TEL Gene Is Involved in Myelodysplastic Syndromes With Either the Typical t(5;12)(q33;p13) Translocation or its Variant t(10;12)(q24;p13)


A t(5;12)(q33;p13) translocation is a recurrent chromosome abnormality in a subgroup of myeloid malignancies with features of both myeloproliferative disorders and myelodysplastic syndromes (MDSs). The molecular consequence of a t(5;12) is a fusion between the platelet-derived growth factor receptor-B gene on chromosome 5 and a novel ETS-like gene, TEL, on chromosome 12. We report on three patients with a t(5;12)(q33;p13) diagnosed as chronic myelomonocytic leukemia, and one case of a t(10;12)(q24;p13) in a progressive MDS, with eosinophilia and monocytosis. Involvement of the TEL gene in these chromosome translocations was investigated by fluorescence in situ hybridization (FISH) with cosmid probes containing selectively the 5’ end or 3’ end of TEL. Hybridization of these cosmid probes to a der(5)/der(10), respectively, demonstrated a rearrangement of TEL in both translocations, showing that the t(10;12) is a variant translocation of the t(5;12). Cloning of the fusion cDNA of one case of t(5;12) showed that the breakpoint occurred at the RNA level at exactly the same position as reported by Golub et al (Cell 77:307, 1994). In addition, the TEL gene on chromosome 12 could be localized between two probes previously mapped to 12p13, namely KRT81 and D12S178, leading to a better definition of the position of TEL in this chromosome region. Moreover, in the case involving chromosome 10, the breakpoint occurred between eKTN206 and eKTN312/LYT-10 at 10q24. Clinicohematological data in these studies as well as the restriction mapping of chromosomal breakpoints strongly suggest that (1) common features in MDSs involving the TEL gene are monocytosis and eosinophilia, (2) chromosomes other than no. 5 may be involved and at least a t(10;12)(q24;p13) variant chromosome translocation does exist in these MDSs, and (3) both standard and variant 12p/TEL translocations may be identified by FISH with appropriate probes.

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We report here three patients with a typical t(5;12)(q33;p13) diagnosed as CMML according to the French-American-British criteria and one case of refractory anemia (RA) with eosinophilia in the bone marrow (BM) and increasing monocytosis, associated with what appeared to be a variant t(10;12)(q24;p13) translocation. We investigated the TEL gene in both types of translocations with Southern blotting and fluorescence in situ hybridization (FISH) analysis with cosmid specific for the 5’ end and the 3’ end of the TEL gene. Moreover, 10q was further analyzed with a panel of probes to better localize the breakpoint on 10q24.

MATERIALS AND METHODS

Patients. The four patients involved in this study were collected from three centers: Centre for Human Genetics (Leuven, Belgium), case no. 1; Hospital de Sant Pau (Barcelona, Spain), case no. 2; and the Institute of Hematology (Perugia, Italy), cases no. 3 and 4. Clinical and hematologic findings are summarized in Table 1.

Cytogenetics. Cytogenetic analyses from peripheral blood (PB) and BM were performed after 3 days using synchronized cultures without stimulation. R- and G-chromosome banding was used. Karyotypes are presented in accordance with ISCN.14

DNA analysis. The TEL cDNA was amplified from a liver cDNA sample using a 5'C-TCCAGAGGCCCAGTGCCGAGGT oligonucleotide as forward primer and a 5'-TCCAGACGGTCTCGGCTCTGTC ACT oligonucleotide as reverse primer. The resulting 1.241-bp cDNA that contains the complete coding sequence of TEL was cloned into pGEM-3Z and was sequenced.

DNA was isolated from BM samples of patients no. 3 and 4 with conventional methods. DNA (15μg) was digested with EcoRI or Pst I, separated on an agarose gel, blotted onto a nylon membrane, and probed with the complete TEL cDNA.

Cosmids were isolated from the Lawrence Livermore (Livermore, CA) chromosome 12 library LL12NC01 with the TEL cDNA as a probe. High-density filters of the library were kindly grown by Dr E. Schoenmakers (Department of Molecular Oncology, Center for Human Genetics). Ten cosmids were isolated. Southern analysis of the cosmids was performed with four fragments of the TEL cDNA ranging from bp 79 to 371, bp 372 to 678, bp 679 to 1038, and bp 1039-1329 (numbering according to Golub et al15) and generated...
with Xmn I, Sac I, and Acc I, respectively. Two cosmids were selected for further analysis, LL12NCO1N50F4 (cTEL5') and LL12NCO1N148B6 (cTEL3'). Cycle sequencing using fluorescein isothiocyanate end-labeled primers derived from the TEL cDNA sequence and a commercially available sequencing kit (BRL, Gaithersburg, MD) on an automated laser fluorescence (ALF) sequencer (Pharmacia, Uppsala, Sweden) confirmed the identity of the cosmids.

In situ hybridization was performed as previously described.13 Probes cKTN206, cKTN420, and cKTN312 were kindly provided by Dr J. Nishisho (Osaka University Medical School, Yamadaoka, Japan); the LYT-10-specific probe PMd10001, by Dr U.H.Weier (University of California, Berkeley, CA); and D12S119, by Dr K. Montgomery (AECOM, New York, NY). All other 12p cosmids were isolated and ordered by FISH in our laboratory.16

FISH experiments were evaluated on a Zeiss Axioplan fluorescence microscope (Zeiss, Oberkochen, Germany) and a Leitz DMRB (LDMRB) fluorescence microscope (E. Leitz Inc, Wetzlor, Germany) equipped with a cooled CCD camera (Photometrics, Stuttgart, Germany) run by Imagegenetics software (Imagegenetics, Stuttgart, Germany). Eight to twenty-five informative chromosome spreads were analyzed for each experiment.

RNA analysis. Total RNA was isolated from BM cells of patient no. 3 by the guanidinium/acid phenol method. Total RNA (5µg) was used for the synthesis of first-strand cDNA, primed with oligo(dT), using Moloney murine leukemia virus reverse transcriptase (GIBCO-BRL) in a volume of 20 µL. Five microliters of the reaction was used for polymerase chain reaction (PCR) analysis. The TEL/PDGFRB fusion cDNA was amplified by nested PCR. For the TEL gene the primers were 5'-CCTCCAGAGAGCCCAGTGCCGAGT and 5'-TCAGGATGGAGGAAGACTCGA. For the PDGFRB gene the primers described by Golub et all3 were used. The PCR products were cloned into SmI-digested pUC18, and 5 clones were sequenced on an automated sequencer (ALF; Pharmacia, Uppsala, Sweden).

RESULTS

Nature of the disease. Based on clinical and laboratory findings (splenomegaly, monocytosis, eosinophilia, and absence of Ph chromosome and breakpoint cluster region rearrangement), three of the four cases (no. 1 to 3) presented were diagnosed as CMML. The fourth case presented as MDS with pancytopenia and only 2% of blasts in the BM (RA). However, an increasing number of monocytes (20%) was detected in the PB 20 months after diagnosis, when blast cells increased to 25%. BM eosinophilia was also present from the beginning.

Cytogenetics. Cytogenetic analysis was performed at the time of diagnosis in three cases (no. 2 to 4) and 2 years after the first observation in patient no. 1. The t(5;12)(q33;p13) was found as the sole structural abnormality in two patients; one of them also had a t(5;12)-positive subclone with trisomy 21 (case no. 1). The karyotype from the third patient showed a more complex rearrangement concerning chromosomes 6 and 16, in addition to the t(5;12). The fourth case was characterized by a t(10;12)(q24;p13) as the sole chromosomal aberration detected in two consecutive BM samples, analyzed at the time of RA and 2 years later, when evolution of the disease to a CMML-like disorder was observed.

DNA and RNA analysis. The involvement of the TEL gene in the t(5;12) and t(10;12) was studied by Southern analysis and FISH with cosmids containing the 5' end or the 3' end of the TEL gene. To isolate these probes a TEL cDNA
was obtained by reverse transcription PCR and was cloned. A 1,241-bp TEL cDNA was obtained containing the complete coding sequence. The sequence of this cDNA was identical to the published one. The cDNA was used to screen a chromosome 12 cosmid library. Ten cosmids were obtained and characterized by Southern hybridization with restriction fragments of the TEL cDNA. Two cosmids were selected for further analysis. Clone cTEL5' yielded a hybridization signal at high stringency, with a cDNA fragment spanning bp 79 to 371 of the TEL sequence but yielded no signal with any TEL probe derived downstream from bp 372 (results not shown). Direct sequencing showed that cTEL5' contained a TEL exon which ends at bp 187 of the TEL cDNA sequence, and no sequence derived downstream from this exon. Thus, the TEL breakpoint must be at least 40 kb.

Southern hybridizations of genomic DNA isolated from a BM sample of patients no. 3 and 4 and digested with EcoRI and Pst I with the TEL cDNA did not show any rearranged fragments. FISH with cosmid probes hybridizing to the chromosome region 12p was performed on leukemic cells from all patients. Patient no. 1 was further investigated with cosmid specific probes that showed cTEL3' was present on the normal chromosome 12p and the der(5) and der(12) all in analyzed cases and was absent on the der(5) (patients 1 through 3) or the der(10) (patient no. 4). The reverse was observed with probe cTEL5'. Signals were present on the normal chromosome 12p and the der(5) for patients 1 through 3 or the der(10) for patient no. 4, but never on the der(12) (Fig 2). Among the other 12p cosmids used, von Willebrand factor and PRBl hybridized to the der(5);t(5;12), whereas hybridization signals from the KRAS2, D12S119, and D12S178 were found on the der(12)t(5;12). For chromosome 10, two cosmids cKTN420 (10q22.1) and cKTN206 (10q22.3-q23.1) mapped proximal to the breakpoint. The third probe, cKTN312 previously assigned by FISH to 10q24.3-25.1, as well as a probe for LYT-10 mapped distal to the breakpoint.

A fusion transcript of TEL and PDGFRB could be amplified from cDNA of patient no. 3 for which an RNA sample was available. Sequencing of the resulting cDNA showed that in this case the fusion occurred at the RNA level at exactly the same position as reported by Golub et al. for their t(5;12) case.

**DISCUSSION**

We showed TEL gene involvement in four cases of MDSs characterized by a t(5;12)(q33;p13) or a variant t(10;12)(q24;p13). In three cases diagnosis was compatible with CMML, whereas the fourth case with the cytogenetic variant t(10;12) had a RA, which later evolved to a CML-like disorder with eosinophilia and monocytosis. The 5;12 translocation was described for the first time by Keene et al. in two patients characterized by eosinophilia and 12p abnormalities, diagnosed as eosinophilic leukemia and Ph-negative CML. An additional case was reported by Srivastava et al. in which CMML was characterized by overexpression of the KRAS2 oncogene, assigned to 12p12. Six other cases of atypical CML, CMML with eosinophilia or myeloproliferative disorder with dyserythropoiesis and eosinophilia, and t(5;12) have been published by Berkowicz et al., Martiat et al., Wessels et al., and Baranger et al. It was postulated that the translocation represents a clinical entity with features of both CML and CMML and eosinophilia. However, an additional observation was published by Lerza et al. as a
INVolvEMENT OF TEL IN MDS WITH t(5;12) OR t(10;12)

Fig 2. FISH with cosmids specific for the 5' end (cTEL5') and the 3' end (cTEL3') of TEL gene performed in t(5;12) and t(10;12) cases. Chromosome 12 and a der(12) are indicated by arrows; a der(5) and der(10) are indicated by arrowheads.

The t(5;12) was recently cloned by Golub et al. The investigators showed that the translocation results in expression of a fusion transcript between a novel gene at 12p13, called TEL (for translocation, ets, leukemia), which is a member of the ETS family, and the PDGFRB gene at 5q33. The TEL-PDGFRB fusion includes the 5' HLH domain of TEL and the transmembrane and tyrosine kinase domains of PDGFRB. The mechanism of transformation by the TEL-PDGFRB fusion is not known. However, it is of interest that the reciprocal fusion transcript containing the TEL DNA-binding domain is not expressed in leukemic cells; therefore, the chimeric gene shows mainly the case of RA with monocytosis and fibrosis. Altogether, data from the literature, from our patients no. 1, 2, and 3, and the clinical evolution of case no. 4 allow us to conclude that the malignant disorder associated with a structural rearrangement on 12p13/TEL gene is characterized by a high number of monocytes (more than 8% of the differential count in PB) and BM eosinophilia (more than 5% in BM) and is consistent with diagnosis of CMML in the majority of cases. Cytogenetically the 12p13 involvement is usually because of a 5;12 translocation. Moreover, for the first time a cytogenetic variant t(10;12)(q24;p13) translocation is shown.

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oncogenic potential of PDGFRB. Our experiments with a TEL
cDNA probe could not show a rearrangement on Southern blot-
ing. Indeed, although Golub et al.13 mentioned detection of TEL
rearrangement by Southern blotting and ribonuclease assays, it
is not clear whether they used genomic or cDNA probes for
the former analysis. Our results suggest that the breakpoint on
chromosome 12 may occur in an intron sequence of the TEL
gene not detected by the applied combination of restriction en-
yzymes and the TEL cDNA. The large genomic size of the TEL
gene supports this hypothesis. In case no. 3, however, sequencing
of the fusion cDNA showed that exactly the same fragments of
the TEL and PDGFRB genes were fused as described by Golub
et al.,13 further supporting the hypothesis that in these cases the
HLH domain of TEL might be of functional importance.

The FISH investigations in this study and the cDNA analysis
of one case definitely proved that the breakpoints in both t(5;12)
and t(10;12) occurred within the TEL gene. Indeed, the 3′ end
of the TEL gene is maintained on the der(12) chromosome,
whereas the 5′ end of TEL is translocated to the der(5) or the
der(10) in both the t(5;12) and the t(10;12) cases, respectively
(Fig 2). Thus, hematologic features, karyotypic results, and mo-
lecular data are in keeping with the definition of 10;12 transloca-
tion as a variant of t(5;12). The involvement of another hypotheti-
cal gene within an intron of TEL in the case with t(10;12)
appears to be extremely unlikely. These results also show the
usefulness of isolated TEL cosmids as probes for the analysis
of the involvement of TEL gene in MDS. In addition, data are
consistent with a pter-5′TEL-3′TEL-cent orientation for the TEL
gene on chromosome 12 and a qter-5′PDGFRB-3′PDGFRB-
cent orientation for the PDGFRB gene on chromosome 5.

Further analysis of the t(5;12) and a t(10;12) with a panel of
d2p-specific cosmids allowed us to determine the position of the
breakpoint, as well as of the TEL gene, between D12S178 and
PRB1 loci at 12p13. With respect to chromosome 10, FISH
analysis showed that the breakpoint occurred in a region flanked
by cKTN206 and cKTN312/LYT-10 at 10q24. Although the 10q24
chromosome region carries some genes that may play an
important role in leukemogenesis and lymphomagenesis (namely
tCL3 (HOX11),18 LYT-10,19 and APO-1/FAS20), no recurrent associations of 10q24 chromosomal abnormalities with myeloid
disorders have been reported before.

In summary, the findings discussed here show that the TEL
gene may be activated not only by fusion with the PDGFRB
gene on chromosome 5q33.1, but also by unknown sequences
on 10q24, located upstream to LYT-10. Involvement of the TEL
in pathogenesis of MDS may be easily analyzed by FISH with
cosmids containing the 5′ end and 3′ end of TEL.

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