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Open

High-throughput sequencing identifies an *NFIA/CBFA2T3* fusion gene in acute erythroid leukemia with t(1;16)(p31;q24)

In a previous publication of ours[1] we showed the involvement of the myeloid translocation gene-related protein 2 gene (*CBFA2T3*) in a case of acute erythroid leukemia with the translocation t(1;16)(p31;q24). Because of lack of material available for analysis, we could not with certainty determine the leukemogenic mechanism, whether it be generation of a fusion gene with *CBFA2T3* as one of the partners or loss of tumor suppressor activity, in which case genes *KANK1* and *L1TD1*, both homozygously lost, might be of the essence.

Some months after our article was published we got hold of 2 ml coagulated blood with 3% of abnormal cells from the same patient. As sequencing technology has also developed very fast lately, we decided to extract RNA from the sample and try to use it for high-throughput sequence analysis. The RNA was extracted and its quality checked by the Experion automated electrophoresis system (Bio-Rad Laboratories, Hercules, CA, USA). A total of 3 µg of RNA was sent for high-throughput pair-end RNA-sequencing to the Norwegian Sequencing Center at the Ullevål Hospital (http://www.sequencing.uio.no/). The Illumina software pipeline was used to process image data into raw sequencing data and only sequence reads marked as 'passed filtering' were used in the downstream data analysis. A total of 107 million reads were obtained. The FASTQC software was used for quality control of the raw sequence data (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). We used the fusion discovery software FusionMap (release date 16-04-2012)[2] and the pre-built Human B37 and RefGene from the FusionMap website (http://www.omicsoft.com/fusionmap/). A list of over 500 possible fusion genes was obtained. A specific fusion involving the *CBFA2T3* gene, which maps to chromosome band 16q24, and the nuclear factor I/A (*NFIA*) gene, which maps to chromosome band 1p31, was identified as number 10 in the list (seed count-rank 48; Supplementary Table 1). The involvement of the *NFIA* gene fits well with the fluorescence *in situ* hybridization (FISH) data on chromosome 1 previously obtained and published.[1] The presence of the *NFIA/CBFA2T3* fusion was verified by PCR using the NFIA-956 F and CBFA2T3–622 R primer combination (Supplementary Table 2). A specific PCR product of about 500 bp was identified and directly sequenced (Figure 1a). The specific fusion occurs between exon 6 of the *NFIA* gene

(accession number NM_001134673.3) and exon 3 of the *CBFA2T3* gene (accession number NM_005187.5; Figure 1b).

The *NFIA/CBFA2T3* fusion gave an open reading frame and is expected to lead to a chimeric protein containing 208 amino-acid residues from NFIA (according to NP_001128145.1) and 603 residues from CBFA2T3 (according to NP_005178.4). The predicted fusion protein should thus consist of 811 amino acids (Figure 1c).

The *NFIA* gene encodes a member of the NFI family of transcription factors (http://genome.ucsc.edu). Interestingly, it has been found that *NFIA* exhibits a marked lineage-specific expression pattern in normal human hematopoiesis; it is upregulated in the erythroid lineage but fully suppressed in granulocytopoiesis.[3] It has been shown that in early hematopoiesis, the NFIA expression level acts as a factor channeling hematopoietic progenitor cells into either the erythroid or granulocytopoietic lineage.[3] The NFI proteins have a DNA-binding and dimerization domain in their N-terminal half, which contains four cysteine residues, and a transactivation and repression domain in their C-terminal half.[4] The *NFIA* gene was found involved in an *NFIA/EHF* chimeric fusion in one breast cancer cell line out of 24 breast tumors analyzed (nine cell lines and 15 primary tumors).[5] However, its role as either a passenger event or a direct, albeit infrequent, contributor to breast cancer development remains uncertain.

CBFA2T3 encodes an ETO myeloid translocation gene family protein, which interacts with DNA-bound transcription factors and recruits a variety of corepressors to facilitate transcriptional repression.[6–8] The t(16;21)(q24;q22) translocation is one of the less common karyotypic abnormalities specifically associated with acute myeloid leukemia (AML). The translocation produces a chimeric gene made up of the 5′-region of the runt-related transcription factor 1 (*RUNX1*) gene fused to the 3′-region of *CBFA2T3* (Figure 1d). In AMLs with either t(8;21) or t(16;21), the transcription factor RUNX1 is juxtaposed to one of the zinc finger nuclear proteins CBFA2T1 and CBFA2T3, respectively, resulting in transcriptional repression of *RUNX1* target genes.[6] Lately, its involvement as a partner in fusion genes was underlined by the identification of a *IGH/CBFA2T3* fusion in a case of Burkitt lymphoma and a diffuse large B-cell lymphoma.[9] This gene is also a putative breast tumor suppressor.[10,11] Interestingly, *CBFA2T3* is downregulated during erythroid differentiation, and it has been
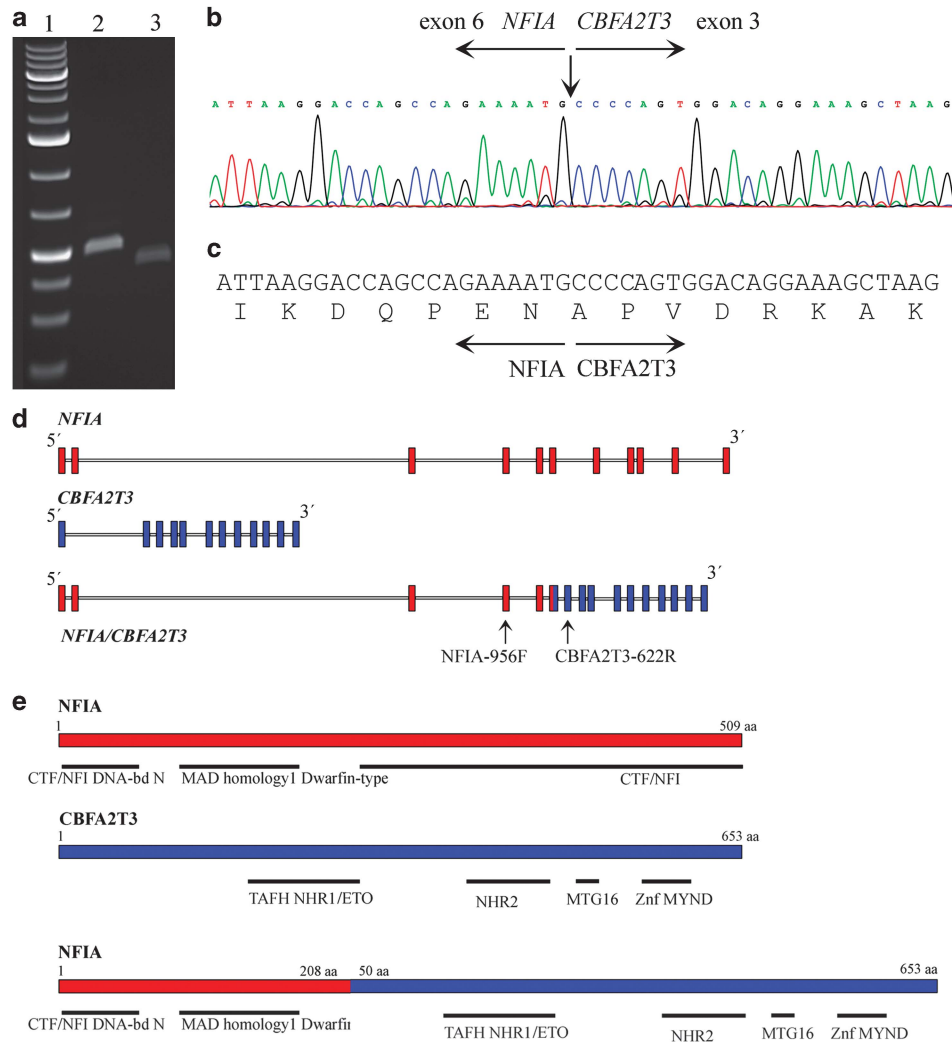
Figure 1. Detection of the *NFIA/CBFA2T3* fusion. (**a**) Gel picture showing the amplified fragment. Lane 1: ladder, lane 2: PCR product obtained with primer combination NFIA-924 F and CBFA2T3–622 R, lane 3: product of the NESTED-PCR obtained with primer combination NFIA-956 F and CBFA2T3–598 R. (**b**) Partial chromatogram showing the junction of the *NFIA* and *CBFA2T3* genes. (**c**) Deduced amino acid sequence of the fusion transcript. (**d**) Schematic overview of the breakpoint region of the *NFIA* and *CBFA2T3* genes. The exons are not in scale. Arrows point to primer positions. (**e**) Schematic overview of the position of the different domains of the NFIA and CBFA2T3 proteins and the NFIA/CBFA2T3 chimeric protein, according to ensembl (http://www.ensembl.org/index.html).

suggested to have a repressive role in early, as well as late human erythroid differentiation.[12] Hildebrand *et al.*[6] demonstrated that the nuclear protein ETO (eight-twenty-one, a family to which also CBFA2T1, CBFA2T2 and CBFA2T3 belong) does not show reduced repressor activity even if it lacks the first 236 amino acids. As in the present fusion the altered CBFA2T3 protein lacks only the first 50 amino acids, we assume that its repressor activity is still retained (Figure 1e). More specifically, we hypothesize a pathogenetic parallel between AML showing a t(8;21) or t(16;21) and the present erythroleukemia with the 1;16-translocation with transcriptional repression of the *NFIA* target genes in the present case.

As the karyotype was described as 46,XY,der(1)t(1;1)(p31;q21),-del(1)(p11p31),der(16)t(1;16)(p31;q24), that is, presented additional rearrangement besides the 1;16-translocation, we decided to screen the list of possible fusion genes in search of genes located in karyotypic breakpoints to see if those were involved in fusions as well. We identified four possible fusions (seed count-rank >12) where one of the genes mapped to a breakpoint position on chromosome 1. An analysis of the hypothetical fusions using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) showed in one of the two genes high-sequence identity with several genes and/or numerous repetitive sequences (for example, SINE). Hence, the reality of the putative fusions was seriously called into question and no further investigations were undertaken.

In addition to the present case, two more cases of erythroleukemia showing a t(1;16)(p31;q24) in their karyotype[13,14] can be found in the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer.[15] All three patients (including ours) were very young children, and clinical outcome was poor. We assume that a *NFIA/CBFA2T3* fusion existed also in these leukemias, but no evidence is at hand to corroborate or falsify this assumption.

In summary, we describe the first fusion gene identified in acute erythroleukemia. Knowledge of its specific functions in the neoplastic context is still incomplete, but pathogenetic similarities with other leukemic fusion genes are readily discernible. As for other leukemias characterized genetically by fusion genes, one may assume that the detailed pathogenetic knowledge now emerging may eventually form a starting point from which therapeutic attempts may begin.

CONFLICT OF INTEREST

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Mutations of epigenetic regulators and of the spliceosome machinery in therapy-related myeloid neoplasms and in acute leukemias evolved from chronic myeloproliferative diseases

Therapy-related myeloid neoplasms (t-MN), including acute myeloid leukemias (t-AML) and myelodysplastic syndromes (t-MDS), indicate myeloid disorders developing in patients treated with radiotherapy and/or chemotherapy for cancer or autoimmune diseases. t-MN represent a unique model of *in vivo* poison-induced leukemogenesis. It displays a high incidence of monosomies, complex karyotypes and *p53* mutations, but other AML-associated mutations, as *FLT3*, *NPM1*, *CEBPA* and *TET2* (ten-eleven-translocation 2), are usually rare.[1–3] In addition, t-MN have been shown to display significant changes in gene expression, including upregulation or downregulation of pathways involved in major cellular processes, which are present long before the development of the t-MN and may be expression of early progenitor damage.[4] These changes are due to the synergistic negative effects of previous treatments, probably in the presence of individual predisposing factors, and finally result into leukemic transformation.

Epigenetic deregulation of gene expression is one of the main pathogenetic processes in AML and MDS. High rates of gene-specific methylation have been reported in these diseases,[5–7] but the underlying mechanisms are poorly understood. The identification of mutations in several genes involved in epigenetic regulation has given new insights. Among these, recurrent somatic mutations of enzymes involved in methylation, including *DNMT3A* (DNA methyltransferase 3A), *IDH1* and *IDH2* (isocitrate dehydrogenase 1 and 2), *TET2* and *EZH2* (enhancer of