Two clonal occurrences of tetrasomy 21 in an atypical chronic myeloid leukemia with wild-type RUNX1 alleles. Additional support for a gene dosage effect of chromosome 21 or RUNX1 in leukemia

Atypical chronic myeloid leukemia (aCML) is a rare leukemic disorder with no specific genetic lesion. Here we demonstrate clonal occurrences of tetrasomy for the long arm of chromosome 21 in a patient with aCML, and a thorough review of the literature provides evidence that this chromosomal anomaly is a so far not recognised recurrent finding in aCML. Further, the timely association of the occurrence of the tetrasomy 21q with acceleration of the leukemia suggests a role for chromosome 21 in leukemic disease progression. The chromosome 21 gene most strongly implicated in both normal and abnormal hematopoiesis is *RUNX1*. Also, RUNX1 haploinsufficiency due to *RUNX1* point mutations characterises the familial platelet disorder with propensity to develop leukemia, and thromboytopenia was a leading feature in the present case. Therefore, an extensive molecular analysis of RUNX1 was performed. However, these analyses did not reveal a mutation, and the results support a gene dosage effect for *RUNX1* in myeloid disease similar to observations in lymphoid disease. Patients with aCML and a tetrasomy 21 may form a karyotypically and phenotypically defined subgroup of aCML.

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Studies of recurrent somatic or germline chromosomal abnormalities such as translocations, inversions and point mutations have identified many genes involved in the multistep process of cellular transformation and leukemia progression. Such an approach initially led to the original identification of the transcription factor RUNX1 as one fusion gene partner in the translocation t(8;21) that is recurrent in acute myeloblastic leukemia (AML) with maturation.[1,2] RUNX1 (or AML1, or CBF?2, or PEBP2?B) plays a major role in in both normal and abnormal hematopoiesis (reviewed in[3]). It is frequently translocated in acute leukemia, and point mutations are found in sporadic and familial leukemia[3] as well as in the transient myeloproliferative disorder (TMP).[4] Remarkably, RUNX1 point mutations are predominantly found in sporadic and familial myeloid leukemia but in one case only of pediatric acute lymphoblastic leukemia (ALL),[3,5] whereas *RUNX1* gene amplification is a recurrent finding in pediatric sporadic ALL but rare in adult myeloid disease. No mutation was found in the multiple *RUNX1* copies, indicating a gene dosage effect of *RUNX1* in lymphoid leukemogenesis.[5] Such patients also show increased RUNX1 transcript levels, a result in line with a gene dosage effect.[6] As the RUNX1 gene maps to chromosome 21q22, the number of RUNX1 copies is also increased in patients that have a karyotype with extra 21q chromosomes. Acquired additional chromosomes 21 are frequent in pediatric hyperdiploid ALL,[7,8] but rare as a sole karyotypic abnormality in acute myeloid leukemia (reviewed in9,10). In the 3 reports that have performed a mutation analysis of the *RUNX1* gene in karyotypes with acquired tri- or tetrasomy of chromosome 21,[5,11,12] 7/32 adult patients and 2 pediatric cases showed a point mutation in *RUNX1*; 4 of these 9 patients had additional chromosomal changes and all patients had a myeloid phenotype. Interestingly, 2 patients with mutated RUNX1 alleles, having a sole tri- and tetrasomy, respectively, had a diagnosis of atypical chronic myeloid leukemia (aCML).[11] Also, among the[11] patients with tri- or tetrasomy 21 and a diagnosis of ALL, no one had a RUNX1 mutation. Therefore, *RUNX1* gene analyses so far suggest that alteration of *RUNX1* function by point mutations predispose to myeloid leukemia in adults, and that *RUNX1* overexpression may be preferentially associated with pediatric lymphoid leukemias.

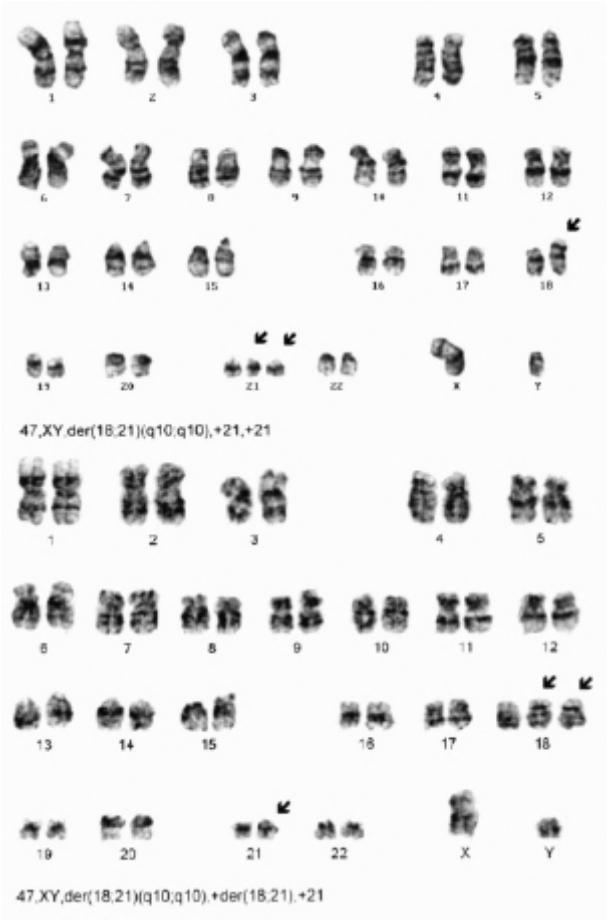

Figure 1. Bone marrow cells obtained at disease acceleration were cultured and chromosomes were prepared as described[29] All metaphases analysed were abnormal and could be subdivided into 2 related clones: 47,XY,der(18;21)(q10;q10),+21,+21[14]/47,XY,der(18;21)(q10;q10),+der(18;21),+21[4]. Both show a whole arm translocation involving the long arm of chromosome 18 and the long arm of chromosome 21: der(18;21)(q10;q10). They differ by the presence of 2 additional chromosomes 21 in one clone, and an additional der(18;21) and an additional chromosome 21 in the other. Defects observed generated a tetrasomy 21q associated with a monosomy 18p in both clones and an additional trisomy 18q in one of them. Arrows indicate derivatives and supernumerary chromosome 21.

Here we provide an extensive molecular genetic analysis of RUNX1 in a patient with aCML with a normal karyotype at disease presentation, but at disease acceleration chromosome defects in 2 clones, both resulting in tetrasomy 21q, and we suggest an association between the phenotypic and genetic findings.

Case report

This 50 year old male patient presented with diffuse

Table 1. Analyses of peripheral blood and bone marrow performed at diagnosis of aCML and during disease progression.

peripheral blood	*at diagnosis*	*at acceleration (15 months)*	*at blast phase (17 months)*
hemoglobin g/L	122	80	
normoblasts/100 leukocytes	4		
leukocytes x10^9/L	83	130	200
neutrophils %	65		
eosinophils %	1,5		
basophils %	0,5		
monocytes %	2		
lymphocytes %	7,5		
metamyelocytes %	13		
myelocytes %	9,5		
blasts %	1	10	30
thrombocytes x10^9/L	250	25	20
vitamine B12 pmol/L	2500		
leukocyte alkaline phosphatase score	362		
bone marrow			
cellularity	packed	hypercellular	packed
hematopoiesis	increased and dysplastic	increased, dysplastic megakaryo- and myelopoiesis, diminished erythropoiesis	increased myelopoiesis, reduced megakaryo- and erythropoiesis
blast count %	<5	<5	30
reticulin content	slightly diffuse and increased		
cytogenetic analysis	normal	see Figure 1	
multiplex PCR	no bcr-abl product		

skeletal pain and unexplained body weight loss. Physical examination was otherwise unremarkable. Blood and bone marrow analyses led to the diagnosis of aCML (Table 1): hematopoiesis was increased with dysplastic megakaryopoiesis and myelopoiesis and a blast count below 5% in the bone marrow, increased neutrophils and immature myeloid precursors in peripheral blood and bone marrow, moderate dysplasia of the neutrophils, no basophilia, only minimal monocytosis, and no bcr-abl product by Multiplex-PCR. Because the patient was alcoholic and there was echocardiographic evidence for ethylic congestive heart disease, allogeneic stem cell transplantation was excluded. Hydroxyurea therapy was started, followed by interferon-α (average dose 6 Mio IE per day); after 5 months, interferon-α was stopped because of marked thrombocytopenia (20x10^9/l), without platelet recovery. 15 months after diagnosis, acceleration of the disease was noted with progressive fatigue, weight loss and splenomegaly. The bone marrow showed markedly increased and dysplastic myelopoiesis and a blast count below 5%. Karyotypic analysis of bone marrow cells revealed the new occurrence of 2 abnormal clones, both generating a tetrasomy 21q (Figure 1). Blast crisis occurred 2 months later (Table 1), and cutaneous dissemination of myelogenous blasts was noted. A trial of low-dose cytarabine was stopped after a few days because of worsened thrombocytopenia, and the patient died within days of generalized tumor spread, marked pericardial effusion and kachexia. Disease evolution further reflected some characteristics of aCML: monocytosis never became prominent, and the patient rapidly developed thrombocytopenia and failure to treatment.

Results and Discussion

We investigated the patient's bone marrow obtained at disease acceleration and showing the new occurrence of tetrasomy 21q. Because the gene coding for RUNX1 maps to chromosome 21 and is on this chromosome the most frequently involved gene in leukemia, showing many genetic alterations in a number of leukemic phenotypes, we performed a molecular analysis of all exons of RUNX1 by using denaturing high pressure liquid chromatography (DHPLC; Varian Instruments, Melbourne, Australia). This technique represents a reliable screening method for detection of a mutation in a tetrasomic sample.[13] After amplification of all RUNX1 exons as described,[14] the patient's sample alone, or mixed in an equimolar ratio with normal reference DNA to detect homozygous mutations, were run on the DHPLC according to instructions of the manufacturer and at melting temperatures as calculated (http://insertion.stanford.edu/melt.html) and at 1 or 2°C higher. In addition, exon 5 that was found mutated in a previous report in 2 aCML cases11 was sequenced from the amplified DNA as well as from 24 clones obtained after subcloning the amplified exon into the TOPO TA cloning system (http://www.invitrogen.com). These extensive analyses did not reveal any sequence variation, demonstrating the molecular diversity of the RUNX1 gene in phenotypically and cytogenetically similar cases of aCML.

Several important observations appear from the study of the clinical phenotype and the cytogenetic and molecular analyses. First, karyotypic analysis revealed a tetrasomy 21q. The molecular pathogenesis in the rare aCML is not well understood, and no specific genotype is known. Also, phenotypic heterogeneity has been noted.[15,16] Surprisingly and not described so far, a thorough review of the literature revealed that tri- or tetrasomy 21 are recurrent findings in aCML,[11,16-20] in 6 aCML patients being the only cytogenetic abnormality,[11,16-19] and in 2 patients being present in conjunction with trisomy 8, or trisomy 8 and trisomy 19, respectively.[18,20] Together with the present case, these findings strongly suggest an important role of a gene on this chromosome in this rare disorder. Second, our report demonstrates a close association between occurrence of tetrasomy 21 and disease acceleration. This finding is in contrast to a previous report where disease progression was linked to trisomy 8,

whereas tetrasomy 21 was linked to the chronic disease.20 However, conclusions of that report were hampered by the simultaneous occurrence of tetrasomy 21 and trisomy 8 at blast crisis. Of interest, similar to the present case, in 2 more reports the tetrasomy 21 was noted at the time of disease acceleration or blast crisis.[16,17] In 2 other cases, information about the time of karyotyping is not given,[11] and in 2 more patients trisomy 21 was present during the chronic phase of the disease as a minor clone with no information about its evolution, the majority of metaphases presenting a normal karyotype (88% and 89%, respectively).[18] Thus, cumulative data strongly suggest that a gene on chromosome 21q may play an important role in the disease evolution in at least a subgroup of the phenotypically heterogenous group of aCML. A predominant contribution to the phenotype by the chromosome 18 abnormalities in our case seems less likely, as such abnormalities are uncommon and part of complex aberrations in myeloid disease and not described in aCML;[21-23] moreover, in contrast to trisomy 21 patients, trisomy 18 patients do not show haematological abnormalities.[24,25]

Exclusion of a *RUNX1* mutation in this patient supports a gene dosage effect for *RUNX1* in myeloid leukemia similar to findings in ALL. Due to lack of material, we cannot assess the expression of *RUNX1*. However, the fact that both *in vitro* overexpression of *RUNX1*[26] and multiple *RUNX1* gene copies in ALL6 give rise to elevated RNA levels supports elevated *RUNX1* gene expression in our patient too. *In vivo* observations demonstrate that tight control of RUNX1 expression is necessary in normal hematopoiesis, as both overexpression and haploinsufficiency of RUNX1 expression are associated with and/or predispose to the development of leukemia;[3,14,27] these findings are also supported by in vitro overexpression experiments.[26] Whether a myeloid or lymphoid leukemic phenotype are determined by the expression level of RUNX1, or by the stage of differentiation alone of the cell that is affected by the mutation, or by additional mutation(s) in other gene(s) as part of the multistep leukemogenesis[3] remains speculative.

A mutation in another gene of chromosome 21 cannot be excluded. However, a role for RUNX1 in this disease is strongly suggested not only by its known role in hematopoiesis and leukemia, but additionally by the presence of a platelet count low enough to stop chemotherapy in this patient. Indeed, thrombocytopenia is a feature of the familial platelet disorder that is due to germline RUNX1 mutations and is also frequent in newborns with Down syndrome and in patients with TMP, and in all these situations an increased incidence of leukemia is observed.[14,28] In 4 cases of aCML with tri- or tetrasomy 21, thrombocytopenia was a leading feature, and the cause of death was cerebral hemorrhage.[18-20] Therefore, one might speculate that marked thrombocytopenia in some aCML patients may be due to a dysregulation in RUNX1 expression, and this functional phenotype may characterise this distinct subgroup of aCML patients. Prognosis in this subgroup is poor (mean survival, 17 months). However, to assess the impact of multiple RUNX1 copies on the prognosis of this subgroup of aCML, on its disease activity, and on thrombopoiesis, larger studies are still necessary, and special attention should be given to the platelet counts during disease evolution.

In conclusion, the present study shows that additional wild-type RUNX1 copies are found in myeloid leukemia, supporting a gene-dosage effect of RUNX1 in myeloid leukemogenesis. We have identified additional chromosome(s) 21 as a recurrent chromosomal anomaly in aCML, possibly defining a new subgroup of aCML patients. The recognition of this aCML subgroup may help the understanding of this disease and aid the clinician in the management of these patients.

Robert Escher,[1,3] Dominique Mühlematter,[2] Hamish S. Scott,[3] Martine Jotterand[2] Andreas Tobler[1]

[1]Central Haematology Laboratory, University Hospital, Berne, Switzerland, [2]Division of Medical Genetics, University Hospital, Lausanne, Switzerland, and [3]Division of Genetics and Bioinformatics, The Walter and Eliza Hall Institute, Melbourne, Australia

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Correspondence to: Robert Escher, MD
Division of Genetics and Bioinformatics
The Walter and Eliza Hall Institute of Medical Research
Royal Parade 1G Parkville, Victoria 3050 Australia
Phone: ++613.9345.2488 Fax: ++613.9347.0852
E-mail: escher@wehi.edu.au
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