  L  R  D  L  L  V  Q  T  L  E  N  S  G  V  L  N  R
```

```
CATCAAGGCTGAACTCCGAGCAGCTGTGTTTTTAGCACTAGAGGAGCAAGAAAAAGTAGAGAACAAAACTCCTTTAGTTAATGAGAGCCT
 I  K  A  E  L  R  A  A  V  F  L  A  L  E  E  Q  E  K  V  E  N  K  T  P  L  V  N  E  S  L
```

```
GAAAAAGTTTTTAAATACCAAAGACGGTCGTTTAGTGGCTAGTCTTGTTGCAGAATTTCTTCAGTTTTTTAACCTTGACTTTACTTTGGC
 K  K  F  L  N  T  K  D  G  R  L  V  A  S  L  V  A  E  F  L  Q  F  F  N  L  D  F  T  L  A
```

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TGTTTTTCAACCTGAAACTAGCACACTGCAAGGTCTCGAAGGTCGAGAGAATTTAGCCCGAGATTTAGGTATAATTGAAGCAGAAGGTAC
 V  F  Q  P  E  T  S  T  L  Q  G  L  E  G  R  E  N  L  A  R  D  L  G  I  I  E  A  E  G  T
```

```
TGTGGGTGGACCCTTATTATTAGAAGTGATCAGGCGCTGTCAACAGAAAGAAAAAGGGCCAACCACTGGGGAAGGTGCACTTGATCTATC
 V  G  G  P  L  L  L  E  V  I  R  R  C  Q  Q  K  E  K  G  P  T  T  G  E  G  A  L  D  L  S
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TGATGTACATTCTCCACCAAAGTCACCAGAGGGAAAAACAAGTGCACAGACAACACCAAGTAAGATACCAAGGTATAAAGGACAAGGTAA
 D  V  H  S  P  P  K  S  P  E  G  K  T  S  A  Q  T  T  P  S  K  I  P  R  Y  K  G  Q  G  K
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GAAGAAGACAAGCGGGCAGAAGGCTGGTGACAAGAAGGCCAATGATGAGGCCAATCAGAGTGATACAAGTGTCTCCTTGTCAGAACCCAA
 K  K  T  S  G  Q  K  A  G  D  K  K  A  N  D  E  A  N  Q  S  D  T  S  V  S  L  S  E  P  K
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GAGCAAAAGCAGCCTTCACTTACTGTCCCATGAAACAAAAATTGGATCTTTTCTAAGCAACAGAACTTTAGATGGCAAAGACAAAGCTGG
 S  K  S  S  L  H  L  L  S  H  E  T  K  I  G  S  F  L  S  N  R  T  L  D  G  K  D  K  A  G
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CCTTTGTCCAGATGAAGATGATATGGAAGGAGATTCTTTCTTTGATGATCCCATTCCTAAGCCAGAGAAAACTTACGGTTTGAGGAAGGA
 L  C  P  D  E  D  D  M  E  G  D  S  F  F  D  D  P  I  P  K  P  E  K  T  Y  G  L  R  K  E
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ACCTAGGAAGCAAGCAGGAAGTCTGGCCTCGCTCTCGGATGCACCCCCCTTAAAAAGTGGACTCAGCTCCCTGGCGGGAGCCCCTTCTTT
 P  R  K  Q  A  G  S  L  A  S  L  S  D  A  P  P  L  K  S  G  L  S  S  L  A  G  A  P  S  L
```

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AAAAGACTCTGAGAGTAAAAGGGGAAATACAGTTTTGAAAGATCTGAAATTGATCAGTGATAAAATTGGATCACTTGGATTAGGAACTGG
 K  D  S  E  S  K  R  G  N  T  V  L  K  D  L  K  L  I  S  D  K  I  G  S  L  G  L  G  T  G
```

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AGAAGATGATGACTATGTTGATGATTTTAATAGTACCAGCCATCGCTCAGAGAAAAGTGAGATAAGTATTGGTGAAGAGATAGAAGAAGA
 E  D  D  D  Y  V  D  D  F  N  S  T  S  H  R  S  E  K  S  E  I  S  I  G  E  E  I  E  E  D
```

```
CCTTTCTGTGGAAATAGATGACATCAATACCAGTGATAAGCTTGATGACCTCACACAAGATCTGACTGTATCCCAGCTCAGTGATGTTGC
 L  S  V  E  I  D  D  I  N  T  S  D  K  L  D  D  L  T  Q  D  L  T  V  S  Q  L  S  D  V  A
```

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GGATTATCTGGAAGATGTTGCATAGacacgaagaaggaagtattctaattaacaaggacagaggactgaccggttccattttttttttt
 D  Y  L  E  D  V  A  *
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tccagacaatcactcagctggaatgtctgctctctattggtgccttgcatttcaaaaacactgcagatattttttaaaagtaattttcat
tttactaaacaaatacttcctatttgagcccatgtgtggaagatttaatattcttaatttaactgtacatttctttatggaaattgatt
atctacactcagtttcattacagggaaggaacccatgaaaacatcagtgttaagagcatgatgaaaggtgtcaataaagccgtaggatcg
cgcaaaaaa
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Fig 1. Sequences of the human *FOP* **gene. A full-length** *FOP* **cDNA clone is shown with the predicted amino acid sequence of an open reading frame. Leucine residues that may correspond to a novel type of leucine-rich motifs are in bold. Putative α-helices are underlined. Arrowheads indicate exon/intron boundaries. The arrow corresponds to the t(6;8) breakpoint. The asterisk indicates the stop codon.**
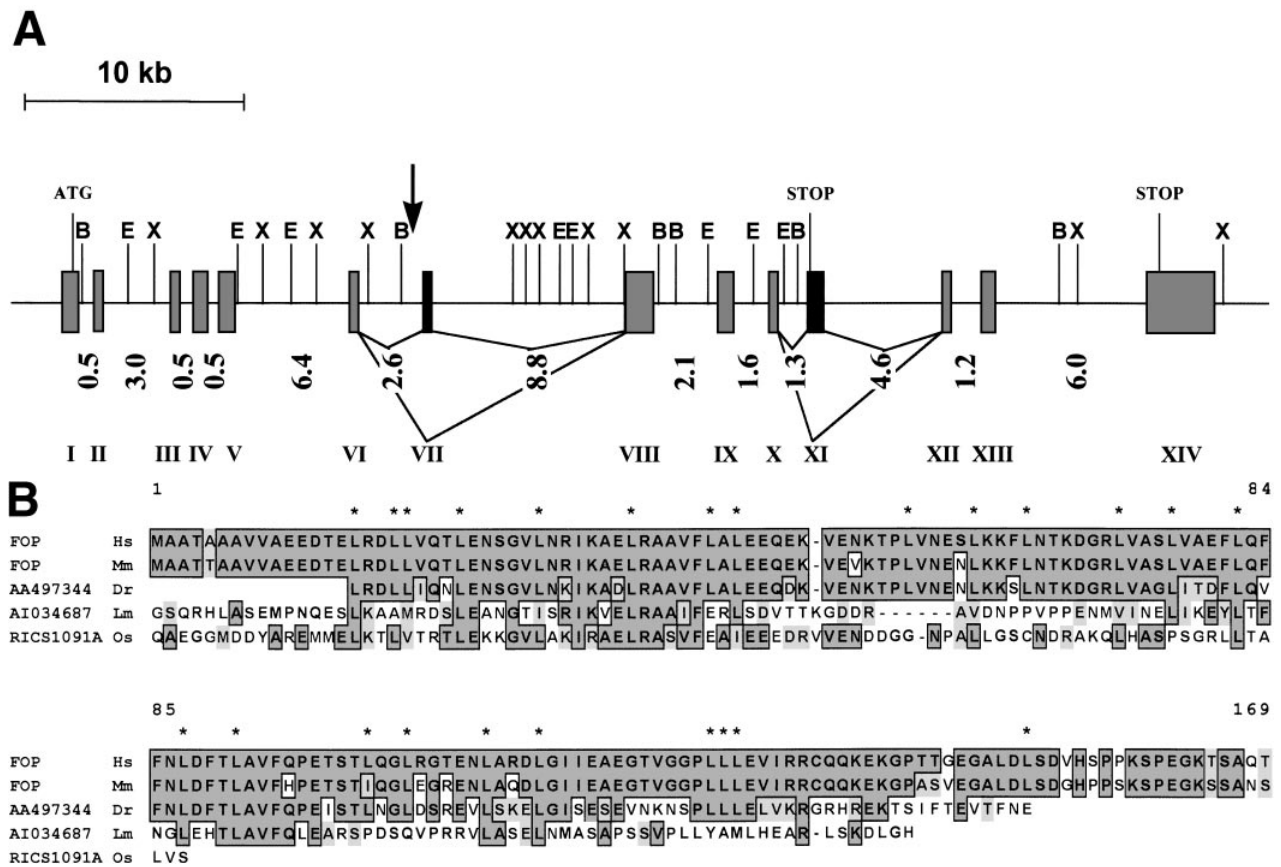
Fig 2. (A) Schematic representation of the genomic structure of the *FOP* gene. All exons are represented in the 5′ to 3′ order of genomic DNA: shaded and solid boxes represent constant and alternatively spliced exons, respectively. Arabic and roman numbers indicate the size in kilobases of introns and the position of exons, respectively. The arrow indicates the breakpoint. Restriction enzyme sites are *Bam*HI (B), *Eco*RI (E), and *Xba* I (X). **(B)** Similarities of the deduced human FOP protein (Hs) with EST protein translation from *Mus musculus* (Mm), *Danio rerio* (Dr), *Leishmania major* (Lm), and *Oryza sativa* (Os). Shading indicates common (boxed) and similar amino acid residues. Numbers at the top of each alignment indicate amino acid positions in FOP. Asterisks show conserved leucine residues.

tion involved in the myeloproliferative syndrome linked to the 8p11-12 chromosomal region. Two novel leukemia-specific fusion genes, *FGFR1-FOP* and *FOP-FGFR1,* were identified as the result of the translocation. In contrast to many chromosome translocations, both chimeric gene derivatives are expressed in patient cells. This observation is consistent with two works in

which the expression of both *FIM-FGFR1* and *FGFR1-FIM* RNAs in cells with t(8;13) associated with the same syndrome.[18,22] In both t(8;13) and t(6;8) translocations, the breakpoints in the *FGFR1* gene lie in intron 8.

To date, two of the three chromosomal translocations consistently associated with myeloproliferative disorder linked to the
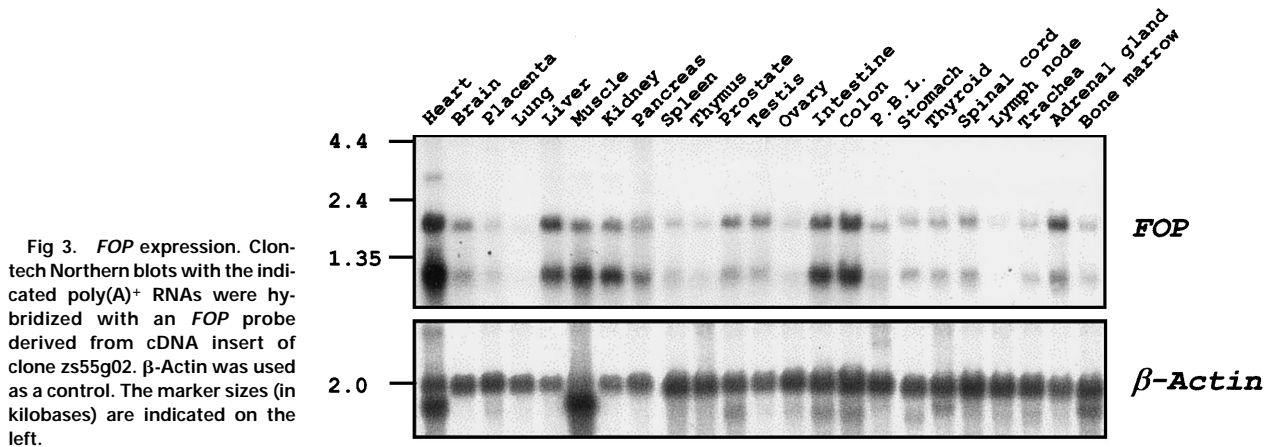

Fig 3. *FOP* expression. Clontech Northern blots with the indicated poly(A)⁺ RNAs were hybridized with an *FOP* probe derived from cDNA insert of clone zs55g02. β-Actin was used as a control. The marker sizes (in kilobases) are indicated on the left.
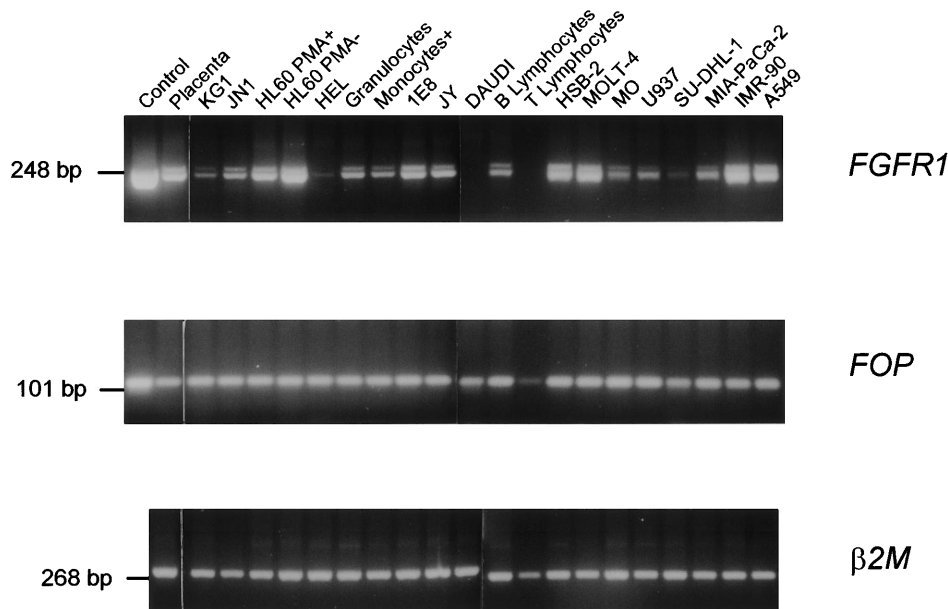
Fig 4. Expression of *FGFR1* and *FOP* genes. RT-PCR products were obtained from a variety of tissues and normal and tumoral hematopoietic cells using specific primer pairs of each gene. Each panel is a photograph of the ethidium bromide-stained agarose gel in which PCR products were electrophoresed. Labels at the top of rows indicate the source of the material. The first row corresponds to cDNA controls: a 2.5-kb *FGFR1* cDNA (pFLG-16) containing the complete coding sequence (upper panel)[49] and the *FOP* cDNA clone zs55g02 (middle panel), respectively. The other rows correspond to RNAs from a variety of lympho-hematopoietic cell lines (listed in Materials and Methods) and mature peripheral (T lymphocytes, B lymphocytes, and monocytes) blood cells purified as indicated in Materials and Methods. β2 microglobulin (β2M) amplification was used as a control.

8p11-12 chromosomal region have been cloned. The *FGFR1* partners are unrelated novel genes. Our preliminary results from the molecular study of the t(8;9) translocation demonstrate that the 9q32-34 gene is also a novel gene that is different from the two others (Popovici et al, manuscript in preparation). In each fusion, the N-terminal region of the novel proteins, postulated to trigger protein dimerization, is fused to the catalytic domain of FGFR1. In the t(8;13), the 13q12 gene encodes a member of a novel family of zinc finger proteins.[18-21] In the present work, we show that the t(6;8) translocation is associated with a leukemic aberrant protein in which a putative leucine-rich N-terminal region encoded by the widely expressed chromosome 6 *FOP* gene. Indeed, *FOP* encodes a protein that has, in its N- and C-terminal parts, some structural features involving putative α-helices. The comparison of aligned internal *FOP* sequences show considerable conservation of leucines that may correspond to a novel type of leucine-rich repeats. Because these motifs are different from the consensus leucine-rich repeats present in a large family of proteins involved in various functions and found in a great variety of species,[35,36] we

postulate that FOP may define a novel family of leucine-rich proteins. In addition, we retrieved from databases several ESTs with sequence similarities from humans and from distant phyla. Altogether, these results suggest that *FOP* belongs to a multigenic family and is highly conserved during evolution.

To date, proteins containing leucine-rich repeats are thought to be involved in protein-protein interactions, and at least half of them take part in signal transduction.[35] We previously showed that the putative oncogenic fusion gene involved in the t(8;13) translocation has a constitutive tyrosine kinase activity.[18] Thus, the oncogenic role of the *FOP-FGFR1* fusion gene product may be mediated by constitutive phosphorylation of the FGFR1 kinase domain triggered by dimerization of FOP leucine-rich motif.

Distinct species of human *FOP* cDNAs derived from differential splicing were identified. This raises the possibility that, although the *FOP* gene is expressed ubiquitously in various tissues and organs, the expression of its alternative splicing products may be regulated in a tissue-specific fashion.

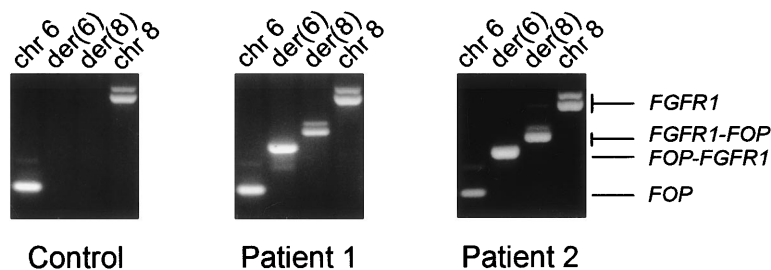The *FGFR1* gene encodes a tyrosine kinase receptor for

Fig 5. Expression of the fusion transcripts. RT-PCR were performed using RNAs from the erythroleukemia HEL cell line used as a control (left panel) and from the two t(6;8) patients' malignant cell samples and specific primers located near the translocation breakpoint. The respective chromosomal positions and the transcripts identified in each reaction are indicated at the top and on the right, respectively.
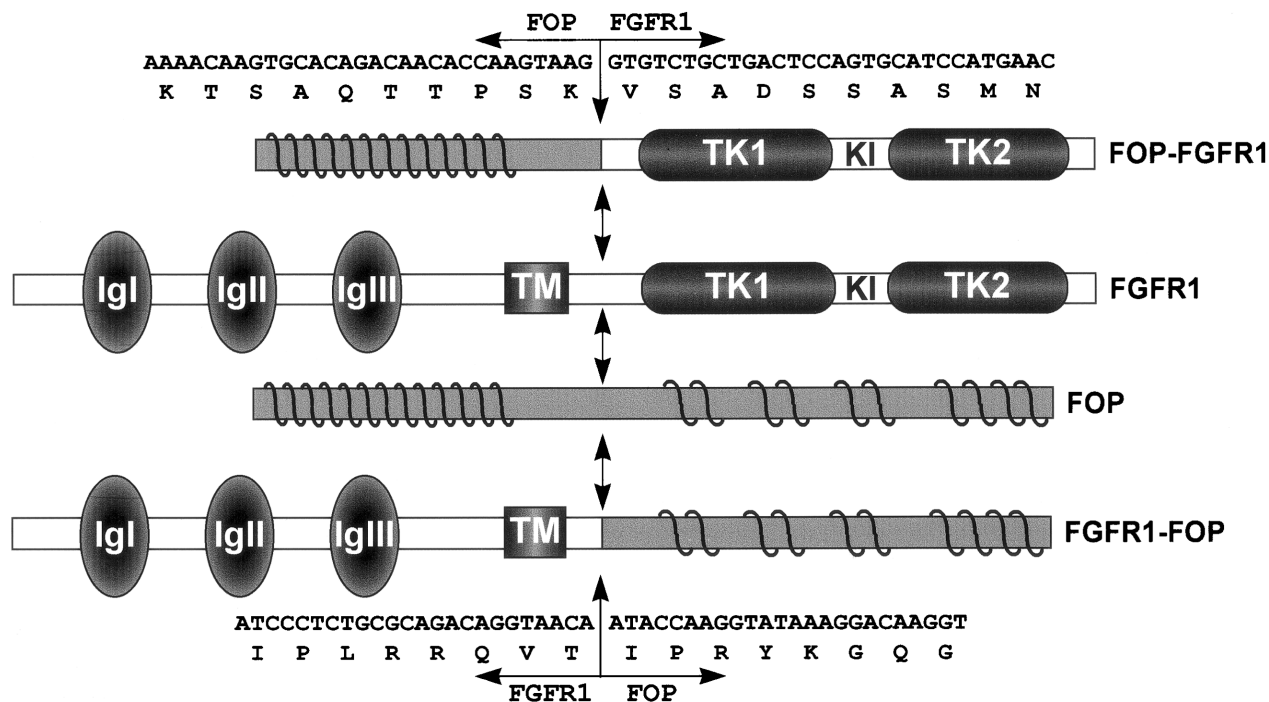
Fig 6. Schematic representation of FGFR1, FOP, and both FOP-FGFR1 and FGFR1-FOP fusion proteins. FGFR1 domains are indicated as follows: IgI, IgII, and IgIII, the three Ig-like domains; TM, the transmembrane domain; and TK1 and TK2, the tyrosine kinase 1 and 2 subdomains interrupted by a kinase insert (KI). Waved lines in the N- and C-terminal parts of FOP represent the leucine-rich repeats. FGFR1-FOP retains the extracellular and transmembrane domains of FGFR1, whereas FOP-FGFR1 fusion protein contains the FGFR1 catalytic domain. Double arrows indicate the t(6;8) breakpoint. Nucleotide and amino acid breakpoint sequences are indicated for both resultant chimeric products.

members of the fibroblast growth factor family.[17] FGFR1 is one of the four FGF receptors responsible for mediating cellular responses to fibroblast growth factor signaling, which include mitogenesis, mesoderm induction, angiogenesis, chemotaxis, and neuronal survival.[37,38] Signals from FGFRs appear to control differentiation as well as proliferation. FGFR1 is specifically involved in a hereditary craniosynostosis syndrome[39] and may contribute to tumorigenesis in human cancers. FGFR2 constitutive activation after chromosomal rearrangement was shown in rat osteosarcoma cells.[40] Activating mutations associated with translocation involving *FGFR3* and the IgH locus were described in some cases of multiple myeloma.[41] *FGFR1* may be the key gene for driving the 8p11 amplification process in breast cancers.[42-45] Its likely oncogenic role in the 8p11-12 MPD has been documented in previous works[16,18-21] and is strengthened by this study. These data therefore provide a new example of an oncogene implicated in both solid tumor formation and leukemogenesis.

Our present data suggest not only that alteration of transcriptional cascades mediated by FGFR1 signaling may be a mechanism of leukemic transformation, but also that FGFR1 may play a role in normal hematopoietic development. The finding of acquired chromosomal translocations that are consistently associated with specific tumor types supports the hypothesis of lineage-specific mechanisms of tumorigenesis.[46] In the case of 8p11-12 MPD, the crucial event for transformation is likely to take place in the hematopoietic stem cell, as previously postulated,[6] leading to the disruption of normal hematopoiesis of both lymphoid and myeloid lineages. Increased expression of

FGF2 and its receptors has been recently shown in CD34$^+$ hematopoietic progenitors from patients suffering from an MPD with myelofibrosis and myeloid metaplasia.[47] Molecular reagents derived from t(6;8) or t(8;13) may be used to facilitate the expansion of stem cells by stimulating their growth and/or survival and by overcoming negative regulatory signals.

In MPD, proliferative activity in the bone marrow is seldom limited to a single lineage, but often involves the erythroid, granulocytic, and/or megakaryocytic series.[48] It may be of interest to note that, whereas overlapping clinical features are found in all patients with MPD linked to 8p11-12,[6] a polycythemia vera was diagnosed in both patients with a t(6;8) translocation. This suggests that the chromosome 6 gene, *FOP*, may be an important gene for normal proliferation and differentiation of the erythroid lineage. A better understanding of the molecular processes affected by the fusion of the genes involved in such an MPD may shed light on the role of *FGFR1* and its multiple fusion partners in leukemogenesis and on the biology of the normal hematopoietic stem cell.

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