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Short communication

Molecular cytogenetic findings in a four-way t(1;12;5;12)(p36;p13;q33;q24) underlying the ETV6-PDGFRB fusion gene in chronic myelomonocytic leukemia

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Abstract Fluorescence in situ hybridization (FISH) and reverse-transcription polymerase chain reaction (RT-PCR) detected the *ETV6-PDGFRB* fusion in a patient with chronic myelomonocytic leukemia characterized by bone marrow and peripheral blood eosinophilia and a four-way t(1;12;5;12)(p36;p13;q33;q24) on bone marrow cells. The patient consequently underwent imatinib mesylate therapy and achieved hematologic, FISH, and molecular remission. The FICTION technique (fluorescence immunophenotyping and interphase cytogenetics as a tool for the investigation of neoplasms) demonstrated that eosinophils and $CD13^+$ and $CD14^+$ cells belong to the neoplastic clone bearing the *ETV6-PDGFRB* rearrangement.

Molecular cytogenetics is the most reliable approach to detect the involvement of promiscuous genes, such as *PDGFRB*, and to properly classify genetic entities for which targeted therapies are available. Assessment of cell lineages harboring the genomic lesion may contribute in the understanding of leukemogenic pathways.

1. Introduction

PDGFRB (platelet-derived growth factor β receptor), a class III receptor tyrosine kinase, rearranges with several gene/chromosome partners and identifies a subgroup of hematologic diseases classified as myeloproliferative disorders/myelodysplastic syndromes (MPD/MDS) in the World Health Organization classification [1]. They are characterized by bone marrow and/or peripheral blood eosinophilia, monocytosis, splenomegaly, and male predominance. It was first identified by Golub et al. as the partner of *ETV6* (also called *TEL*) in the rare t(5;12)(q33;p13), which produces the ETV6-PDGFRβ fusion protein [2]. ETV6-PDGFRβ contains the first 154 amino acids of ETV6 (corresponding to the helix-loop-helix domain) linked to the tyrosine kinase domain of PDGFRB and acts as a constitutive activated tyrosine kinase that transforms hematopoietic cells by stimulating proliferation and reducing apoptosis. In transgenic mice, ETV6-PDGFRβ induces a myeloproliferative proliferation resembling human myeloproliferative disease [3].

ETV6-PDGFRβ activity is ihnibited by tyrosine kinase inhibitors such as imatinib mesylate either in vitro or in vivo, and patients with *ETV6-PDGFRB*–positive MPD/MDS achieve long-lasting hematologic and molecular remission [4].

We report molecular cytogenetic findings in an *ETV6-PDGFRB*–positive chronic myelomonocytic leukemia (CMML) with a complex four-way variant of the t(5;12)(q33;p13).

2. Materials and methods

2.1. Case report

A 45-year-old man was referred to the Hematology Department of the University of Florence, Italy, because

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of fever and leukocytosis. Peripheral blood counts were as follows: white blood cells 40.3 $\times$ 10^9/L (65% neutrophils, 10% myelocytes, 5% metamyelocytes, 8% eosinophils, 7% lymphocytes, 5% monocytes); hemoglobin 14.3 g/dL; and platelets 187 $\times$ 10^9/liter. Bone marrow aspirate and biopsy revealed proliferative features mainly involving the myeloid lineage with blasts less than 5% and no fibrosis. The patient had a stable disease for 9 months when he was treated with α-interferon 6,000,000 IU/day for 1 month for increased leukocytosis and anemia. Since FISH revealed *PDGFRB* involvement, imatinib mesylate was started (400 mg/day). Hematologic, cytogenetic, and FISH remission were documented after 1 month of therapy, and molecular remission was documented after 25 months. The patient was still in remission as of the last follow-up (after 40 months of therapy).

2.2. Cytogenetics

Karyotyping was performed after G-banding on bone marrow samples at diagnosis and was described according to the International System for Human Cytogenetic Nomenclature (ISCN 2005).

2.3. Fluorescence in situ hybridization (FISH)

Multicolor FISH (M-FISH) was done with the 24XCyte human multicolor FISH probe kit (MetaSystem, Zeiss, Altlussheim, Germany) following the manufacturer's instructions. Dual-color split FISH assays were applied to study the *PDGFRB*/5q33 breakpoint (cosmid 9-4 in red and cosmid 4-1 in green) and the *ETV6*/12p13 breakpoint (cosmid 179A6 in red and cosmid 148B6 in green) as already described [5,6]. The 1p36 breakpoint was investigated with 12 DNA clones ordered from telomere to centromere as follows: RP1-163G9, RP11-193J6, RP4-785P20, RP1-187A9, RP5-863N7, RP5-967O8, RP4-703E10, RP5-1014E24, RP3-330O12, RP11-56N19, RP5-934G17, and RP5-1077B9. To study the breakpoint at 12q, the following DNA clones for bands q14~q24 were used: centromere, RP11-877M13, RP11-578A18, RP11-478C19, RP11-421J14, RP11-115H15, RP11-18C24, RP11-512M8, RP11-214K3, and telomere [PAC and BAC clones belong to the Roswell Park Cancer Institute, libraries RCPI-1, RCPI-3, RCPI-4, RPCI-5 and RCPI-11, (http:www.chori.org/bacpac/), and were kindly provided by Dr. Mariano Rocchi, DIGEMI, University of Bari, Bari, Italy). At least eight abnormal metaphases were analyzed for each experiment. One-color interphase FISH with DNA clones for *PDGFRB* and/or *ETV6* was done to monitor the disease at 6, 25, and 40 months of therapy on 500 nuclei for each experiment. Normal bone marrow samples were used as controls, and the cut-off for splitting (three signals) was the highest normal value (2% for *PDGFRB* and 2.5% for *ETV6*, respectively), as established by our laboratory standard [6].

2.4. Fluorescence immunophenotyping and interphase cytogenetics as a tool for the investigation of neoplasms (FICTION)

Mononuclear frozen cells from bone marrow were used to prepare cytospins. The following monoclonal antibodies were used: anti-CD13, anti-CD14, anti-CD3, anti-CD19, anti-CD20, anti-Glycoforin A (Dako, Milan, Italy), and anti-CD34 (Becton Dickinson, Milan, Italy). Evaluation of eosinophils was based on autofluorescence of cytoplasmic granules [6]. Immunostaining was performed with a three-step technique: Cy3-conjugated polyclonal goat anti-mouse, rabbit anti-goat, and donkey anti-rabbit (Jackson-Immunoresearch/ListarFISH, Milano, Italy), and hybridization was done with cosmids 9-4, 4-1, 12-A, and 4-6 for *PDGFRB*/5q33 and/or with cosmids 179A6 and 148B6 for *ETV6*/12p13 labeled with biotin [5,6]. The cut-off for *PDGFRB* and *ETV6* splitting (i.e., percentage of cells with three signals), was established at the highest value in one normal bone marrow sample for each antibody (Fig. 2). Analyses were done on 10–83 positive cells with a fluorescence microscope (Provis; Olympus, Milano, Italy) equipped with a CCD camera (Sensys; Photometrics, Tucson, AZ) run from a image analysis software (Abbot Molecular/Vysis, Stuttgart, Germany).

2.5. RT-PCR

RT-PCR was performed on bone marrow samples at diagnosis and during imatinib therapy as described previously [7].

3. Results

3.1. Cytogenetics

The karyotype was 46,XY,add(1)(p36),del(5)(q33q35), der(12)del(12)(p13)add(12)(q22~q24)[18]/46,XY[2] (Fig. 1A).

3.2. FISH

M-FISH identified the four-way reciprocal t(1;12;5;12) (p36;p13;q33;q24). Experiments with cosmid 9-4/*3′PDGFRB*, retained on der(5), and cosmid 4-1/*5′PDGFRB*, translocated to the long arm of der(12), indicated distruption of *PDGFRB* (Fig. 1B). Cosmid 179A6/*5′ETV6*, translocated to the der(5), and cosmid 148A6/*3′ETV6*, retained on the short arm of der(12) (Fig. 1B), indicated that *ETV6* was also involved and likely juxtaposes to *PDGFRB* on der(5), as observed in simple balanced t(5;12)(q33;p13). One signal on normal chromosomes 5 and 12 with clones for *PDGFRB* and *ETV6*, respectively, was present.

The 1p breakpoint fell at band 36.2 within a 4.5-megabase region. At the telomeric side, it was flanked by clone RP11-1014E24, which was the first clone translocated to the short arm of der(12), and while at the