A case of acute myeloid leukemia with inv(16)(p13q22) reveals a novel MYH11 breakpoint and a new CBFβ-MYH11 transcript variant

We present a case of acute myeloid leukemia (AML) with a cytogenetically typical inv(16)(p13q22), M4 morphology and eosinophilia. However, studies revealed a CBFβ-MYH11 fusion transcript which did not correspond to any of the 10 known variants. Subsequent sequencing revealed a new in-frame transcript variant resulting from a novel MYH11 exon 32 breakpoint and a seven base insertion at the fusion point. The patient remains in complete remission following standard protocols. Prognostic implications cannot, therefore, be evaluated.

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The pericentric inversion of chromosome 16 (inv(16)(p13q22)) and the related t(16;16)(p13;q22) are among the most common rearrangements in acute myeloid leukemia (AML). They are closely associated with AML type M4 with abnormal bone marrow eosinophils and a favourable clinical course.[1]

The inversion generates an in-frame chimeric fusion gene formed from 5' sequence from the core binding factor β gene (CBFβ) at 16q22 with 3' sequence from the myosin heavy chain gene (MYH11) at 16p13[2]. Literature describes 10 possible CBFβ-MYH11 transcripts (types A to J). Eight are in-frame and involve 2 CBFβ breakpoints (at nt 495 or 399) and 8 MYH11 breakpoints (at nt 1921, 1528, 1201, 994, 1098, 1591, 2143 or 1306).[3,4] Type A is the most frequent, being found in > 80% of cases and Types D and E in approximately 5%. Other transcript variants are rare.[5]

We describe a new CBFβ-MYH11 transcript involving a novel MYH11 breakpoint within exon 32 (previously numbered exon 11[3]) in a case of AML.

A 49-year old female presented with a 10 day history of purpura and a 24 hour history of gum bleeding. The full blood count revealed hemoglobin of 9.7g/dL, a white cell count of 50×10^9/ L, neutrophil count of 1×10^9 and a platelet count of 7×10^{12}/L. Peripheral blood showed an excess of blasts and eosinophils. The bone marrow aspirate was grossly hypercellular with normal hematopoiesis replaced by a population of blasts, accounting for 47% of nucleated cells and eosinophils, and their precursors, accounting for 44% of nucleated cells. No Auer rods were seen although some blasts contained fine granules. Cytochemically, the blasts were positive for Sudan black and chloracetate esterase and 25% of nucleated marrow cells showed weak staining with non-specific esterase. Immunophenotyping showed positivity for myeloperoxidase, CD33, CD34, HLA-DR, CD117, CD13, CD64 and CD15, and B and T cell markers were negative. In addition, reticulin staining was diffusely increased at grade 3. Given this, a diagnosis of AMMLeo was made.

The patient was randomized on the MRC AML 15 trial to receive induction therapy with daunorubicin and ara-C (3+10). The neutrophil count recovered 15 days after finishing the first course of chemotherapy and the patient is currently in complete morphologic and genetic remission. Cytogenetic analysis of diagnostic bone marrow revealed a typical inv(16)(p13q22) as the sole abnormality and fluorescent *in situ* hybridization (FISH) using a CBFβ break-apart probe (Vysis, UK) showed a signal pattern consistent with a CBFβ rearrangement with deletion of 3' sequence. This has been reported in some inv(16) cases with no apparent clinical impact.[6,7] CBFβ-MYH11 fusion transcript was confirmed by an established qualitative reverse transcriptase polymerase chain reaction (RT-PCR) method.[8] This generated an unexpected 550 bp product inconsistent with any of the recognised MYH11 or CBFβ breakpoint variants. Direct sequencing showed a homogeneous product made up of an in-frame fusion transcript with a novel MYH11 breakpoint within exon 32 at nt 1795 (*www.ensembl.org* accession # OTTHUMT00000155641) plus the insertion of a TTTAATT sequence at the fusion point (Figure 1), with the typical CBFβ breakpoint at nt 495. The homogeneous nature of the PCR product strongly suggested a genomic breakpoint variant rather than an alternatively spliced product.

Figure 1. Sequence of the novel CBFβ-MYH11 transcript breakpoint region showing the insertion of additional bases at the fusion junction (in bold).

Figure 2. Genomic sequence of MYH11 exon 32 with putative heptamer and nonamer sites underlined. The corresponding sequences from the IgH locus (in italics) are aligned to them, with homologous bases in bold. The genomic breakpoint is indicated by an arrow.

Van Reijden *et al.*[9] reported the insertion of short, apparently random sequences at the genomic fusion points in 2 out of 24 cases[9] together with possible immunoglobulin heavy chain locus (IgH) V(D)J recombinase recognition sites adjacent to many of the genomic MYH11 breakpoints. Their conclusion was that V(D)J recombinase-mediated recombination may be responsible for some CBFβ-MYH11 rearrangements. As we also found an inserted junctional sequence, the published germline MYH11 exon 32 sequence was examined and revealed both heptamer and nonamer upstream sequences with >70% homology to known sequences from the human IgH locus[10] (Figure 2). Furthermore, they were separated by a 23 bp interval in keeping with the organisation of V(D)J rearrangement events.[10] It should be underlined that the evidence is interesting but largely conjectural and further studies would be needed to fully clarify any mechanism.

Although the case we describe expressed a new variant CBFβ-MYH11 transcript, the patient presented with a typical AML M4eo clinical picture and a typical inv(16) as

a sole abnormality. Response to therapy was good and the patient entered and remains in full clinical and cytogenetic remission. The study by Schnittger *et al.*[10] suggested that rare variant CBFβ-MYH11 cases may be biologically distinct from typical inv(16) AML but this appears not to be the case. Longer follow-up will be needed before we can draw any conclusions on the impact of this particular transcript variant.

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