t(3;21)(q22;q22) leading to truncation of the *RYK* gene in atypical chronic myeloid leukemia

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ABSTRACT

The analysis of a small number of patients with atypical chronic myeloid leukemia showing balanced chromosomal translocations has revealed diverse tyrosine kinase fusion genes, most commonly involving *FGFR1*, *PDGFRA*, *PDGFRB*, *JAK2*, and *ABL*. We present a case of aCML with a 3q22;21q22-translocation that led to truncation of the receptor-like tyrosine kinase (*RYK*) gene and its juxtaposition with sequences from chromosome 21 including the *ATP5O* gene coding for a mitochondrial ATP synthase. The resulting fusion was not in frame, however, which is why we speculate that an abrogated *RYK* gene product rather than a chimeric protein might be the leukemogenic result.

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1. Introduction

Atypical chronic myeloid leukemia (aCML) demonstrates myelodysplastic as well as myeloproliferative features at the time of diagnosis. The disease is characterized by principal involvement of the neutrophil series with leukocytosis comprising immature as well as mature dysplastic neutrophil granulocytes. Multilineage dysplasia is common, reflecting the stem cell origin of aCML. As another defining feature, the neoplastic cells do not harbor a Philadelphia (Ph) chromosome with the resulting *BCR/ABL1* fusion gene [1]. The relative incidence of aCML is reported to be 1–2 cases for every 100 cases of Ph + *BCR/ABL1* + CML [2].

The Mitelman database of chromosome aberrations in cancer [3] presently lists 51 aCML cases with an abnormal karyotype. Although cytogenetic abnormalities, including +8 and +21, have been described in up to 80% of patients with aCML, the aberration pattern has shown none of the specificity typical of CML [4–8]. Recently, however, fusion genes were repeatedly described in aCML. Reiter et al. [9] and Bousquet et al. [10] simultaneously identified a *PCM1/JAK2* in seven aCML patients carrying a t(8;9)(p22;p24), and also an *H4/PDGFβR* fusion has been reported in a few cases of aCML carrying a t(5;10)(q33;q22) [11–13]. Both these translocations target tyrosine kinases, perhaps hinting at a general pathogenetic mechanism in this disease.

We report a new case of aCML with a 3;21-translocation leading to fusion of the receptor-like tyrosine kinase (*RYK*) gene from 3q22 with sequences from chromosome 21 including the mitochondrial ATP synthase (*ATP5O*) gene from 21q22. Since the resulting fusion was not in frame, we speculate that truncation of *RYK* was the central pathogenetic outcome of the translocation.

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2. Material and methods

2.1. Case history

A 77-year-old man presented in May 2005 with anemia. His medical history was remarkable for recurrent venous thromboembolism, hypothyroidism, and rheumatoid arthritis for which he received warfarin, thyroxine, and methotrexate, respectively. Methotrexate (10 mg/m^2 once weekly), which had been prescribed for the last year, was discontinued upon admission. The diagnostic procedures revealed anemia, thrombocytopenia, and leukocytosis (Table 1).

No BCR/ABL1 fusion transcript was detected by PCR and a diagnosis of aCML was made. Treatment was started with hydroxyurea. The anemia improved within short and the patient was well till the end of 2005. In January 2006, he was again admitted to hospital due to increasing fatigue, weight loss, and unexplained low-grade fever. Blastic transformation was now diagnosed (white blood cells were 90×10^9/L with 90% blasts). Mercaptopurine was substituted for hydroxyurea and his condition improved, but in June 2006 he was readmitted to hospital unconscious. An intracerebral hemorrhage had occurred and the patient died within hours of admission. An autopsy was not performed.

2.2. G-banding and karyotyping

The bone marrow aspirate intended for cytogenetic analysis at diagnosis (May 2005) was short-term cultured according to standard techniques [14]. Chromosome preparations were G-banded using trypsin (DIFCO Laboratories, Detroit, MI, USA) and Leishman staining (BDH, Poole, England). The subsequent cytogenetic analysis and karyotypic description followed the recommendations of the International System for Human Cytogenetic Nomenclature [15].

2.3. Fluorescence in situ hybridization (FISH) analyses

Multiplex FISH (M-FISH) was performed to verify the composition of the der(21)t(3;21) (see below) detected by G-banding and whose presence had also been confirmed using FISH with painting probes specific for chromosome 21. FISH with locus-specific probes for RPN1 (3q21; BAC probe, see below), EVI1 (3q26; BAC probe, see below), and RUNX1 (AML1; 21q22; TEL/AML1 dual color translocation probe from Vysis, Downers Grove, IL, USA) was performed to check for possible involvement of these candidate genes in the rearrangement. Furthermore, FISH with the BCR/ABL1 Dual Color, Dual Fusion Translocation Probe Set (Vysis) was also performed to make sure no cryptic rearrangement between 9q34 and 22q11 had gone undetected. When none of the above analyses showed involvement of the genes tested for, a panel of locus-specific probes derived from bacterial artificial chromosomes (BAC) and fosmids was prepared and hybridized to detect the exact breakpoint of the der(21)t(3;21)-rearrangement (Table 2). The BAC clones were retrieved from the RPCI-11 Human BAC library and the CalTech human BAC library D (P. de Jong libraries, http://bacpac.chori.org/home.htm). The probes were selected according to their physical and genetic mapping data on chromosomes 3 and 21 as reported by the Human Genome Browser at the University of California, Santa Cruz webside (http://genome.ucsc.ed/). The clones initially used were, from centromere to telomere, RP11-662G11, RP11-772C17 (containing the RPN1 gene), CTD-2255O24, CTD-2034E12, RP11-1055J3, RP11-656L2, RP11-482M18, RP11-99C6, RP11-831F10, RP11-454D11, RP11-57D18, CTD-2509E11, and RP11-33A1 (containing the EVI1 gene) on 3q and, from centromere to telomere, RP11-32A2, RP11-54F16, RP11-67E8, RP11-369E2, RP11-116F22, RP11-1033C16, RP11-829G14, CTD-3223F18, RP11-8P19, CTD-3022C14, and CTD-2590G13 on 21q. FISH using fosmid clones was then performed to find which genes were split at the breakpoints. The clones used were G248P89202D10 and G248P83817E1 on 3q and G248P88418G5, G248P86186G5, and G248P87594B4 on 21q. All clones were grown in selective media and DNA was extracted according to standard methods [16]. DNA probes were directly labelled with a combination of fluorescein isothiocyanate (FITC)-12-deoxycytidine triphosphate (dCTP) and FITC-12-2-deoxyuridine triphosphate (dUTP), Texas Red-6-dCTP and Texas Red-dUTP (New England Nuclear, Boston, MA, USA), or indirectly with Biotin-dUTP (Molecular Probes, Invitrogen, Carlsbad, CA, USA) by nick translation and detected with streptavidin-diethylaminocoumarin (DEAC; Invitrogen). The subsequent hybridization conditions as well as the detection procedure were according to standard protocols [17]. The hybridizations were analyzed using a CytoVision system (Applied Imaging, Newcastle, UK).

2.4. Molecular genetic investigations

Total RNA was extracted from bone marrow cells frozen and stored at −80 °C using the Trizol reagent according to the manufacturer's instructions (Invitrogen). The primers used for PCR amplification and sequencing are listed in Table 3. cDNA was synthesized using 5 μg of total RNA in 20 μL reaction mixture [50 mmol/L Tris–HCl (pH 8.3) at 25 °C, 75 mmol/L KCl, 3 mmol/L MgCl$_2$, 10 mmol/L DTT, 1 mmol/L of each deoxynucleotide triphosphate, 20 units RNase inhibitor (RNA guard, Amersham, Biosciences, Piscataway, NJ, USA), 0.5 pmol/L random hexamers, and

Table 1
Blood and bone marrow assessment at diagnosis.

Blood		Bone marrow	Hypercellular (%)
Hgb (g/dL)	7.2	Erythropoiesis	5
Leukocytes ($\times 10^9$/L)	60.3	Granulocytopoiesis	88
Thrombocytes (x10^9/L)	81	Granulocytes[a]	20
Lymphocytes	8%	Myelocytes	64
Monocytes	6%	Promyelocytes	4
Granulocytes[*]	73%	Blasts	4
Eosinophils	<1%	Lymphocytes	3
Basophils	<1%	Thrombopoiesis	Decreased
Myelocytes	9%		
Promyelocytes	2%		
Blasts	2%		

[a] Dysgranulopoiesis was present. Bizarrely segmented nuclei were prominent, and abnormal cytoplasmic granularity, both hyper–and hypogranularity, was seen in the neutrophils.