A Novel Gene, *MDS2*, Is Fused to *ETV6/TEL* in a t(1;12)(p36.1;p13) in a Patient With Myelodysplastic Syndrome

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ETV6/TEL is the first transcription factor identified that is specifically required for hematopoiesis within the bone marrow. This gene has been found to have multiple fusion partners of which 16 have been cloned. Fluorescence in situ hybridization (FISH) analysis in a patient with myelodysplastic syndrome (MDS) revealed a t(1;12)(p36;p13) involving *ETV6,* with the breakpoint in this gene between exon 2 and exon 3. We report here the cloning of a novel *ETV6* partner located on 1p36.1, involved in the t(1;12). 3′ RACE-PCR from RNA identified a novel sequence fused to exon 2 of *ETV6*. Database searches localized this sequence in a bacterial artificial chromosome (BAC) mapped to 1p36 by fingerprint analysis. This result was confirmed by FISH using this BAC as probe. 5′ and 3′ RACE experiments with primers from this novel sequence were carried out on RNA from a healthy donor and identified a novel full-length mRNA, which we named *MDS2* (myelodysplastic syndrome 2). RT-PCR experiments were performed on a panel of human cDNAs to analyze the expression pattern of this gene and they revealed four splicing variants. RT-PCR analysis showed that *ETV6-MDS2,* but not the reciprocal *MDS2-ETV6* fusion transcript, was expressed in the bone marrow of the patient. The product of the *ETV6-MDS2* fusion transcript predicts a short ETV6 protein containing the first 54 amino acids of ETV6 plus four novel amino acids, lacking both the PTN and the DNA-binding domains. Possible mechanisms to account for the development of MDS in this patient are discussed.

INTRODUCTION

ETV6/TEL is the first transcription factor identified that is specifically required for hematopoiesis within the bone marrow (Wang et al., 1998). This gene is rearranged in half of the patients with 12p13 translocations, in both myeloid and lymphoid leukemias (Sato et al., 1997; Odero et al., 2001). *ETV6* has been found to have multiple fusion partners; using fluorescence in situ hybridization (FISH), more than 40 different chromosome bands have been reported to be involved in *ETV6* translocations (reviewed in Odero et al., 2001), and 16 partner genes have been cloned: *ARNT* (1q21), *ARG* (1q25), *MDS1/EVI1* (3q26), *CHIC2/BTL* (4q12), *ASC2* (5q13), *PDGFRB* (5q33), *STL* (6q23), *MNX1* (7q36), *PAX5* (9p13), *JAK2* (9p24), *SYK* (9q22), *ABL* (9q34), *CDX2* (13q12), *TRKC* (15q25), *CBFA2* (21q22), and *MN1* (22q11) (Golub et al., 1994, 1995; Romana et al., 1994; Buijs et al., 1995; Papadopoulos et al., 1995; Peeters et al., 1997a,b; Suto et al., 1997; Cazzaniga et al., 1999, 2001; Chase et al., 1999; Cools et al., 1999; Eguchi et al., 1999; Yagasaki et al., 1999; Iijima et al., 2000; Salomon-Nguyen et al., 2000; Beverloo et al., 2001; Kuno et al., 2001). The *ETV6* gene codes for a ubiquitously expressed nuclear protein that contains two critical domains, the 5′ pointed (PTN) domain (coded for by exons 3 and 4) and the 3′ E26 transformation-specific (ETS) DNA-binding domain (exons 6–8). The translocation breakpoints are distributed throughout the gene, and *ETV6* contributes to the pathogenesis of leukemia by remarkably diverse molecular mechanisms that are only partially understood. Molecular characterization of chromosomal translocations involving *ETV6* showed that many of them result in the expression of chimeric proteins; the most common involve fusion between the amino-terminal part of *ETV6* and either unrelated transcription factors or protein tyrosine kinases. However, in a number of cases,

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including a t(6;12)(q23;p13) (Suto et al., 1997), the recurrent t(5;12)(q31;p13) (Yagasaki et al., 1999), and some t(4;12)(q11–12;p13) (Cools et al., 2002), functionally significant fusions could not be detected.

We report here the identification and characterization of a novel gene, *MDS2* (myelodysplastic syndrome 2), involved in a rearrangement with *ETV6* in a t(1;12)(p36;p13), in a patient with myelodysplastic syndrome (MDS).

MATERIALS AND METHODS

Case Report

A 66-year-old Caucasian woman was admitted to our institution because of weakness lasting for a year. There were no signs or symptoms at admission, except for the presence of mild gingival bleeding. The patient reported no fever or infectious episodes during the previous weeks, and there was no history of hematological malignancy. Blood count showed Hb 8.8 g/dL, white cell count 3.3×10^9/L with 3% blast cells, and platelet count 104×10^9/L. No liver or kidney dysfunction was detected on serum biochemical determinations. Prothrombin time and activated partial thromboplastin time were within normal ranges. The bone marrow aspirate was hypercellular, showing 23% blast cells and red cell hyperplasia with dysplastic changes in erythroblasts (intercytoplasmatic bridges, binucleated cells, and megaloblastosis). Flow cytometry of bone marrow cells showed that the blast cells were CD34, CD13, CD33, and HLA-DR positive. The patient was diagnosed with MDS (RAEBT). She refused any treatment other than transfusion therapy. Disease evaluation after 6 months showed Hb 8.1 g/dL, white cell count 4.2×10^9/L with 5% blast cells, and platelet count 45×10^9/L. Bone marrow aspiration showed 52% blast cell infiltration with the same characteristics as those observed at diagnosis. Conditions deteriorated further, and the patient died 8 months after diagnosis.

Cytogenetic and FISH analyses of this patient were described previously (Odero et al., 2001). The karyotype showed a single clone with two reciprocal translocations, a t(1;12)(p36;p13) and a t(9;11)(q34;q13). The sample was obtained at relapse, with informed consent. Only fixed cells were available for cytogenetic and molecular studies.

Fluorescence In Situ Hybridization

Cosmid clones for the *ETV6* locus were described previously (Odero et al., 2001). BACs and PACs located on chromosomes 1, 9, and 11 were identified by database searches, obtained from libraries from the Roswell Park Cancer Institute (Buffalo, NY), and used to map the breakpoints in the two translocations present in the patient. Three BACs and three PACs located in chromosome 1 were selected to map the breakpoint on 1p36. Eleven BACs located in chromosome 9 and 10 in chromosome 11 (all from the RPCI-11 library) were used in FISH experiments to map the breakpoint in the t(9;11)(q34;q13). The order of the probes in chromosome 9 is: centromere–696J10–498E2–282P20–545E17–98H23–247A12–492E3–138E2–7M2–17L7–253A1–telomere. The order of the BACs in chromosome 11 is: centromere–77M17–163K24–877I6–669D23–142N13–699D24–259D17–154D10–699M19–300I6–telomere.

Nucleid Acid Isolation

Total RNA was extracted from fixed cells using TriReagent (Molecular Research Center, Cincinnati, OH). cDNA was generated with the First-strand cDNA Synthesis kit (Clontech, Palo Alto, CA) and was used as template for PCR. Previous experiments using fixed cells from the THP-1 and U-937 cell lines were done to confirm the feasibility of the technique and to adjust the concentration of DNA needed in the PCR reactions. The quality of cDNA in the patient sample was confirmed by amplification of the normal *ETV6* gene. In subsequent experiments, RNA was extracted using the GenElute Mammalian Total RNA Isolation kit (Sigma Chemical, St. Louis, MO), and cDNA was prepared using SuperScript II RNase H^- Reverse Transcriptase (Life Technologies, Gaithersburg, MD).

Rapid Amplification of cDNA Ends (RACE) Polymerase Chain Reaction (PCR)

For 3′ RACE, nested PCR was performed using the primers TELex2-F1 and TELex2-F2, both in exon 2 of *ETV6* (3′ RACE System for Rapid Amplification of cDNA Ends; Life Technologies). For 5′ RACE, nested PCR was performed using the primers TELex3-R2 and TELex3-R1 (5′ RACE System for Rapid Amplification of cDNA Ends; Life Technologies). Both 3′ and 5′ RACE in the normal donor were performed using the GeneRacer kit (Invitrogen BV, Groningen, The Netherlands), which selects for RNAs containing the cap structure. Primers NGex6-F1 and NGex6-R1 were used for initial 3′ RACE and 5′ RACE amplifications, and primers NGex6-F2 and NGex6-R2 for the respective nested reactions. These primers

TABLE 1. Oligonucleotide Primer Sequences

Primer	Gene	Oligonucleotide sequences (5′ → 3′)
TELex2-F1	*ETV6*	CAGGAACGAATTTCATATACACCT
TELex2-F2	*ETV6*	CCAGTGCCGAGTTACGCTTCCT
TELex3-R1	*ETV6*	CTTTGGTCAGCAGCAGGAGAGC
TELex3-R2	*ETV6*	CTGAATGAGGAGATCGATAGCG
TELex1-F1	*ETV6*	TCCTGATCTCTCTCGCTG
TELex2-F3	*ETV6*	TCCAGAGAGCCCAGTGCC
NG-R1	*MDS2*	GCGACAGCCAGGTCTTAA
NGex1-F1	*MDS2*	CCGCCTGTATCCGTGCCTGTT
NGex6-F1	*MDS2*	AGCAGTCCAGCTATGAGGGGAGGT
NGex6-R1	*MDS2*	TGGAGGCGACAGCCAGGTCTTAA
NGex6-F2	*MDS2*	GACCCTGGAGAGGCCATTAAGACCT
NGex6-R2	*MDS2*	GTCTTAATGGCCTCTCCAGGGTCAG
RP-F	*RPL11*	AGGTGCGGGAGTTGGAGTTA
RP-R	*RPL11*	TGTCCTGCGCTTCTTGTCTG
ELA-F	*TCEB3*	TCCGGAGGAGGCAGGAAAAGTT
ELA-R	*TCEB3*	CCCCAACCCCCACTGTCCTTGTA
X69111-F	*ID3*	CCCCGGTATCAGCGCTTCCTCATT
X69111-R	*ID3*	CGCCTTCATGCTGGGGAGTGAGT
E2F2-F	*E2F2*	ACGCCAGGGTCGGGGACAGGAA
E2F2-R	*E2F2*	GCACCCAAGGGCCCATCGAAGTC
G3PDH1-F	*G3PDH*	TGAAGGTCGGAGTCAACGGATTTGGT
G3PDH2-R	*G3PDH*	CATGTGGGCCATGAGGTCCACCAC

were carefully designed to be compatible with the primers included in the kit. All primer sequences are shown in Table 1.

Reverse Transcriptase–PCR

RT-PCR reactions were carried out with AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA) after optimizing cycling conditions for each primer pair. To confirm the presence of the fusion product, nested RT-PCR was performed on patient RNA with the TELex1-F1 and TELex2-F3 forward primers in *ETV6* exon 1 and exon 2, respectively, and the NG-R1 reverse primer in the new gene.

Analysis of the expression pattern of *MDS2* was performed by RT-PCR using primers NGex1-F1 and NGex6-R1 on a set of normalized, first-strand cDNA from 16 different human tissues (Multiple Tissue cDNA Panels I and II; Clontech, Basingstoke, UK), following the manufacturer's protocols. These primers are located in exon 1 and exon 6 of *MDS2*, respectively. All amplifications were performed for 35 cycles with an annealing temperature of 60°C.

Semiquantitative RT-PCR

Expression levels of several genes were compared by semiquantitative RT-PCR. For each reaction, serial dilutions of a control cDNA from peripheral blood lymphocytes were included to check that all samples analyzed were within the linear range of the amplification reaction. All reactions were normalized to *G3PDH* expression levels in the same samples (also obtained in the linear phase of amplification) after densitometric analysis of the gels. The primer sequences are shown in Table 1.

DNA Cloning and Sequencing

Products were cloned either into the pMOS (Amersham Pharmacia Biotech UK, Buckinghamshire, UK) or into the pCR 4-TOPO vector (TOPO TA Cloning Kit for Sequencing; Invitrogen BV) and were sequenced with ABI-PRISM d-Rhodamine Terminator Cycle Sequencing Kit (Applied Biosystems) in an ABI 377 DNA Sequencer (Applied Biosystems).

RESULTS

Cloning of the t(1;12)

The karyotype of this patient was described previously (Odero et al., 2001). G-banding, FISH, and spectral karyotyping (SKY) showed a single clone with two translocations: t(1;12)(p36;p13) and t(9;11)(q34;q13) (Fig. 1A). FISH analysis with *ETV6* probes localized the breakpoint in this gene between exon 2 and exon 3 (Odero et al., 2001).

To identify the fusion partner of *ETV6*, 3′ and 5′ RACE-PCR experiments were performed on re-

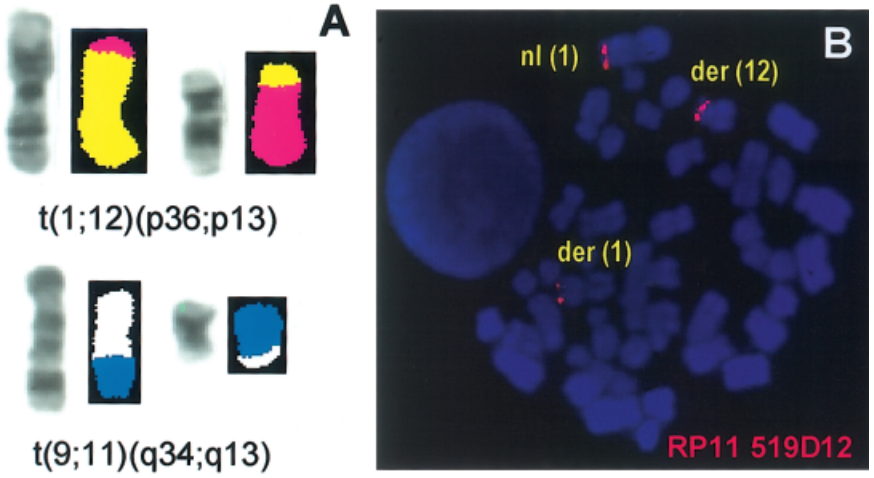

Figure 1. G-banding, SKY, and FISH analyses of the patient. **A:** G-banding and SKY analyses showing the t(1;12) and t(9;11). **B:** RP11 519D12 probe (red) from chromosome 1 labels normal chromosome 1, and both der(1) and der(12).

verse-transcribed RNA obtained from bone marrow of the patient. Because the FISH results had indicated that the breakpoint was between *ETV6* exons 2 and 3, nested primers located in exons 2 and 3 of *ETV6* were used for 3′ and 5′ RACE-PCR experiments, respectively. All 3′ RACE-PCR products were cloned, and 11 clones were sequenced. Six clones represented the normal *ETV6* allele, three clones were possible genomic sequences (exon 2 followed by intron 2), and two clones contained 116 bp of novel sequence immediately after *ETV6* exon 2. BLAST searches against the HTGS database localized this sequence to BAC 223J15 from the RPCI-11 library (accession no. AC068847), which maps to 1p36.1. This finding was consistent with the initial cytogenetic data and revealed that it could be an exon belonging to a novel gene. 5′ RACE-PCR from *ETV6* exon 3 failed to find any novel sequences.

FISH Analysis

To confirm the position of the breakpoint, three BACs and three PACs located on 1p36.1 were selected from RPCI libraries, including clone 223J15. The exact position of the clones along 1p36.1 was still tentative at the beginning of the analysis, given that sequencing of this region by the HGP is in progress. Our FISH studies were consistent with the following order: telomere-RP1–150O5-RP11–519D12-RP11–223J15-RP4–633K13-RP11–509F14-RP3–462O23–centromere.

FISH analysis on bone marrow cells from the patient showed that clone 223J15 hybridized to both the normal chromosome 1 and the der(1), whereas clone 150O5 hybridized to the normal chromosome 1 and the der(12). Clone 519D12, which overlaps with 223J15, was present in the normal chromosome 1 and in both der(1) and der(12), indicating the localization of the breakpoint in this case (Fig. 1B).

To determine whether the breakpoint is recurrent in other cases, we checked samples from 10 patients with hematological malignancies and reciprocal translocations involving 1p36. The clones used as probes were 150O5 and 223J15, which flank the clone that splits in the patient with t(1;12). In all cases, both probes hybridized to the same chromosome, either chromosome 1 or the other chromosome involved in the translocation (data not shown), suggesting that the breakpoints were not within the region covered by the probes.

Eleven BACs on chromosome 9 and 10 BACs on chromosome 11 were used in metaphase FISH experiments, to map the breakpoint in the t(9;11)(q34;q13). The breakpoint in chromosome 9 was mapped to 9q34.13, between RP11 98H23 and 247A12, centromeric to *ABL*. Surprisingly, there were two breakpoints on 11q13.4: 142N13 shows one signal in the normal chromosome 11, whereas the other signal splits and hybridizes to both the der(11) and chromosome 21 (weaker signal); 699D24 shows one signal on normal chromosome 11, and another signal splits on both der(21) and der(9) (weaker signal), indicating that this is a complex translocation involving not only chromosome 21 but also chromosomes 9 and 11 (data not shown). Further studies are required to know the genes involved in this translocation.

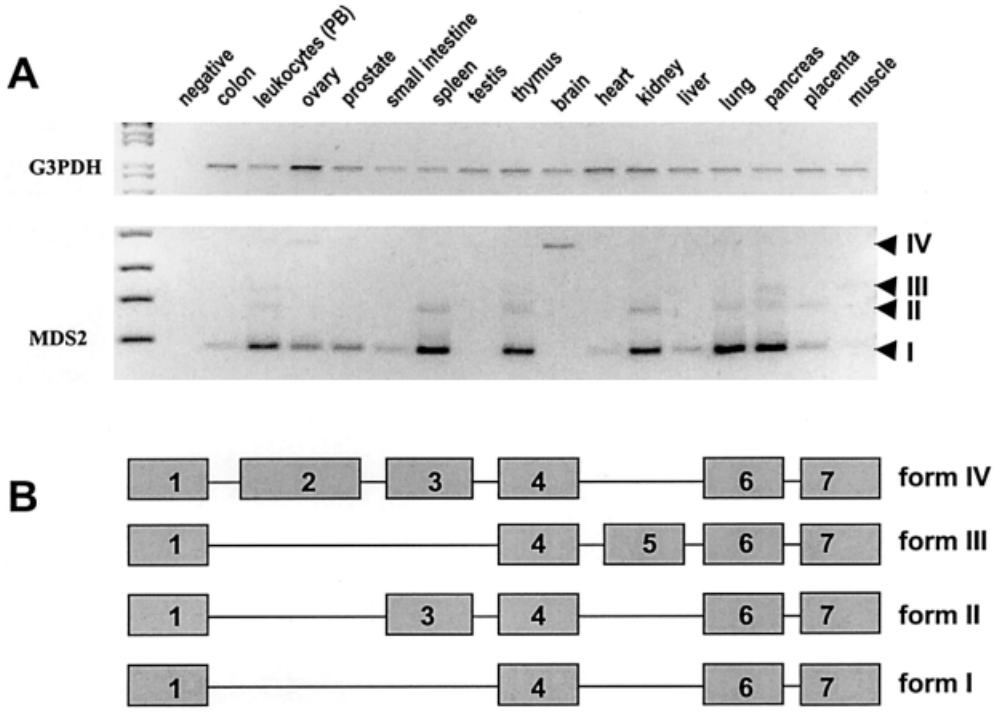

Figure 2. Analysis of the expression of *MDS2* in a panel of human cDNAs. **A:** RT-PCR amplification of *MDS2,* using primers in exon 1 and exon 6, reveals a main band present in most tissues. Minor bands were cloned and sequenced, and shown to represent alternatively spliced forms (arrowheads and roman numerals). **B:** Schematic representation of the four splicing variants detected in A.

Characterizing the New Gene

Our finding that the 116 bp of novel sequence were included in the region of overlap between clone 223J15 and clone 519D12, which splits in the translocation, prompted us to check whether this sequence could be part of a gene, given that alignments with genomic sequences had shown that the novel sequence was flanked by consensus GT and AG splice signals. For this purpose, 5′ and 3′ RACE reactions were performed on RNA from a normal donor, using primers located in the new sequence, and yielded various bands that were cloned and sequenced (not shown). These sequences were assembled and analyzed against the human genome sequence database using various resources, and they also matched the same BAC clone as the original 116-bp sequence. Furthermore, alignment of these sequences with genomic DNA revealed the presence of novel exons, all of them flanked by consensus splicing sequences.

From these experiments, we were able to identify three exons upstream of the original sequence, plus one downstream exon that contained a poly-A tail. The longest 5′ fragment identified an upstream exon flanked by a consensus GT splicing site at its 3′ end but lacking a consensus AG splicing site at its 5′ end. Instead, a canonical TATA box was located 25 bp upstream of the first nucleotide of this exon, indicating that this is probably the first exon of a novel gene, and further pinpointing the location of the putative promoter. The presence of both a first exon and a poly-adenine tail confirmed that the various 5′ RACE and 3′ RACE fragments encompassed a novel, full-length mRNA that we named *MDS2* (myelodysplastic syndrome 2).

MDS2 Gene

We analyzed the expression of this novel mRNA on a panel of human cDNAs using an RT-PCR designed to amplify the 5′ half of the novel gene (primers NGex1-F1 and NGex6-R1). This experiment revealed a major band of 281 bp, which was stronger in peripheral blood leukocytes (PBLs), spleen, thymus, kidney, pancreas, and lung. This band was also present in other tissues, although at lower abundance, and was completely absent in brain and testis (Fig. 2A). Additional bands of weaker intensity were obvious in most tissues; we did a Southern blot of this gel and hybridized the filter with a probe consisting of an internal fragment of the major 281-bp band, from which the primer sequences had been removed. All the minor

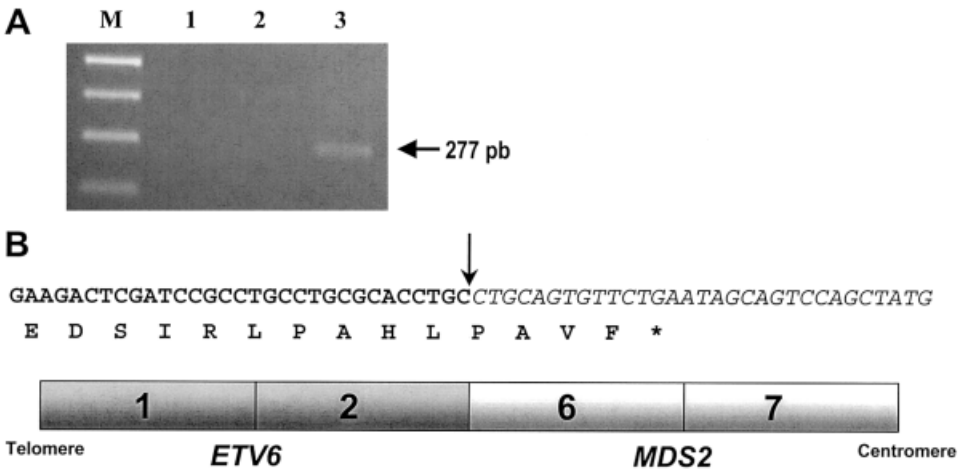

Figure 3. Detection of the *ETV6-MDS2* fusion in the patient. **A:** RT-PCR showing amplification of the fusion product in the bone marrow from the patient (lane 3) but not in normal bone marrow (lane 2). M = 100-bp ladder. Lane 1 = water control. **B:** Schematic representation of the breakpoint (arrow) and the result of the fusion transcript. *ETV6* exon 2 sequence (bold) is followed by *MDS2* exon 6 (italics).

bands hybridized with the probe, indicating that they were specific products, probably representing alternatively spliced forms of the gene. We cloned and sequenced all the minor bands from these reactions and confirmed that they corresponded to alternatively spliced products. This analysis also revealed two new exons that had not been found in the previous RACE experiments (exons 3 and 5) and provided a complete picture of the splicing patterns present in different human tissues.

Putting together all the data from RACE and RT-PCR experiments, we identified a novel gene containing seven exons (accession no. AJ310434). The *MDS2* gene spans approximately 13 kb and is flanked by *E2F2* and *ID3* at its 5′ end (telomeric) and by *RPL11* and *TCEB3* at its 3′ end (centromeric) and gives rise to four alternatively spliced products (Fig. 2B). The major mRNA (referred to as form I) is made up of exons 1, 4, 6, and 7 and is expressed mainly in thymus, spleen, PBLs, pancreas, lung, kidney, and at lower levels in ovary, prostate, liver, small intestine, placenta, colon, and skeletal muscle. It is not expressed in brain and testis. The second most frequently found mRNA (form II) is the result of splicing together of exons 1, 3, 4, 6, and 7. This form was found predominantly in spleen, thymus, and kidney, and at much lower levels in lung, pancreas, placenta, and PBLs. Form III contains exons 1, 4, 5, 6, and 7 and is present in pancreas, skeletal muscle, and PBLs. Form IV is the rarest and largest of all, containing exons 1, 2, 3, 4, 6, and 7; it is present only in brain.

Computer analysis revealed that forms I, II, and IV all have the potential to encode the same peptide of 82 amino acids, starting at an ATG located in exon 6, whereas form III could be translated into a longer protein because of the addition of exon 5. This protein is predicted to have a size of 140 amino acids, including the 82 carboxy-terminal amino acids that are identical to the peptide predicted by the other splicing variants.

RT-PCR Experiments in the Sample From the Patient

Nested RT-PCR reactions were performed on patient RNA with forward primers TELex1-F1 and TELex2-F3 in *ETV6* exon 1 and exon 2, respectively, and reverse primer NG-R1 in exon 6 of *MDS2*. The PCR products were cloned and sequenced, confirming the fusion between exon 2 of *ETV6* and exon 6 of *MDS2* that had been found in the original RACE experiment. The predicted *ETV6-MDS2* fusion product contains 58 amino acids, starting at the first ATG of *ETV6* and ending in a TGA stop codon only four amino acids after *ETV6* exon 2 (Fig. 3). Because the sequences from both genes are not in frame, the fusion transcript encodes the first 54 amino acids of *ETV6*, followed by four additional novel amino acids encoded by *MDS2* sequences. This predicts a truncated ETV6 protein that lacks both the PTN domain and the DNA-binding domain encoded in the C-terminal region of *ETV6*. No RT-PCR amplification products could be obtained using forward primers in *MDS2* exon 6 and reverse primers in *ETV6* exon 4, indicating that the reciprocal *MDS2-ETV6* fusion is probably not expressed in the bone marrow in this patient (data not shown). This would be in agree-

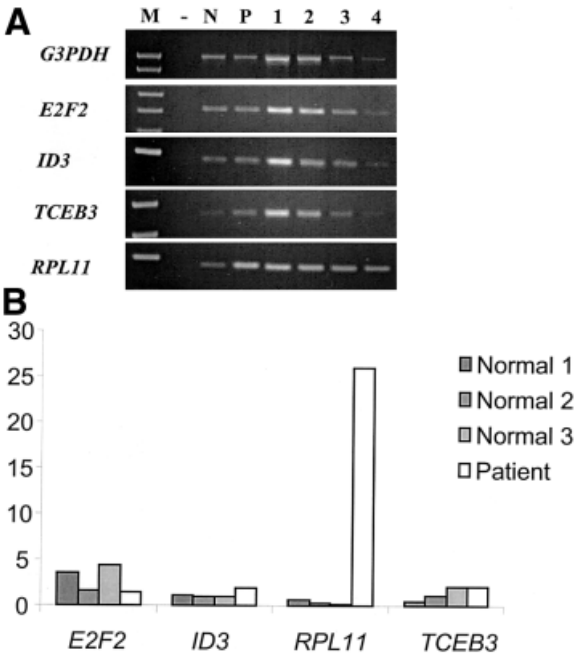

Figure 4. Analysis of the expression levels of genes surrounding *MDS2* in the patient and in three unrelated normal bone marrow samples. **A:** Semiquantitative RT-PCR for *G3PDH, E2F2, ID3, RPL11,* and *TCEB3.* For each PCR, one normal bone marrow (N) and the patient's bone marrow (P) are shown. Lanes 1 to 4 represent serial dilutions of a standard cDNA, showing that all PCR reactions are in the linear phase of amplification. **B:** Graphical representation, including the results obtained for three different normal samples, after densitometric analysis of the gels.

ment with the initial 5′ RACE experiments from *ETV6* exon 3, which failed to identify chimeric transcripts.

Changes in the Expression of Neighboring Genes

Because the t(1;12) did not result in a fusion protein to which an oncogenic function could be attributed, we checked whether the translocation could affect the expression of known genes flanking *MDS2.* We identified candidate genes in the genomic sequence surrounding *MDS2* and designed semiquantitative RT-PCR experiments to compare the expression of *E2F2*, *ID3*, *RPL11*, and *TCEB3* between the patient and three unrelated nontumor samples (all samples were from bone marrow). As shown in Figure 4, the expression of *RPL11* was 26 times higher in the patient than that in controls.

DISCUSSION

We report here the characterization of a novel gene, *MDS2,* fused to *ETV6* in a t(1;12)(p36;p13) in a patient with MDS. The previous mapping of the breakpoint on 12p13 between exon 2 and exon 3 of *ETV6* using FISH (Odero et al., 2001) allowed for the molecular study using fixed cells, the only material that was available. The *MDS2* gene spans around 13 kb and seven exons accounting for the cDNA sequence were identified. RT-PCR experiments on a panel of human cDNAs, to analyze the expression pattern of this gene, showed that it is widely expressed in tissues and further revealed four splicing variants. *MDS2* is located in a GC-rich genomic region that encompasses the terminal 48 Mb of the short arm of chromosome 1. This region is rich in repeated elements, and it is particularly interesting that *MDS2* noncoding exons 1, 2, 3, and 4 are included in consensus SINE or LINE sequences (detected by RepeatMasker), whereas part of exon 7 is embedded in an LTR repeat. The longest open reading frame predicted from *MDS2* has 140 amino acids and shows no significant homology to any known protein.

In the *ETV6-MDS2* fusion, exon 2 of *ETV6* is followed by exon 6 of *MDS2.* This predicts a truncated ETV6 protein that is unlikely to play an important role in the oncogenic process because it lacks both the PTN domain and the ETS DNA-binding domain.

ETV6 is an active repressor of transcription and the ETV6-CBFA2 fusion protein has been suggested to exert its oncogenic properties through a dominant-negative effect on normal CBFA2 function (Lopez et al., 1999; Fenrick et al., 2000). It could be speculated that the ETV6-MDS2 fusion protein interacts with either the *ETV6* gene or the *MDS2* gene. However, the repressor activities seem to be dependent on the integrity of the *ETV6* PNT oligomerization domain and on its self-association properties. Therefore, the fact that the predicted fusion protein lacks both main functional domains of *ETV6* suggests a loss of function rather than a dominant effect.

The role of the fusion protein in the leukemic cells still has to be fully investigated. In the t(1;12) described here, both *ETV6* and *MDS2* genes are expressed from the nontranslocated copies of the gene. Because the reciprocal *MDS2-ETV6* fusion is not expressed, it can be concluded that bone marrow cells from this patient have only one functioning allele of *MDS2.* Haploinsufficiency could account for the leukemogenic process if *MDS2* is a dosage-sensitive gene, but this is unknown at present. Alternatively, the combined loss of one allele of *ETV6* and one allele of *MDS2* could be responsible for the MDS in this patient. *MDS2* could also code for a nontranslated transcript that inhibits the expression of an overlapping gene

through an antisense effect. The short ORF and the lack of strong homology to known protein domains seem to support this hypothesis. In this regard, computer analysis of the region predicts several genes, transcribed from the complementary strand, which overlap the 3′ end of *MDS2*. We are currently analyzing whether any of these predictions represents an expressed sequence.

ETV6 has been shown to be fused to many partner genes, most of them encoding either protein tyrosine kinases or other unrelated transcription factors. However, in a number of cases, including a t(6;12)(q23;p13) (Suto et al., 1997), the recurrent t(5;12)(q31;p13) (Yagasaki et al., 1999), and some t(4;12)(q11–12;p13) (Cools et al., 2002), functionally significant fusions could not be identified. It has been reported that dysregulation of the expression of surrounding genes could be an alternative leukemogenic mechanism for translocations lacking functionally significant fusion transcripts (Cools et al., 2002). With this in mind, we set out to study the expression of genes located around the breakpoint in this patient. Analysis of the April 2001 assembly of the human genome at the UCSC Genome Browser (http://genome.ucsc.edu/) led us to measure the expression of four genes in the bone marrow of the patient and in the bone marrow of three normal subjects. Comparison by semiquantitative RT-PCR revealed that *RPL11*, a gene immediately centromeric to *MDS2* (63.5 kb), shows higher expression levels in the sample of the patient with the t(1;12) than those in normal bone marrow samples. *RPL11* encodes a ribosomal protein that is a component of the 60S subunit and belongs to the L5P family of ribosomal proteins. As is typical for genes encoding ribosomal proteins, there are multiple processed pseudogenes of this gene dispersed through the genome. Although the role that *RPL11* overexpression may play in the development of MDS is not clear at present, our findings support the notion that at least part of the pathogenic process initiated by the translocation could be the result of position effects, placing surrounding genes in a different chromatin context and thereby modifying their expression levels.

MDS2 does not seem to be a gene frequently involved in 1p36 rearrangements. FISH analysis of a further 10 patients with hematological malignancies and reciprocal translocations involving 1p36 showed that the breakpoints on this region are distributed throughout this band, and we found no breakpoints within the *MDS2* locus in any of the cases.

The other translocation found in the patient clone probably is important for the malignant transformation. Using FISH, we showed that this is a complex translocation involving chromosomes 9, 11, and 21. We localized the breakpoint in 9q34.13, between the clones RP11 98H23 and RP11 247A12; although the distance between these two BACs is less than 100 kb, there are several candidate genes in this region. The two breakpoints in chromosome 11 are in 11q13.4. There are two candidate genes in this region, *C11ORF23* and *LRP5*, a low-density lipoprotein receptor-related protein 7.

In conclusion, a novel *ETV6* partner gene, *MDS2*, located in 1p36.1 is reported. Determining the function of this gene will help to delineate the pathogenic mechanisms triggered by this translocation. Likewise, the changes in the expression of surrounding genes that we have shown support the notion that position effects could be partly responsible for the development of MDS in this patient.

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