HCMOGT-1 Is a Novel Fusion Partner to PDGFRB in Juvenile Myelomonocytic Leukemia with t(5;17)(q33;p11.2)

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Abstract

PDGFRB, a transmembrane tyrosine kinase receptor for platelet-derived growth factor, is constitutively activated by gene fusion with different partners in myeloproliferative/myelodysplastic disorders with peculiar clinical characteristics. Six alternative partner genes have been described thus far. In this study, we report the molecular cloning of a novel translocation t(5;17)(q33:p11.2) in a case of juvenile myelomonocytic leukemia. The novel partner gene was identified as HCMOGT-1 using 5′-rapid amplification of cDNA ends; fluorescence in situ hybridization and reverse transcriptase-PCR analyses confirmed that the translocation resulted in PDGFRB/HCMOGT-1 fusion. We show that the breakpoint of PDGFRB occurred at the same site of all previously reported PDGFRB translocations.

Introduction

Chronic myeloproliferative disorders and myelodysplastic/myeloproliferative diseases characterized by splenomegaly, monocytosis, and marked eosinophilia in peripheral blood (PB) and/or bone marrow are often associated with chromosome rearrangements at 5q31-33 (1–3). This region harbors the PDGFRB gene, which encodes a tyrosine kinase receptor, and its fusion with different partner genes results in its constitutive activation (2). Six partner genes have been identified thus far: ETV6 in t(5;12)(q33;p13) (Refs. 4, 5); CEV14 in t(5;14)(q33;q32) (Ref. 6); HIP1 in t(5;7)(q33;q11.2) (Ref. 7); H4/D10S170 in t(5;10)(q33;q21-q22) (Refs. 8, 9); RAB5 in t(5;17)(q33; p13) (Ref. 10); and PDE6DIP in t(1;5)(q23;33) (Ref. 11). Disruption of PDGFRB has also been demonstrated in patients with other 5q33 rearrangements, but the partner gene in these cases has yet to be identified (3). We report a case of juvenile myelomonocytic leukemia presenting a novel translocation (5;17)(q33;p11.2) with involvement of the PDGFRB gene; the cloning of the translocation breakpoint demonstrated its fusion with a sequence from HCMOGT-1 gene coding for a sperm antigen.

Materials and Methods

An 18-month-old boy was admitted with mild anemia (hemoglobin, 9.1 g/dl), thrombocytopenia (platelet count, 39 × 10^9/liter), leukocytosis (WBC, 25.4 × 10^9/liter with 50% immature and mature granulocytic cells, 4% monocytes, 11% eosinophils, 35% lymphocytes) and marked hepatosplenomegaly. Fetal hemoglobin was 5.3%. Bone marrow aspiration resulted in a dry tap. BM trephine biopsy showed reduced cellularity with prevalence of the myeloid compartment, absence of megakaryocytes, and degree III myelofibrosis. In vitro PB cultures showed spontaneous growth of myeloid/macrophage colony stimulating factor was also documented. The patient was diagnosed with juvenile myelomonocytic leukemia and underwent an allogeneic BMT from an unrelated donor, after informed consent was obtained. Conditioning regimen included Busulphan, Cyclophosphamide, Melphalan and horse anti-thymocyte serum. The patient is alive and well after 3 years.

Cytogenetic and Fluorescence in Situ Hybridization Studies. Chromosome analyses were repeated on unstimulated and phytohemagglutinin-stimulated PB cultures and on fibroblast cultures from a skin biopsy by quinacrine-bandining technique. Fluorescence in situ hybridization was carried out on PB metaphases according to the manufacturer’s protocols with the following probes: whole chromosome 17 painting probe (Appelgene Oncor-Qbiogene, Illkirch, France); dual color LSI SMS/RARA probe (Vysis, Downers Grove, IL) recognizing Smith-Magenis syndrome region at 17p11.2 and RARA locus at 17q21.1; dual color LSI CSF1R probe (Vysis), which maps to 5p15.2 (DSS23, DSS721), and to 5q33 spanning a region of ~160 kb comprising DTDS, CSF1R, and PDGFRB genes. Using bioinformatic resources available at the University of California at Santa Cruz 4, bacterial artificial chromosome clones RP11-7904 and RP11-81D5 were selected as telomeric and centromeric probes flanking the HCMOGT-1 locus, respectively. For dual color fluorescence in situ hybridization, bacterial artificial chromosome probes were differentially labeled by nick translation with Digoxygenin- or Biotin-Nick Translation Mix (Roche, Mannheim, Germany) and visualized using streptavidin-Cy3 (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom) and sheep-anti-digoxygenin-FITC (Roche).

Molecular Cloning of Breakpoints. Total RNA was isolated from patient PB using Trizol solution (Invitrogen, Carlsbad, CA). 5′-Rapid amplification of cDNA ends-PCR was performed according to Frohman (12) using PDGFRB primers, as described in detail by Golub et al. (4). The obtained 468-bp fragment was cloned using an Original TA Cloning kit (Invitrogen). Vector inserts from selected colonies were sequenced by an automated sequencer. The DNA sequences were analyzed using the Blast algorithm. 5

Reverse Transcriptase-PCR of the Wild-Type and Fusion Genes. RNA of the patient and of a healthy control was reverse transcribed using a cDNA synthesis kit (Clontech, Palo Alto, CA). To amplify the 5′-HCMOGT-1/PDGFRB-3′ and the 5′-PDGFRB/HCMOGT-1-3′ fusion genes, we used a couple of primers, which encompass the breakpoint region of the derivative chromosomes. To identify the first fusion gene, 30 PCR cycles were performed with the HCMOGT-1 forward primer (5′-AGACATGAAAGACCATCATA-3′) and the 1848 PDGFRB reverse primer (4). To amplify the 5′ PDGFRB/HCMOGT-1 3′ fusion gene, the 1827PF PDGFRB forward primer (6) was paired with the HCMOGT-1 reverse primer (5′-TGCAATCGCGCTTGAAAC-3′). Primer 1827PF was coupled with primer 1848 PDGFRB to analyze the native PDGFRB gene, whereas the native HCMOGT-1 gene was amplified by HCMOGT-1F and HCMOGT-1R primers.
Results and Discussion

The karyotype of PB blasts was 46,XY,t(5;17)(q33;p11.2), whereas the constitutional karyotype was normal. Hybridization by whole chromosome 17 painting probe confirmed the reciprocal translocation with chromosome 5; the CSF1R probe gave a signal split between der (5) and der (17) (Fig. 1A), in contrast to Magnusson et al. (10), who found the 5q breakpoint telomeric to the LSI CSF1R probe spanning region. The LSI SMS probe showed that the breakpoint on chromosome 17p was centromeric to the Smith-Magenis syndrome region (Fig. 1B) because the hybridization signal was translocated onto the derivative chromosome 5. Hybridization signals for HCMOGT-1 probes were split, with localization of the telomeric part of HCMOGT-1 on der (5) and the centromeric signal retained on the derivative chromosome 17 (Fig. 1C).

Previous studies (4–11) have demonstrated that all partner cDNA sequences are fused in-frame to PDGFRB at nucleotide 1936 (GenBank accession no. NM002609). On this assumption, we performed a rapid amplification of cDNA ends-PCR on the patient’s RNA to identify the PDGFRB partner gene on chromosome 17 using a set of PDGFRB primers lying near the presumed breakpoint. The 468-bp fragment obtained was cloned and sequenced: sequence analysis performed on 20 clones revealed a partial PDGFRB sequence fused in an open reading frame, at nucleotide 1936, with a non-PDGFRB sequence. A Blast search on the novel sequence revealed 100% homology with the HCMOGT-1 gene (GenBank accession no. NM152904). To confirm this finding, we analyzed the patient’s leukocytes using reverse transcriptase-PCR for the presence of the chimeric mRNA: an expected 180-bp fragment was detected and sequenced (Fig. 2A), whereas the reciprocal fusion product 5’-PDGFRB/HCMOGT-1-3’ was not detected (data not shown). Noteworthy is that this and all other cloned PDGFRB breakpoints thus far reported are located, at the RNA level, at exactly the same bp. This finding derives from the fact that the genomic breakpoint falls on an intron of the PDGFRB gene and that the chimeric transcript is spliced across this breakpoint to the splice acceptor site upstream of exon 10 of PDGFRB. The six currently known partners fused to PDGFRB gene encode proteins different in function and sequence, but all contribute to a dimerization domain of the fusion protein that is essential for its constitutive activity, enabling self-association and mimicking the normal process of receptor activation after ligand binding (13). The same mechanism

Fig. 2. Reverse transcriptase-PCR analysis and the t(5;17) breakpoint resulting in HCMOGT-1/PDGFRB. A, reverse transcriptase-PCR analysis. RNA from leukemic cells carrying the t(5;17) and from a normal donor were reverse transcribed and amplified using 1827PF and 1848R PDGFRB primers and HCMOGT-1F and HCMOGT-1R primers. Amplified fragments of 193 and 210 bp, corresponding to the PDGFRB and HCMOGT-1 native genes, respectively, visualized on 2% agarose gel, are shown. A 180-bp amplified fragment of the HCMOGT-1/PDGFRB chimeric transcript (obtained with HCMOGT-1F and 1848R primers) was detected in the patient but not in the normal control. A 100-bp DNA ladder was used as marker. B, schematic representation of the hypothesized fusion protein HCMOGT-1/PDGFRB. The functional protein domains and the breakpoints (arrowheads) from the two involved proteins, HCMOGT-1 and PDGFRB, are shown. The nucleotide and amino acid sequences flanking the t(5;17) breakpoint of the resulting fusion product HCMOGT-1/PDGFRB are shown at the bottom of the figure.

also likely occurs for the predicted fusion protein of the novel rearrangement described herein, with ligand-independent dimerization of PDGFRB (1) through coiled-coil domains of HCMOGT-1 (Fig. 2B).

Our case presented all of the features common to patients with PDGFRB rearrangement: male sex; splenomegaly; monocytosis; and eosinophilia. Nevertheless, peculiar and noteworthy are both age at presentation (18 months) and diagnosis (juvenile myelomonocytic leukemia): most reported cases with demonstrated PDGFRB involvement present a median ages of 50–60 years (2), and pediatric cases reported thus far are rare.

In conclusion, HCMOGT-1 has been reported for the first time as a translocation partner of PDGFRB in an hematological malignancy, namely juvenile myelomonocytic leukemia. To our knowledge, HCMOGT-1 has never been implicated in any neoplasia. Our results confirm the key role of the fusion process of the PDGFRB gene in malignant proliferation.

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References

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