Fusion of *ETV6* to *GOT1* in a case with myelodysplastic syndrome and t(10;12)(q24;p13)

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The *ETV6* gene on 12p13 is involved in different hematologic malignancies through a variety of chromosomal translocations. Here we report the analysis of a t(10;12)(q24;p13) identified in a patient with myelodysplastic syndrome. Our results show that the t(10;12) results in the generation of an in-frame fusion between *ETV6* and GOT1, a gene encoding a cytosolic glutamic-oxaloacetic transaminase enzyme. In addition, two other fusion transcripts involving exons from yet unidentified genes were detected, as well as expression of an uncharacterized gene from the 10q24 region. This work identifies *GOT1* as a novel fusion partner of *ETV6* in myelodysplastic syndrome.

Key words: fusion gene, leukemia, translocation, GOT1, NKX2-3.

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Molecular analyses of translocations involving the *ETV6* gene in various hematologic malignancies have shown that these translocations can have different consequences. The majority of translocations involving *ETV6* result in the generation of fusion genes, encoding chimeric tyrosine kinases or chimeric transcription factors, directly linking these translocations to clear oncogenic events. Important examples include the t(5;12)(q33;p13) with the *ETV6-PDGFRB* fusion, the t(9;12)(p24;p13) with the *ETV6-JAK2* fusion, and the *ETV6-RUNX1* fusion associated with t(12;21)(p13;q22).[1-3] In a small number of translocations with breakpoints in introns 1 or 2 of *ETV6*, the primary oncogenic event is believed to be the deregulated expression of genes on the partner chromosomes, due to a promoter swap or the effect of enhancers within the *ETV6* gene. This was described for cases with t(5;12)(q31;p13) with deregulated *IL3* expression, t(4;12)(q12;p13) with deregulated GSH2, and t(3;12)(q26;p13) with *ETV6-MDS1/EVI1* expression.[4,5] We describe here the molecular findings in a case with myelodysplastic syndrome (MDS) and a t(10;12)(q24;p13).

Design and Methods

Case report

The patient (a 77-year old male) presented with signs of MDS at the Institute of Hematology in Perugia (Italy) and was further diagnosed as having refractory anemia (RA) type MDS, according to the French-American-British classification. His disease was characterized by pancytopenia and 2% of blasts in the bone marrow. Bone marrow eosinophilia was present from the beginning, with 3% eosinophils in the peripheral blood. Peripheral blood monocytes were present at diagnosis (5%) and had increased to 20% by 20 months later, when blast cells increased to 25%. Cytogenetics, performed at the time of diagnosis (RA) and 2 years later when evolution to RA with excess blasts in transformation (RAEB-t) was observed, indicated the presence of a t(10;12)(q24;p13) as the sole chromosomal aberration in the majority of cells analyzed.

Fluorescence in situ hybridization (FISH) and molecular analysis

FISH was performed as described previously.[6] RACE experiments were performed with the primers ETV6F1b (5'-ACTCCTGCTCAGTGTAGCATTAAG) and ETV6F2 (5'-CCTCCAGAGAGCCCAGTGCCGAGT) for 3'-RACE, and the primers ETV6R4 (5'-CCAGGGTGGAAGAATG) and ETV6R3b (5'-TCCCTGCTCCAGTAAATTGGCTGCAAG) for 5'-RACE, in combination with the general RACE primers, as described previously.[7] Taqman polymerase chain reactioin (PCR) was performed as described previously.[4] The sequences were submitted to Genbank with accession numbers: DQ372721, DQ372722, DQ372723, and DQ372724.

Results and Discussion

We studied a patient with MDS and a t(10;12)(q24;p13) as the single chromosomal abnormality. Two other cases of a t(10;12)(q24;p12) were reported in chronic myeloid leukemia and acute lymphoblastic leukemia, but no molecular studies were performed.[8,9] In our case, FISH analysis using probes spanning the *ETV6* gene confirmed that *ETV6* was interrupted by the translocation in intron 2.[6] To identify the possible fusion

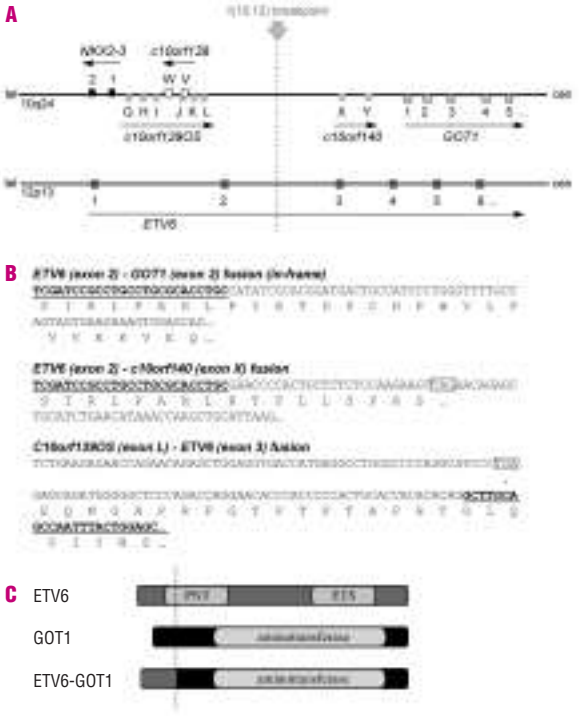

Figure 1. Schematic representation of the 10q24 and 12p13 regions, and sequence of the fusion transcripts. A. Schematic representation of the 10q24 and 12p13 regions. Exons are indicated by boxes and numbered. The orientation of the genes is indicated by the arrows. B. Different fusion transcripts were identified in this patient. Only *ETV6-GOT1* is an in-frame fusion. Sequences from *ETV6* are underlined. Stop codons are boxed. C. Schematic representation of the ETV6, GOT1 and ETV6-GOT1 proteins.

Table 1. Exon structure of the putative genes identified on chromosome 10.

The exon-intron boundaries of the c10orf139 gene

exon	*3'-end of the previous intron*		*exon (bp)*		*5'-end of the next Intron*
V	AGGGAAAC**AG**	GTGGGGAG	258	CTCAAGAG	**GT**AAGAAG
W	TCGTTCTC**AG**	ACGGTTTG	476	ACAGGAAA	–

The exon-intron boundaries of the c10orf139OS gene

exon	*3'-end of the previous intron*		*exon (bp)*		*5'-end of the next Intron*
G	CAAAACCC**AG**	TACTAAGGGT	(170)	AAACCAGGCG	**GT**GAGTCTGC
H	TAAATTAC**AG**	GTCCTTGCTG	(95)	AGATAAAAAG	**GT**AAATTACC
I	TCTCTTCC**AG**	CGCTATCTAC	(316)	GGACGGCCAG	**GT**CTGCGGGC
J	nd	nd	(>148)	CCCGCACAAG	**GT**GGGCCTTT
K	TGACCCTC**AG**	GGCTGCTCAG	(72)	GTTCCAACTG	**GT**AAGACCTC
L	ATTGTGGC**AG**	TCTGAAGAGA	(117)	CCACACACAG	**GT**GAGAGCAA

nd: not determined; Splicing in spleen: GHIKL; splicing in the t(10;12) case: JKL-ETV6 (exon 3).

partner of *ETV6*, we performed RACE experiments, and identified two fusion transcripts when using 3'-RACE, and one fusion transcript when using 5'-RACE (Figure 1). One of these fusion transcripts encoded an in-frame fusion between the first two exons of *ETV6* and exon 2 to exon 9 of *GOT1* (glutamic-oxaloacetic transaminase 1) (Figure 1B,C). *GOT1* encodes the cytosolic glutamic-oxaloacetic transaminase enzyme, involved in amino acid metabolism and gluconeogenesis.[10] The other fusion transcripts that were identified contained novel sequences fused to *ETV6*, but these transcripts (*ETV6-XY, ETV6-Y, GJKL-ETV6*) all contained stop codons in the three reading frames, and do not represent in-frame fusions (Figure 1B). These sequences did not show any significant similarity to known sequences, but were present in the genomic sequence directly upstream of the *GOT1* locus at 10q24 (Figure 1A), and are likely to represent exons, as these were flanked by correct splice sites (Table 1). Based on the fusion transcripts, the breakpoint on 10q24 could be mapped to a 100 kb region between *GOT1* and *NKX2-3* (NK2 transcription factor related, locus 3). In addition to *NKX2-3*, an uncharacterized gene (*c10orf139*) is also present directly telomeric to the breakpoint (Figure 1A). An 800 bp cDNA sequence of *c10orf139* was cloned from the spleen, but showed no similarity to any characterized gene. Two additional putative genes were mapped to this region, namely *c10orf139OS* (opposite to *c10orf139*) and *c10orf140*. These putative genes were fused to *ETV6* (Figure 1B). Expression analysis of *c10orf139* and *c10orf139OS* in a variety of tissues and cell lines revealed highest expression in spleen, and undetectable expression in bone marrow cells (*data not shown*).

GOT1, the cytosolic glutamic-oxaloacetic transaminase enzyme,[10] is involved in amino acid metabolism catalyzing the substitution of glutamate to α-ketoglutarate and vice versa by a transaminase reaction. The exact consequences of the *ETV6-GOT1* fusion does, however, remain unclear. The GOT1 enzyme is functional in a dimeric state,[11] in which the free N-terminal part of one of the molecules in the GOT1-GOT1 dimer is required for enzymatic activity.[12] With respect to this, it is possible that the ETV6-GOT1 protein, only lacking a short N-terminal part of GOT1, can still form heterodimers with wild type GOT1, thereby acting as a dominant negative form, resulting in a reduction of GOT1 enzymatic activity in dysplastic cells. Although a link between amino acid metabolism and differentiation of hematopoietic cells has been demonstrated *in vitro*,[13] a clear causative link between the enzymes involved in amino acid metabolism and the generation of hematologic malignancies remains to be investigated. In analogy with previously reported translocations, a major consequence of the t(10;12) could also be deregulation of the expression of the *NKX2-3* homeobox gene or any of the other uncharacterized genes.[4,14,15] To investigate the latter possibility, expression of these transcripts was investigated by quantitative PCR. *NKX2-3* expression was detected at similar levels in the MDS sample as in a control bone marrow sample. *NKX2-3* expression was also detected in spleen, peripheral blood, and all subpopulations of bone marrow obtained by flow sorting, indicating that this gene is expressed at all stages of hematopoiesis (*data not shown*). *NKX2-3* is a

Table 2. Expression levels of *NKX2-3* and *c10orf139* in controls and material from the patient.

tissue	C_T NKX2-3	C_T GAPD expression	ΔC_T	fold	C_T C10orf139	C_T GAPD	ΔC_T	fold expression
BM	24.8	19.9	4.9	1	36.5	19.4	17.1	1
PB	nd	nd	nd	nd	40	22.1	no expr	–
spleen	25.2	19.4	5.8	0.5	32	23.3	8.7	338
t(10;12)	24.1	18.5	5.6	0.6	28.0	20.3	7.7	675
water	40	40	no expr	–	40	40	no expr	–

BM: bone marrow; PB: peripheral blood. Fold expression is compared relative to bone marrow. One representative result out of more than three independent experiments is shown. CT: threshold cycle; ΔCT: difference in CT between NKX2-3 (or c10orf139) and GAPD. Nd, not determined. No expr, no expression.

homeobox gene required for the homing of lymphocytes to the spleen and mucosa-associated lymphoid tissue.[16] Our results were not suggestive of deregulation of *NKX2-3* as a consequence of the translocation. Unlike *NKX2-3*, *c10orf139* expression was detected at very low levels in control bone marrow, with significantly higher expression in spleen (Table 2). Interestingly, expression of *c10orf139* was highly elevated in the MDS sample with the t(10;12), compared to in control bone marrow (table 2). Whether this reflects an upregulation of *c10orf139* or the expansion of a rare bone marrow cell type that expresses this gene cannot be directly concluded from our experiments.

Finally, the potential role of inactivation of *ETV6* needs to be considered. The 12p13 region is frequently deleted in hematologic malignancies, and *ETV6* has been suggested as a candidate tumor suppressor gene.[17] In addition, mutations and loss of expression of ETV6 were recently identified in acute myeloid leukemias.[18] Thus, the consequence of the t(10;12) could also be inactivation of *ETV6*. Additional work is required to determine the functional consequences of the *ETV6-GOT1* fusion and *c10orf139* expression in MDS. Molecular analysis on additional cases with t(10;12)(q24;p13) may also bring new insights into the importance of these different aberrations.

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