ETV6/GOT1 fusion in a case of t(10;12)(q24;p13)-positive myelodysplastic syndrome

The *ETV6/GOT1* fusion, resulting from t(10;12)(q24;p13), has been recently described in a myelodysplastic syndrome. We reported a second case of t(10;12)-positive myelodysplastic syndrome in whom fluorescent *in situ* hybridization confirmed the non-random translocation but molecular biology analyses revealed a *ETV6/GOT1* chimera varying from the first case described.

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ETV6 (ets variant gene 6) is frequently rearranged in both myeloid and lymphoid hematologic malignancies and to date, 24 translocation partner genes with fusion transcripts have been identified.[1-5] These partners are heterogeneous and include kinases as well as transcription factors. Janssen *et al*.[6] recently reported a t(10;12)(q24;p13) in MDS leading to an *ETV6-GOT1* fusion. *GOT1*, glutaminic-oxaloacetic transaminase 1, encoding for a cytosolic enzyme, is involved in amino acid metabolism and in the urea and tricarboxylic acid cycles (Genbank Accession number NM_002079). The consequences of the *ETV6-GOT1* fusion have not yet been established.

We present here a second case of t(10;12)(q24;p13) suggesting a recurrent translocation in MDS, with a breakage hot spot on chromosome 10.

A 71 year old woman with chronic macrocytosis was admitted for pancytopenia. Blood analysis showed the following: hemoglobin 89 g/L, platelets 157×10^9/L and white blood cell count 1.3×10^9/L with 52% neutrophils, 45% lymphocytes, 3% monocytes, no circulating blasts. The bone marrow showed 10% blasts establishing the diagnosis of refractory anemia with excess blasts (RAEB). Five months later, she was admitted with a fever. A bone marrow evaluation confirmed the transformation to an acute myeloid leukemia (AML) M1 according to the FAB classification. The patient died five days later.

At diagnosis, karyotype revealed 46,XX,del(5)(q13q34)[11]/46,idem,t(10;12)(q24;p13)[5]/46,XX[6]. The cytogenetic analysis at transformation displayed:46,XX,der(2)t(2;11)(q34;q14~21),del(5)(q13q34),idic(8)(p12),t(10;12)(q24;p13)[22].

As this case was very similar to the one previously described,[6] we tested the hypothesis of a fusion involving *ETV6* and *GOT1* genes. Hybridization with the *ETV6*/AML1 probe (Abbott), which covers the SAM domain of *ETV6*, and BAC RP11-441O15 (RZPD, Berlin, Germany) for the *GOT1* locus, confirmed fusion signals of *ETV6* and *GOT1* on both derivative chromosomes 12 and 10 (Figure 1A).

A semi-nested RT-PCR was realized, using exon 2 *ETV6* forward primers (5'-ACACCTCCAGAGAGCCCAGT-3' and 5'-TCAGGATGGAGGAAGACTCGA-3', NM_001987) in combination with an exon 3 *GOT1* reverse primer (5'-CACATAGACAGGTGTGTTCTTGT-3', NM_002079). The result of the amplification is shown in Figure 1B. Two strong bands were obtained in the patient sample, contrasting with the unspecific amplification detected in the REC cell line. Sequence analysis of the smaller PCR product showed a transcript encoding an in-frame fusion between *ETV6* exon 3 and *GOT1* exon 2 (in the previous published case, *ETV6* exon 2 was fused to *GOT1* exon 2)(sequence references Ensembl[7]). The larger band was composed of the same *ETV6* sequences fused to genomic sequences of chromosome 10 due to a cryptic splice acceptor site. The location of this site was 22 kb telomeric to *GOT1* and 79 kb centromeric to *NKX2-3* (Figure 2). This 10q24 breakpoint, very close to that of the already published case, was

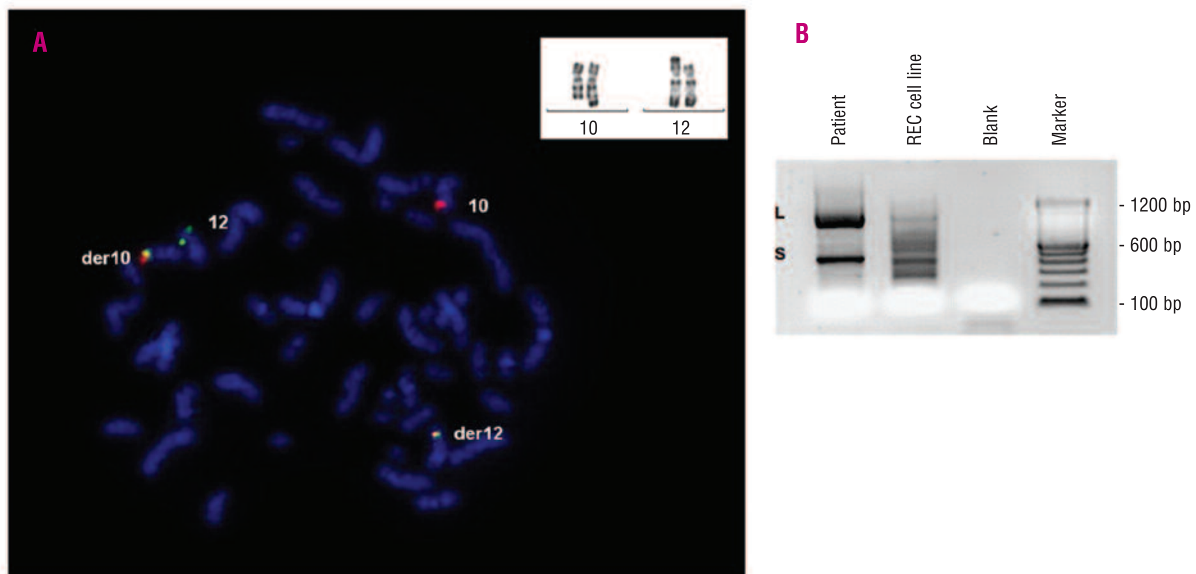

Figure 1. Characterization of the ETV6-GOT1 fusion: (A) Partial RHG bands showed a t(10;12)(q24;p13). Hybridization of ETV6 probe in green and RP11-441O15 (GOT1) in red showed two fusion signals on both derivative chromosomes. (B) cDNA obtained from cytogenetic pellet was amplified using primers located in exon 2 of ETV6 and exon 3 of GOT1. A semi-nested RT-PCR was performed in order to increase the specificity and the amplification of low expressed transcripts. The smaller band (S) corresponded to the ETV6-GOT1 fusion transcript. Sequence analysis of the larger band (L) identified a chimeric transcript containing ETV6 sequence fused to genomic sequences located at 22961 bp 3' to GOT1 and 79407 bp 5' to NK2 transcription factor related, locus 3 (NKX2-3).

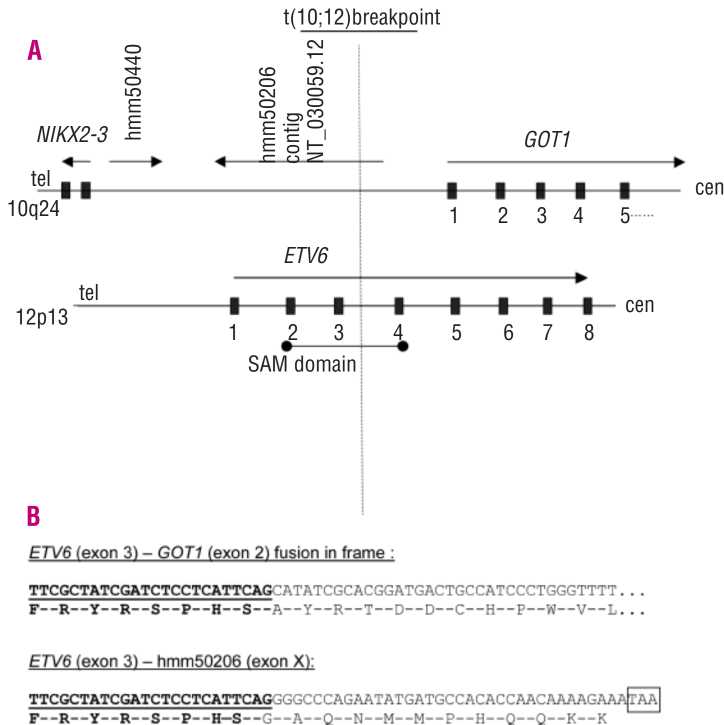

Figure 2. Schematic representation of 10q24 and 12p13 regions implicated in the t(10;12) and transcripts identified. (A) Genomic representation of t(10;12). Only exons of known genes are indicated in boxes. Orientation of the genes are indicated by arrows and breakpoint by dotted line. The Sterile Alpha Motif (SAM) domain (exons 2 to 4), responsible for hetero- and homodimerization with other ETV6 proteins and possibly other ETS family members, is indicated. (B) Transcripts identified. ETV6 sequences are in bold and underlined, and stop codon is boxed. No reciprocal transcript was identified.

located within the hmm50206 putative transcript, predicted by Gnomon algorithm gene prediction analysis on genomic contig NT_030059.12. The corresponding chimeric protein stopped after 11 aminoacids due to a stop codon in the coding sequence. We also verified the reciprocal transcript using exon 4 *ETV6* primer (5'-CGAGGTTTCCTCTGCTTCAG-3') and exon 1 *GOT1* primer (5'-ATATGGCACCTCCGTCAGTC-3'). As expected, RT-PCR did not detect the reciprocal *GOT1-ETV6*. Although the del(5q) was the first abnormality, we can hypothesize that the appearance of t(10;12) in a sub-clone had an accelerator effect on the MDS. During the AML phase, the t(10;12) was observed in all metaphases, the others abnormalities attesting to the acceleration. The chimeric *ETV6-GOT1* predicted protein is composed of a N-terminal part of *ETV6* containing the SAM domain, inducing oligomerization, and the quasi totality of *GOT1*. Only a short N-terminal part of *GOT1* is lacking, necessary for enzymatic activity as described in the only previous case published.[6] The resulting protein can still form homo- or heterodimers that may act as a dominant negative form for *GOT1* but also for *ETV6*. The SAM domain of *ETV6* may result in the inactivation of the protein, and so contribute to leukemogenesis as suggested in acute myeloid leukemia.[8] Another consequence of this translocation might be the transcriptional deregulation of the homeobox gene NKX2-3, which lies adjacent to the breakpoint. The case reported here confirms the presence of a chromosomal breakage hot spot in chromosome 10 and the recurrence of the t(10;12)(q24;p13) in MDS-related AML. Attention should be paid to identify this abnormality in such cases in order to evaluate its frequency and its clinical implications.

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