RAPID COMMUNICATION

Fusion of Huntingtin Interacting Protein 1 to Platelet-Derived Growth Factor β Receptor (PDGFβR) in Chronic Myelomonocytic Leukemia With t(5;7)(q33;q11.2)

By Theodora S. Ross, Olivier A. Bernard, Roland Berger, and D. Gary Gilliland

We report the fusion of the *Huntingtin interactin protein 1* (*HIP1*) gene to the *platelet-derived growth factor βreceptor* (*PDGFβR*) gene in a patient with chronic myelomonocytic leukemia (CMML) with a t(5;7)(q33;q11.2) translocation. Southern blot analysis of patient bone marrow cells with a *PDGFβR* gene probe demonstrated rearrangement of the *PDGFβR* gene. Anchored polymerase chain reaction using *PDGFβR* primers identified a chimeric transcript containing the *HIP1* gene located at 7q11.2 fused to the *PDGFβR* gene on 5q33. HIP1 is a 116-kD protein recently cloned by yeast two-hybrid screening for proteins that interact with Huntingtin, the mutated protein in Huntington's disease. The consequence of t(5;7)(q33;q11.2) is an *HIP1/PDGFβR* fusion gene that encodes amino acids 1 to 950 of HIP1 joined in-frame to the transmembrane and tyrosine kinase domains of the PDGFβR. The reciprocal *PDGFβR/HIP1* transcript is not expressed. HIP1/PDGFβR is a 180-kD protein when expressed in the murine hematopoietic cell line, Ba/F3, and is constitutively tyrosine phosphorylated. Furthermore, HIP1/PDGFβR transforms the Ba/F3 cells to interleukin-3–independent growth. These data are consistent with an alternative mechanism for activation of PDGFβR tyrosine kinase activity by fusion with HIP1, leading to transformation of hematopoietic cells, and may implicate Huntingtin or HIP1 in the pathogenesis of hematopoietic malignancies.
© *1998 by The American Society of Hematology.*

MYELODYSPLASTIC syndromes (MDS) and acute leukemias are disorders of hematopoietic progenitor cells characterized by acquired somatic mutations that confer a proliferative advantage. Cloning of chromosomal translocation breakpoints has been a productive strategy for identification of disease genes in MDS. Examples include the *AML1-EVI1* fusion associated with t(3;21),[1] the *MLL-CBP* fusion with t(11;16) in therapy-related MDS,[2] the *TEL-EVI1* fusion in t(3;12),[3] the *NPM-MLF1* fusion associated with t(3;5) in primary MDS,[4] and the *TEL/JAK2*[5] and *TEL/PDGFβR* fusions that are associated with t(9;12) and t(5;12) in chronic myelomonocytic leukemia (CMML), respectively.[6]

Several of the fusion genes associated with hematopoietic disorders involve tyrosine kinases. These include *BCR/ABL*,[7,8] *TEL/PDGFβR*,[6] *TEL/ABL*,[9] *TEL/JAK2*,[5,10] and *CEV14/PDGFβR*.[11] CMML, associated with the TEL/PDGFβR, is a subtype of MDS characterized by dysplastic monocytosis, variable bone marrow fibrosis, and progression to acute leukemia. The clinical phenotype is similar to chronic myelogenous leukemia associated with constitutive activation of the ABL kinase by fusion with BCR. There is also convincing evidence for contribution of tyrosine kinases to pathogenesis of solid tumors. Noteworthy examples include point mutations that constitutively activate the *RET* tyrosine kinase gene in medullary carcinoma of the thyroid,[12] amplification of the HER2/neu receptor tyrosine kinase in breast cancer,[13] and the *ETV6-NTRK3* gene fusion in congenital fibrosarcoma.[14]

A subset of patients with CMML have a t(5;12)(q33;p13) that results in fusion of the amino terminal portion of TEL, which contains the pointed (PNT) oligomerization domain, to the transmembrane and tyrosine kinase domains of platelet-derived growth factor β receptor (PDGFβR). The consequence of the fusion is constitutive oligomerization and activation of PDGFβR tyrosine kinase activity leading to transformation of cells.[15] PDGFβR kinase activity is required for transformation of Ba/F3 cells as is the PNT domain.[15] We report here a novel PDGFβR fusion associated with CMML and t(5;7)(q33;q11.2) involving the Huntingtin Interacting Protein 1 (HIP1).

MATERIALS AND METHODS

DNA isolation and Southern blots. Leukocytes were isolated by ficoll sedimentation from the peripheral blood and bone marrow of the index patient and normal controls after informed consent had been obtained. DNA was prepared using standard methods.[16] After enzymatic digestion with restriction endonucleases and electrophoretic separation of fragments, the genomic DNA was transferred to HYbond N nylon membranes (Amersham, Arlington Heights, IL). The PDGFβR genomic probe was a 1.1-kb *Hin*dIII-*Xho* I fragment prepared from PDGFβR cosmid B.[17] Probes were labeled with ^{32}P by random priming, and Southern hybridizations were performed as described.[18]

Cloning of the t(5;7) breakpoint. Mononuclear cells were isolated from t(5;7) bone marrow cells by ficoll sedimentation. Anchored polymerase chain reaction (PCR) was performed to clone the human chromosome 7 partner gene according to the method of Frohman[19] with minor modifications. In brief, total RNA was prepared with RNA-STAT reagents according to the manufacturer's recommendations (Tel-Test, Inc, Friendswood, TX). RNA (3 μg) was reverse transcribed using avian

myeloblastosis virus (AMV) reverse transcriptase and PDGFβR oligo-nucleotide primer 1873R (5′-CGTAACGTGGCTTCTTCTGC-3′). A poly(A) tail was appended using terminal transferase and dATP at 37°C for 15 minutes. After a single cycle of amplification (94°C for 1 minute, 50°C for 2 minutes, and 72°C for 40 minutes) using primer Qt (5′-TGAGCAGAGTGACTATTACTCGAGCTCAAGCTTTTTTT-TTTTT-3′) and internal PDGFβR primer 1848R (5′-AGTCTCGAG-CATGATGAGGATGATAAG-3′), 30 cycles of PCR (94°C for 1 minute, 58°C for 2 minutes, and 72°C for 3 minutes) were performed with primers Qo (5′-CCAGTGAGCAGAGTGACG-3′) and 1848R. The PCR products were diluted 20-fold and reamplified with nested primers 1829R (5′-GAGATGATGGTGGAGCACCAC-3′) and Q1 (5′-GAGGACTCGAGCTCAAGC) using the same PCR conditions (30 cycles of 94°C for 1 minute, 58°C for 2 minutes, and 72°C for 3 minutes). Specific bands were not detected by direct visualization after ethidium bromide staining, but were detectable by Southern blot analysis using the ^{32}P–end-labeled PDGFβR 1806R oligo (5′-GGCCAGGATGGCTGAGATCA-3′). The nested PCR product was subsequently diluted 20-fold and reamplified with primer 1806R and Q1 (30 cycles of 94°C for 1 minute, 58°C for 2 minutes, and 72°C for 3 minutes), yielding a specific 500-bp product that was subcloned into pBluescript KS(+) (Stratagene, La Jolla, CA) and sequenced. The DNA sequence was sent via Netscape to the BLAST server at NIH (http://www.ncbi.nlm.nih.gov) to compare to GenBank (blast*n*).

Library screening. In light of the high mRNA levels of HIP1 in tumor cell lines, the cDNA sequence isolated by anchored PCR was used to screen a λgt11 SW480 colon cancer cell line cDNA library (Clontech, Palo Alto, CA) to obtain more 5′ sequence. The 500-bp PCR product obtained from cloning of the breakpoint was labeled with ^{32}P by random priming and plaque lifts were performed.[18] Positive phage clones were subcloned into pBluescript KS(+) and sequenced.

In the longest clone, SW9, there were 2 new potential in frame initiator methionines in the additional 5′ sequence. The first ATG at nucleotides 16-19 has a better Kozak consensus sequence than the second ATG (nucleotides 37-39). The third methionine at position 368-370 (or 248-250 of the previously published sequence[20]) has the best fit of the 3, because it has a purine at position −3. No upstream stop codons were identified. However, native HIP1 migrates as a protein of 116 kD by Western blot analysis,[20,21] which is consistent with a preferred start site at methionine 368-370.

Reconstruction of the fusion cDNA for expression experiments. The chromosome translocation breakpoint was amplified from patient material using primers HIP1301F (5′-CCTGAAACTGCTAAGAACCA-3′) and PDGFβR 1806R, and the product was digested with *Bgl* I and *Nhe* I. The *Bgl* I-*Sac* II fragment of the *PDGFβR* was isolated after *Bgl* I and *Sac* II digestion of the *PDGFβR* cDNA and ligated to the *Nhe* I-*Bgl* I breakpoint fragment. This ligation reaction was amplified with primers containing the *Nhe* I and *Sac* II sites (5′-AAATTGCTGCTAGCA-
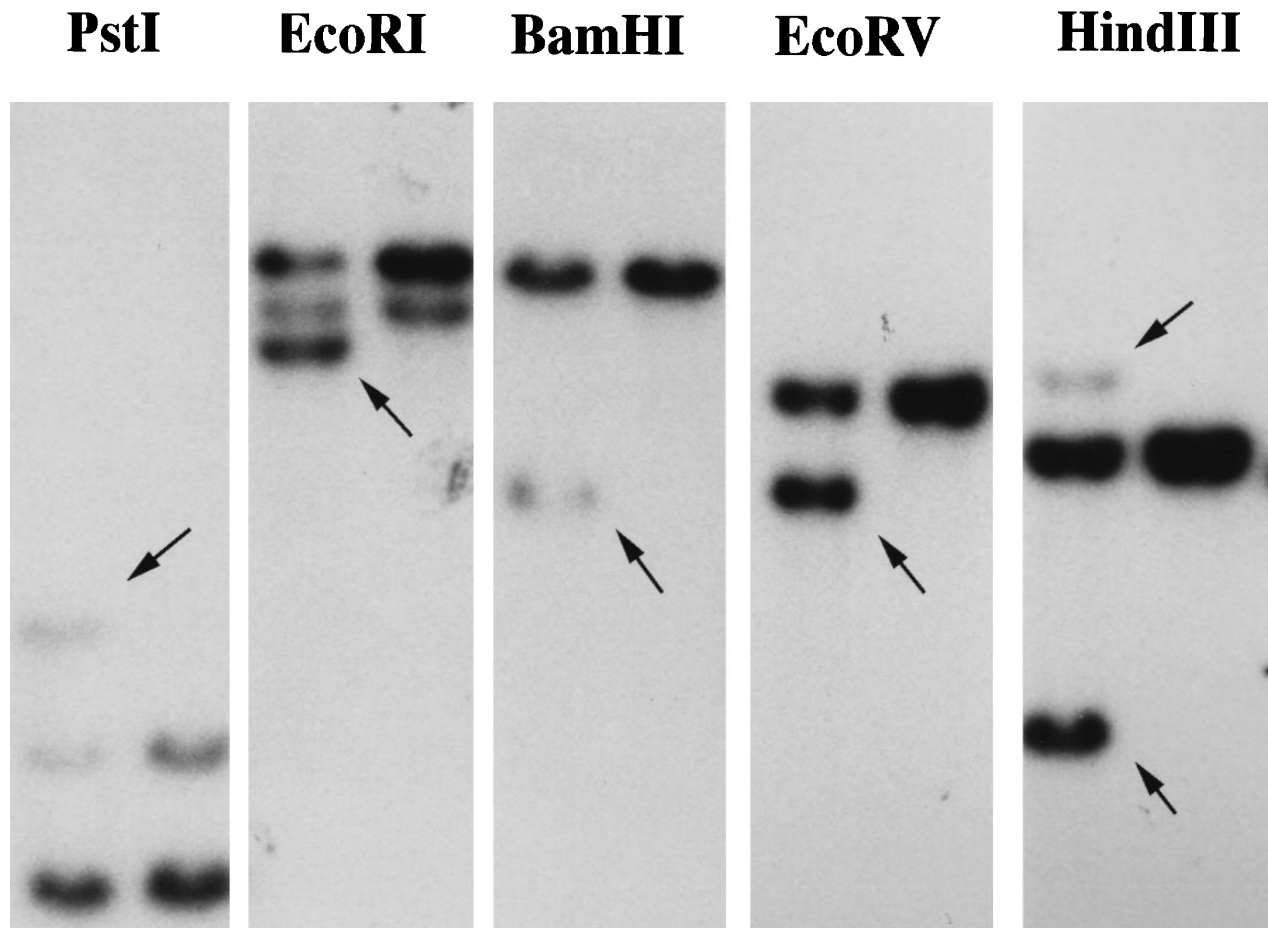
Fig 1. Identification and molecular analysis of t(5;7)(q33;q11.2). Southern blot analysis of the *PDGFβR* gene locus in patient DNA with t(5;7)(q33;q11.2). Genomic DNA of patient t(5;7)-positive cells (lane 1) and control cells (lane 2) was analyzed by Southern blotting with a 1.1-kb *Hin*dIII-*Xho* I *PDGFβR* probe.[6] Arrows indicate the rearranged bands in the *Eco*RI, *Bam*HI, *Pst* I, *Eco*RV, and *Hin*dIII digests.

CAGCCCAGCTTG-3′ and 5′-CTGGTCCCGCGGCAGCTCCCAC-GTGGA-3′ respectively), digested with *Sac* II, and ligated with the 3′ end of *PDGFβR*.[22] The reaction mixture was then digested with *Nhe* I and ligated with the 5′ end of *HIP1* (from SW480 l clone 9) via the unique *Nhe* I site. The region amplified by PCR was confirmed to be void of PCR generated mutations by sequence analysis.

Stable expression of HIP1/PDGFβR. The full-length fusion cDNA was subcloned into the pMSCVneo vector (kindly provided by R.
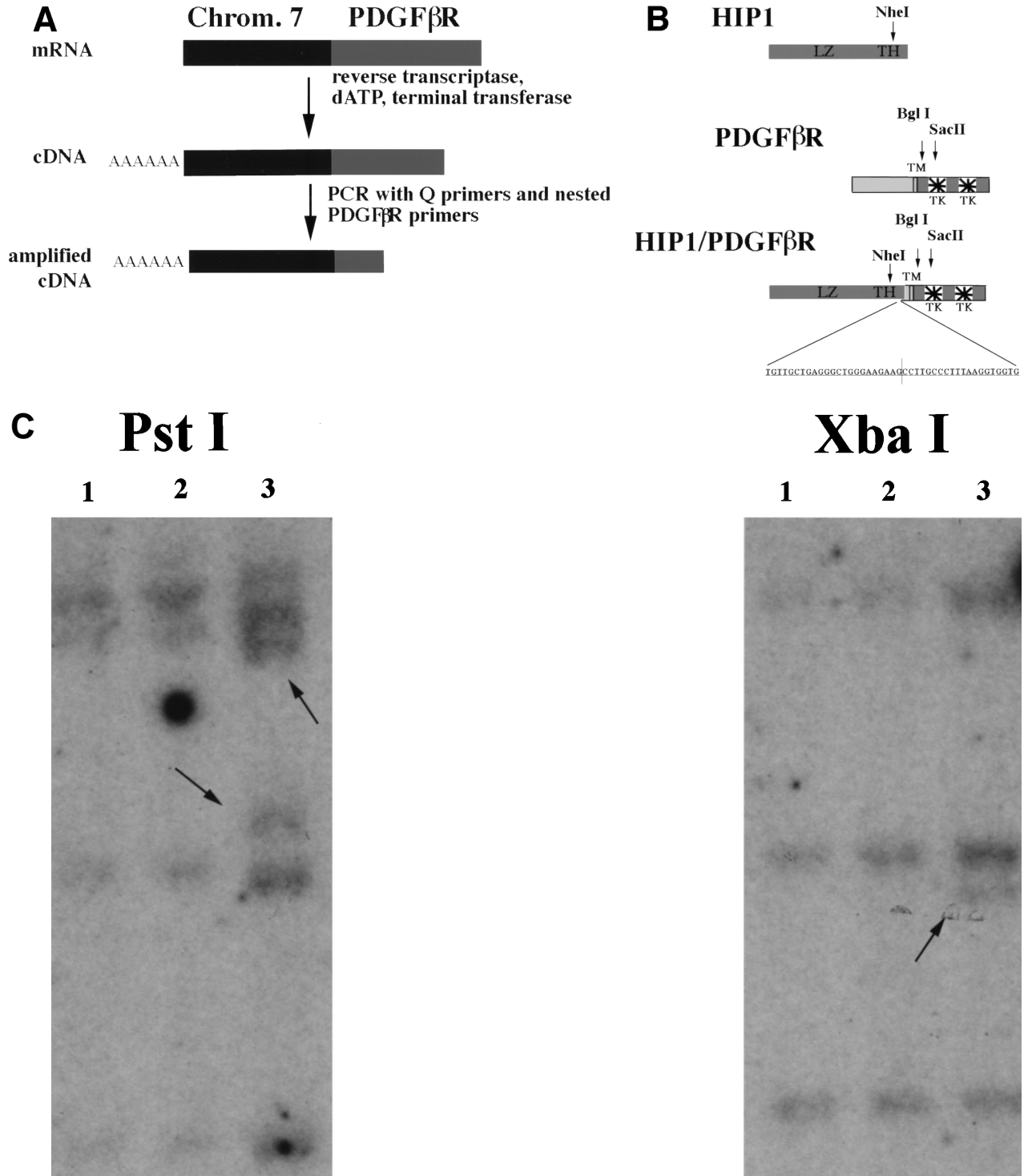
Fig 2. Identification of the chromosome 7 fusion partner. (A) Schematic diagram of anchored PCR.[6,19] (B) Sequence of the *HIP1/PDGFβR* breakpoint and schematic of the fusion protein. (C) Southern blot analysis of *HIP1* gene locus in control DNA (lanes 1 and 2) and patient DNA (lane 3). Arrows indicate rearranged fragments in the *Pst* I and *Xba* I digests.

Hawley, University of Toronto, Toronto, Ontario, Canada). Bosc cells (the kind gift of W. Pear, University of Pennsylvania, Philadelphia, PA) were transfected via the calcium phosphate technique.[18] The 48-hour supernatant (1 mL) was then added to 10^6 Ba/F3 cells (1 mL) in the presence of polybrene (4 μL) as described previously.[23] Cells with stable expression were selected in the presence of G418 and interleukin-3 (IL-3) as described.[15]

RESULTS

Identification of HIP1/PDGFβR in CMML. The HIP1/PDGFbR fusion was cloned from a single patient with a clinical phenotype of CMML. The patient was a 54-year-old man who presented with fatigue, weight loss, and splenomegaly. Laboratory evaluation showed monocytosis, anemia, and peripheral eosinophilia. Bone marrow biopsy showed a hypercellular marrow with increased myeloid:erythroid ratio, eosinophilia, and dysplastic maturation of monocyte lineage cells. Cytogenetic analysis of the bone marrow cells showed t(5;7)(q33; q11.2) (data not shown).

We hypothesized that the PDGFβR on 5q33 was activated as a consequence of fusion to a novel partner on chromosome 7. This region of chromosome 7 is of particular interest because it is frequently deleted in de novo and therapy-related MDS/ AML. Rearrangement of the *PDGFβR* gene was demonstrated by Southern blot analysis of *Eco*RI, *Bam*HI, *Pst* I, *Eco*RV, and *Hin*dIII digests using a 1.1-kb *PDGFβR* genomic probe localized near the *TEL/PDGFβR* breakpoint (Fig 1). These data demonstrated that the chromosome 5 breakpoint was at or near the same intron of *PDGFβR* as for the t(5;12)(q33;p12) breakpoint.[6]

The chromosome 7 fusion partner was identified using anchored PCR with *PDGFβR* primers to amplify the fusion transcript from the patient's bone marrow cell cDNA (Fig 2A). Analysis of the amplified cDNA clones demonstrated 500 bp of non-*PDGFβR* sequence encoding an open reading frame fused to the transmembrane and tyrosine kinase encoding regions of the *PDGFβR* gene (Fig 2B). A database search showed this sequence to be identical to the *HIP 1* gene[20,21] localized by fluorescence in situ hybridization (FISH) to 7q11.2.[20] Southern blot analysis with an *HIP1* cDNA probe (nucleotides 1141-3041) demonstrated rearranged bands in *Pst* I and *Xba* I digests of patient DNA (Fig 2C).

HIP1 is a 116-kD protein that was cloned by yeast two-hybrid screening for proteins that interact with Huntingtin. Huntingtin is the protein mutated in Huntington's disease.[24] HIP1 has a leucine zipper motif and homology to talin, a cytoskeletal associated protein, at amino acids 412-433 and 861-900, respectively.[20]

HIP1 has homology with the *SLA2* gene product (Sla2p) from *Saccharomyces cerevisiae*,[25] an essential cytoskeletal associated protein. The leucine zipper motif and talin homology domain of HIP1 are conserved in Sla2p. HIP1 is also homologous to the *Caenorhabditis elegans* ZK370.3 gene product that has no known function.[26] The degree of homology (40% similarity, 20% identity in both yeast and worm) suggests that HIP1 is the human homologue of these proteins. The highest degree of homology is at the carboxy terminus, where all 3 proteins share homology with talin.[27]

Tissue expression of HIP1/PDGFβR and HIP1. Reverse transcription-PCR (RT-PCR) using PDGFβR 3′ primers and 5′ primers spanning the coding sequence of HIP1 generated the

expected size fragments from patient cDNA (Fig 3), but was not detected in mRNA from normal bone marrow. The reciprocal *PDGFβR/HIP1* fusion could not be detected by RT-PCR analysis (data not shown). Because bone marrow cells from the patient were limited, detection of the fusion transcript required the use of nested PCR primers. In addition, because of this limitation, Northern blot analysis of patient material was not possible. HIP1/PDGFβR protein contained nearly all of the *HIP1* coding sequence, including the leucine zipper and talin homology domains, fused in frame to the transmembrane and tyrosine kinase domain of the PDGFβR (Fig 2B). Only 18 C-terminal amino acids of HIP1 were excluded from the fusion protein.

Northern blot analysis using an *HIP1* cDNA probe demonstrated a previously reported 9.4-kb transcript in all tissues
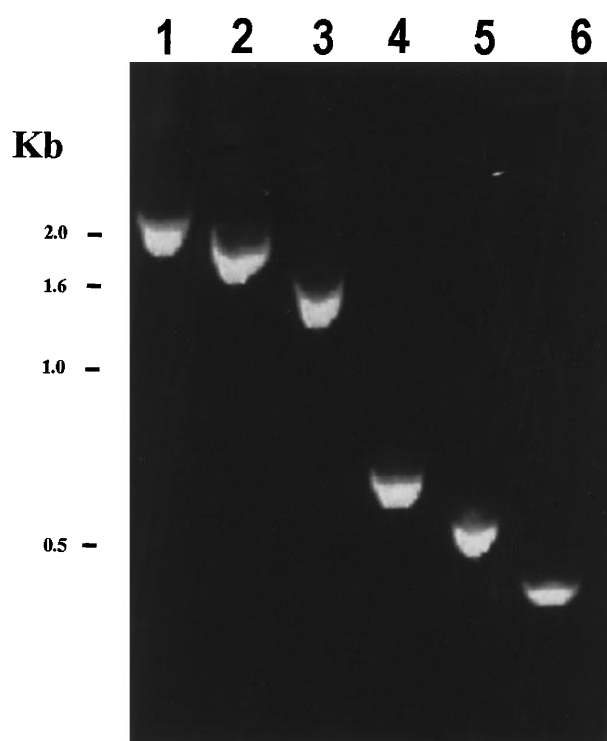
Fig 3. Expression of the chimeric *HIP1/PDGFβR* mRNA in patient bone marrow. RT-PCR analysis of *HIP1/PDGFβR* was performed using total RNA (2 μg) from t(5;7) patient bone marrow that had been reverse transcribed using the Qt primer. PCR was performed using a HIP301F forward primer and the PDGFβR 1848R primer for 30 cycles (94°C for 1 minute, 60°C for 2 minutes, and 72°C for 3 minutes). Nested PCR was performed on PCR products from the first reaction diluted 20-fold and amplified using the same PCR reaction conditions with HIP1 forward primers HIP721F, HIP1141F, HIP1561F, HIP2372F, HIP2494F, and HIP2613F in lanes 1 through 6, respectively, and the *PDGFβR* reverse primer 1806R. HIP1 primer numbers correspond to the first nucleotide of a 20-bp primer of HIP1 sequence according to Kalchman et al.[20] The expected band sizes are 2,279, 1,859, 1,439, 628, 506, and 387 bp for lanes 1 through 6, respectively. Control experiments with no template or in the absence of reverse transcriptase gave no PCR product (not shown). The *HIP1/PDGFβR* fusion was not detected in normal bone marrow and neither was the reciprocal *PDGFβR/HIP1* fusion transcript detected in t(5;7) patient bone marrow using a nested PCR reaction with primers PDGFβR1691F, PDGFβR1711F, HIP13071R, and HIP12966R (primer numbers correspond to the first nucleotide of a 20-bp primer of either *PDGFβR*[22] or *HIP1*[20] sequence).

tested[20] as well as a 2.4-kb transcript present in testis. There are high levels of expression in solid tumor cell lines, including HeLa, SW480, A549, and G861, as well as in testis. Although HIP1 has been implicated in pathogenesis of central nervous system disorders such as Huntington syndrome, there are lower levels of expression in brain and other adult tissues such as bone marrow and peripheral blood (Fig 4 and data not shown).

Transformation of Ba/F3 cells by HIP1/PDGFβR. To characterize the biological properties of the HIP1/PDGFβR fusion, a full-length cDNA encoding *HIP1* was obtained by screening a λgt11 SW480 colon cancer cell line cDNA library. The sequence of the longest clone, SW9, from this library is identical to the published sequence, but incorporates and additional 120 nucleotides of 5′ sequence.

Clone SW9 was then used to reconstruct the full-length *HIP1/PDGFβR* (see the Materials and Methods). Transforming properties of *HIP1/PDGFβR* were tested by subcloning the cDNA encoding the *HIP1/PDGFβR* into the retroviral vector MSCVneo and obtaining stable expression of the fusion protein under control of the LTR in the murine Ba/F3 hematopoietic cell line (Fig 5A). The HIP1/PDGFβR protein was constitutively tyrosine phosphorylated in stably transfected cells (Fig 5B).

To assay the ability of HIP1/PDGFβR to confer IL-3– independent proliferation, Ba/F3-transfected cells were seeded in 96-well trays at a concentration of 2×10^4 cells/well. Cells infected with insert-free virus failed to proliferate in the absence of IL-3 and died. In contrast, HIP/PDGFβR-expressing cells grew at the same rate in the presence or absence of IL-3 (Fig 5C).

DISCUSSION

Involvement of HIP1 in the pathogenesis of leukemia is a novel finding. As for TEL/PDGFβR, fusion of HIP1 to PDGFβR results in constitutive activation of PDGFβR as assessed by tyrosine autophosphorylation and may be mediated by oligomerization through the HIP1 leucine zipper domain. However, other interactions with this fusion may be relevant to the pathogenesis of leukemia in addition to the protein-protein interation mediated by the leucine zipper. For example, the leucine zipper is not necessary for the interaction of HIP1 with Huntingtin.[20] Although the role of HIP1 in Huntingtin's disease is still under investigation, it is conceivable that inhibition of apoptosis mediated by HIP1, as suggested in by Kalchman et al,[20] is also relevant in leukemogenesis mediated by the HIP1/PDGFβR fusion. Thus, there may be an additional role for HIP1 in the leukemogenic pathway.

There are several possible consequences of expression of the HIP1/PDGFβR fusion that may be relevant to leukemogenesis. These could include interference with the function of the native HIP1 protein or the Huntingtin protein through HIP1/PDGFβR heterodimerization. In addition, it is possible that, because the HIP1/PDGFβR is an activated tyrosine kinase, heterodimerization with HIP1 or Huntingtin could modify function or protein
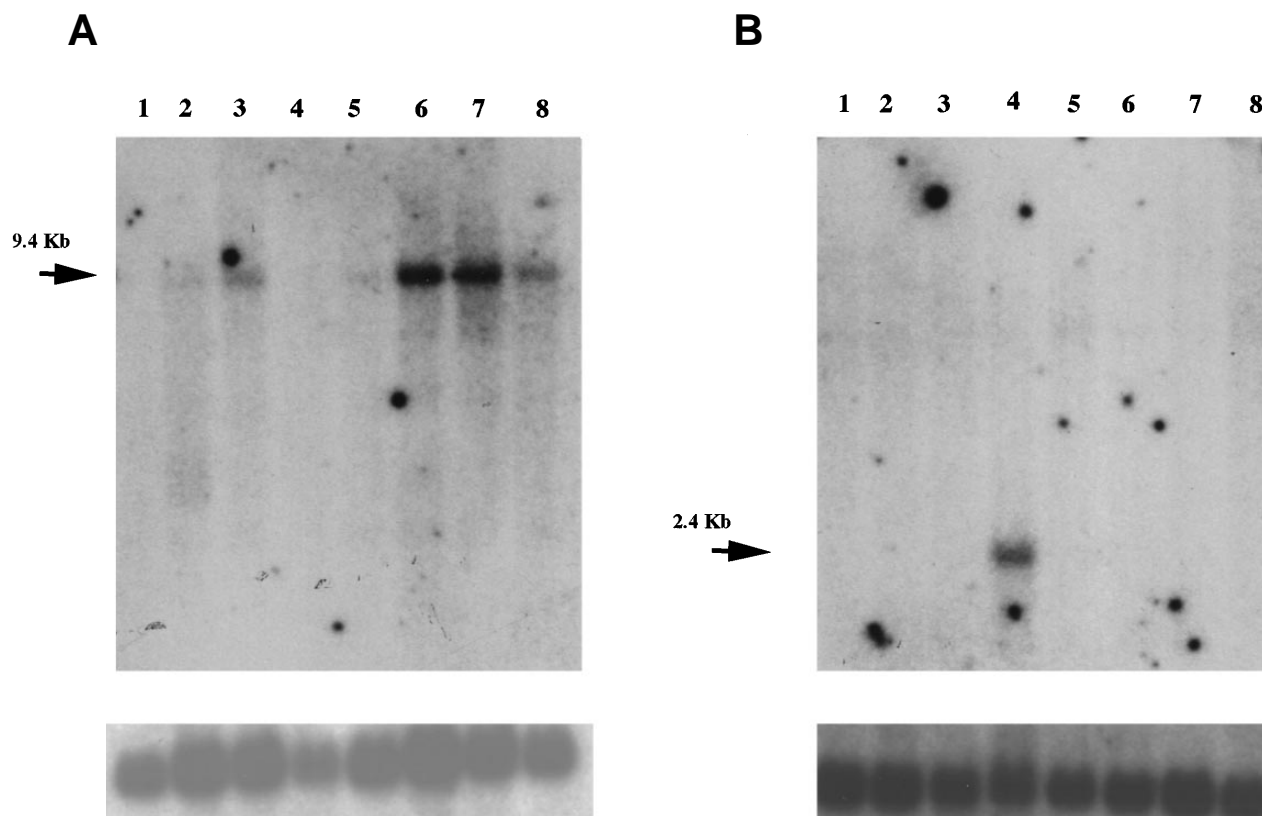
Fig 4.   Northern blot analysis of *HIP1* mRNA in various tissues. Blots (Clontech) were probed with with an ^{32}P–end-labeled probe from HIP1 nucleotides 2890 to 2930.[20] Exposure time was 12 hours. The lower panels are the same blots stripped and reprobed with actin cDNA. (A) Cell lines. RNA sources were HL60, HELA, K562, MOLT4, Raji, SW480, A549, and G361, designated 1 through 8, respectively. (B) Adult tissue RNA sources are spleen, thymus, prostate, testis, ovary, small intestine, colonic mucosa, and peripheral blood, designated 1 through 8, respectively.

A

B

C
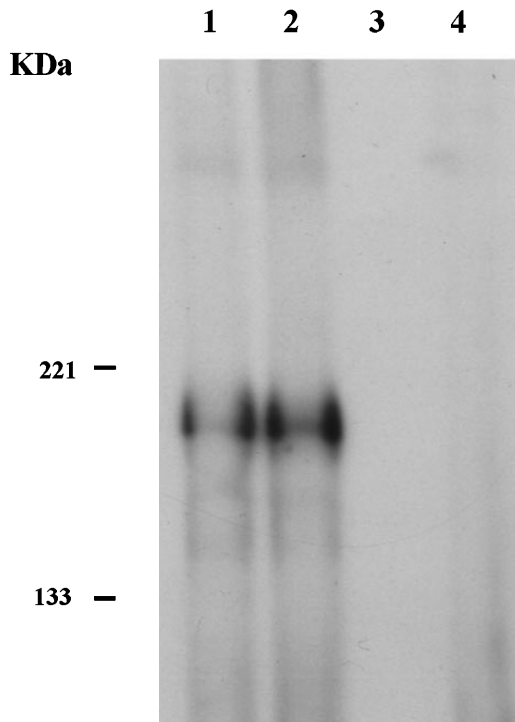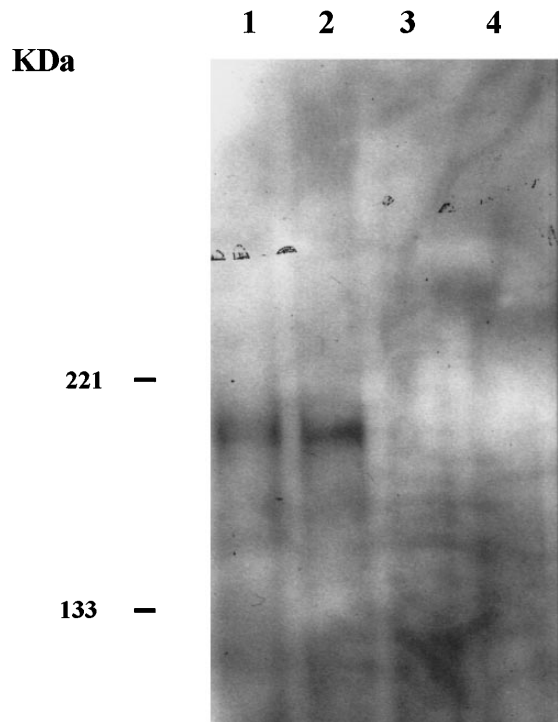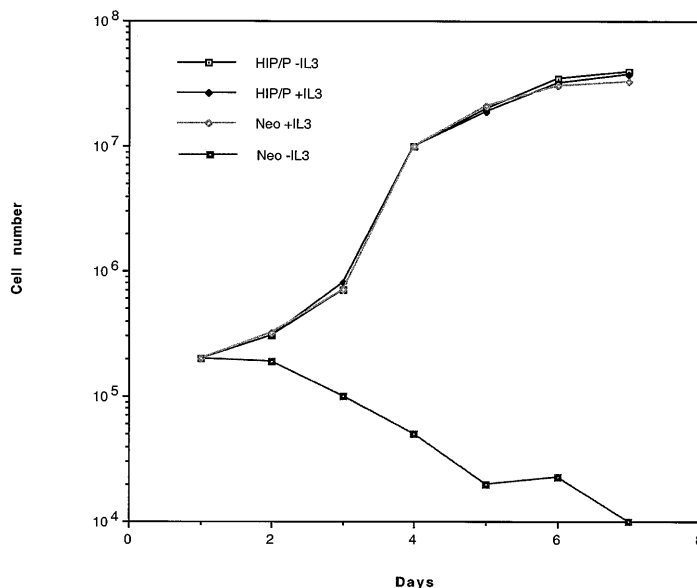
Fig 5. HIP1/PDGFβR transforms Ba/F3 cells to factor independence. Two independent infections of Ba/F3 cells were performed. To assess protein expression and phosphorylation, lysates were immunoprecipitated with anti-PDGFβR antibody (tail; Pharmingen), separated on 8% polyacrylamide gel electrophoresis (PAGE), and blotted onto nitrocellulose. Proteins were detected with anti-PDGFβR peptide antibody directed against the C-terminus (part a) and HRP-conjugated anti-phosphotyrosine 4G10 monoclonal antibody (part b). Lanes 1 and 2 are the HIP1/PDGFβR stable infectants, and lanes 3 and 4 are neomycin-resistant controls. (C) The G418-resistant cells growing in IL-3 were seeded in 96-well trays with 2×10^4 cells per 200 μL per well in RPMI 1640 and 10% fetal calf serum media with or without IL-3. Cells were assessed for number and viability (trypan blue) in triplicate at 24-hour intervals. Each point is the average of the triplicate samples, with standard deviations ranging from 1% to 3% of the number of cells counted each day.

stability of these associated proteins through tyrosine phosphorylation. Because the physiologic roles of HIP1, Huntingtin, and the HIP1/Huntingtin complex are not well understood, it is difficult to speculate at this time about the relevance of these interactions in the mechanism of transformation. However, it should be possible to directly assay for these interactions and

determine whether dysregulation of the apoptotic pathway in hematopoietic cells as a consequence of HIP1/PDGFβR interactions with HIP1 or Huntingtin contributes to leukemogenesis.

Finally, HIP1 localization to 7q11.2 raises the possibility of involvement in 7q− deletions. Loss of chromosome 7 or deletion of the long arm, del(7q), is observed in 10% of MDS or

AML de novo and in greater than 50% of therapy-related AML.[28] 7q− cytogenetics are associated with particularly poor prognosis.[28] It has been hypothesized that deleted chromosomal bands contain as yet unidentified myeloid-specific tumor suppressor loci. Most 7q deletions are interstitial and some have been found to be the result of cryptic translocations.[29-31] The majority of patients with deletions have proximal breakpoints in bands q11-21 and distal break points in q31-36.[29-31] FISH has been used with a panel of YAC clones from 7q to examine patients with deletion breakpoints near 7q22. These data are being used to to narrow down the region of deletion to clone tumor-suppressor genes on 7q involved in myeloid leukemia.[29-31] Other data that may be useful in narrowing the critically deleted region is to characterize translocations in this area, such as the t(5;7) described herein, that could result in inactivation or dysregulaton of tumor-suppressor genes. Examples in which translocations that disrupt the function or expression of tumor-supporssor genes include a translocation that disrupts *p16*.[32] In addition, it has been proposed that disruption of PML, a putative growth suppressor, as a consequence of the t(15;17) chromosomal translocation, may contribute to the pathogenesis of acute promyelocytic leukemia.[33] The possibility that HIP1 is one of the important genes in this area is currently being investigated.

Analysis of HIP1/PDGFβR should contribute to our understanding of the pathogenesis of CMML and may help to elucidate the function of Huntingtin and HIP1 in normal and neoplastic cells. Furthermore, localization of HIP1 to 7q11.2 warrants an investigation of its relevance in pathogenesis of hematopoietic malignancies with 7q deletions.

ACKNOWLEDGMENT

REFERENCES

1. Peeters P, Wlodarska I, Baens M, Criel A, Selleslag D, Hagemeijer A, Van den Berghe H, Marynen P: Fusion of ETV6 to MDS1/EVI1 as a result of t(3;12)(q26;p13) in myeloproliferative disorders. Cancer Res 57:564, 1997

2. Rowley J, Reshmi S, Sobulo O, Musvee T, Anastasi J, Raimondi S, Schneider N, Barredo J, Cantu E, Schlegelberger B, Behm F, Doggett N, Borrow J, Zeleznik L: All patients with t(11;16)(q23;p13.3) that involves MLL and CBP have treatment-related hematologic disorders. Blood 90:535, 1997

3. Raynaud SD, Baens M, Grosgeorge J, Rodgers K, Reid CD, Dainton M, Dyer M, Fuzibet JG, Gratecos N, Taillan B, Ayraud N, Marynen P: Fluorescence in situ hybridization analysis of t(3; 12)(q26; p13): A recurring chromosomal abnormality involving the TEL gene (ETV6) in myelodysplastic syndromes. Blood 88:682, 1996

4. Yoneda-Kato N, Look AT, Kirstein MN, Valentine MB, Raimondi SC, Cohen KJ, Carroll AJ, Morris SW: The t(3;5)(q25.1;q34) of myelodysplastic syndrome and acute myeloid leukemia produces a novel fusion gene, NPM-MLF1. Oncogene 12:265, 1996

5. Peeters P, Raynaud SD, Cools J, Wlodarska I, Grosgeorge J, Philip P, Monpoux F, Van Rompaey L, Baens M, Van den Berghe H, Marynen P: Fusion of TEL, the ETS-variant gene 6 (ETV6) to the receptor-associated kinase JAK2 as a result of t(9;12) in a lymphoid and t(9;15;12) in a myeloid leukemia. Blood 90:2535, 1997

6. Golub TR, Barker GF, Lovett M, Gilliland DG: Fusion of PDGF receptor beta to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. Cell 77:307, 1994

7. de Klein A, van Kessel AG, Grosveld G, Bartram CR, Hagemeijer A, Bootsma D, Spurr NK, Heisterkamp N, Groffen J, Stephenson JR: A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. Nature 300:765, 1982

8. Groffen J, Heisterkamp N, Reynolds FH Jr, Stephenson JR: Homology between phosphotyrosine acceptor site of human c-abl and viral oncogene products. Nature 304:167, 1983

9. Golub TR, Goga A, Barker GF, Afar DE, McLaughlin J, Bohlander SK, Rowley JD, Witte ON, Gilliland DG: Oligomerization of the ABL tyrosine kinase by the Ets protein TEL in human leukemia. Mol Cell Biol 16:4107, 1996

10. Lacronique V, Boureaux A, Della Valle V, Poirel H, Tran Quang C, Maucharffe M, Berthou C, Lessard M, Berger R, Ghysdael J, Bernard O: A TEL-JAK2 fusion protein with constitutive kinase activity in human leukemia. Science 278:1309, 1997

11. Abe A, Emi N, Mitsune T, Hiroshi T, Marunouchi T, Hidehiko S: Fusion of the platelet-derived growth factor receptor beta to a novel gene CEV14 in acute myelogenous leukemia after clonal evolution. Blood 90:4271, 1997

12. Donis-Keller H: The RET proto-oncogene and cancer. J Intern Med 238:319, 1995

13. Heffelfinger SC, Lower EE, Miller MA, Fenoglio-Preiser CM: Plasma membrane phosphotyrosine, Her2-NEU, and epidermal growth factor receptor in human breast cancer. A comparative study. Am J Clin Oncol 19:552, 1996

14. Knezevich SR, McFadden DE, Tao W, Lim JF, Sorensen PHB: A novel ETVˆ-NTRK3 gene fusion in congenital fibrosarcoma. Nat Genet 18:184, 1998

15. Carroll M, Tomasson MH, Barker GF, Golub TR, Gilliland DG: The TEL/platelet-derived growth factor beta receptor (PDGF beta R) fusion in chronic myelomonocytic leukemia is a transforming protein that self-associates and activates PDGF beta R kinase-dependent signaling pathways. Proc Natl Acad Sci USA 93:14845, 1996

16. Bell GI, Karam JH, Rutter WJ: Polymorphic DNA region adjacent to the 5′ end of the human insulin gene. Proc Natl Acad Sci USA 78:5759, 1981

17. Morris SW, Foust J, Valentin M, Roberts W, Shapiro DN, Look AT: Sublocalization of the chromosome 5 breakpoint of the 3;5 translocation in myelodysplastic syndromes and acute myeloid leukemia. Genes Chromosom Cancer 5:385, 1992

18. Sambrook J, Fritsch EF, Maniatis T: Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, 1989

19. Frohman M: Rapid amplification of complementary DNA ends for generation of full-length complementary DNAs: Thermal RACE. Methods Enzymol 218:340, 1993

20. Kalchman MA, Koide HB, McCutcheon K, Graham RK, Nichol K, Nishiyama K, Kazemi-Esfarjani P, Lynn FC, Wellington C, Metzler M, Goldberg YP, Kanazawa I, Gietz RD, Hayden MR: HIP1, a human homologue of S. cerevisiae Sla2p, interacts with membrane-associated huntingtin in the brain. Nat Genet 16:44, 1997

21. Wanker EE, Rovira C, Scherzinger E, Hasenbank R, Walter S, Tait D, Colicelli J, Lehrach H: HIP-I: A huntingtin interacting protein isolated by the yeast two-hybrid system. Hum Mol Genet 6:487, 1997

22. Kazlauskas A, Cooper JA: Autophosphorylation of the PDGF receptor in the kinase insert region regulates interactions with cell proteins. Cell 58:1121, 1989

23. Pear WS, Nolan GP, Scott ML, Baltimore D: Production of high-titer helper-free retroviruses by transient transfection. Proc Natl Acad Sci USA 90:8392, 1993

24. Anonymous: A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group [see comments]. Cell 72:971, 1993

25. Holtzman DA, Yang S, Drubin DG: Synthetic-lethal interactions

identify two novel genes, SLA1 and SLA2, that control membrane cytoskeleton assembly in Saccharomyces cerevisiae. J Cell Biol 122: 635, 1993

26. Genpept an: celzk370.3.

27. Rees DJ, Ades SE, Singer SJ, Hynes RO: Sequence and domain structure of talin. Nature 347:685, 1990

28. Velloso ER, Michaux L, Ferrant A, Hernandez JM, Meeus P, Dierlamm J, Criel A, Louwagie A, Verhoef G, Boogaerts M, Michaux JL, Bosly A, Mecucci C, Van den Berghe H: Deletions of the long arm of chromosome 7 in myeloid disorders: Loss of band 7q32 implies worst prognosis. Br J Haematol 92:574, 1996

29. Tosi S, Harbott J, Haas OA, Douglas A, Hughes DM, Ross FM, Biondi A, Scherer SW, Kearney L: Classification of deletions and identification of cryptic translocations involving 7q by fluorescence in situ hybridization (FISH). Leukemia 10:644, 1996

30. Le Beau MM, Espinosa RR, Davis EM, Eisenbart JD, Larson RA, Green ED: Cytogenetic and molecular delineation of a region of chromosome 7 commonly deleted in malignant myeloid diseases. Blood 88:1930, 1996

31. Fischer K, Frohling S, Scherer SW, McAllister Brown J, Scholl C, Stilgenbauer S, Tsui LC, Lichter P, Dohner H: Molecular cytogenetic delineation of deletions and translocations involving chromosome band 7q22 in myeloid leukemias. Blood 89:2036, 1997

32. Duro D, Bernard O, Della Valle V, Leblanc T, Berger R, Larsen CJ: Inactivation of the P16INK4/MTS1 gene by a chromosome translocation t(9;14)(p21-22;q11) in an acute lymphoblastic leukemia of B-cell type. Cancer Res 56:848, 1996

33. Grimwade D, Solomon E: Characterisation of the PML/RAR alpha rearrangement associated with t(15;17) acute promyelocytic leukaemia. Curr Top Microbiol Immunol 220:81, 1997