Overexpression of *PRDM16* in the Presence and Absence of the *RUNX1/PRDM16* Fusion Gene in Myeloid Leukemias

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To the Editors

In a recent study published in *Genes, Chromosomes & Cancer*, Sakai et al. (2005) reported the identification of a novel *RUNX1* partner gene, *MDS1/EVI1*-like gene 1 (*PRDM16*), in a patient with acute myeloid leukemia (AML) M4 with the t(1;21) translocation. This fusion gene has also been described recently in a case of t(1;21) positive therapy-related AML (Stevens-Kroef et al., 2006). The t(1;21)(p36;q22) is a rare but recurrent translocation associated with de novo and therapy-related AML and with myelodysplastic syndromes (MDS).

We, here, describe the first case of chronic myeloid leukemia (CML) blast crisis cells in which the *RUNX1/PRDM16* fusion gene likely contributed to clonal evolution from CML to AML. A 59-year-old woman was referred in July 2000 for a CML. Cytogenetic analysis showed 46,XX,t(9;22)(q34.1;q11.2) in 20 metaphases (UPN 00-H59). She was treated initially with IFN for 20 months with a minor cytogenetic response and then with imatinib mesylate. After 6 months of imatinib, she developed a blastic transformation with 68% blast cells in the peripheral blood. Cells were obtained after an informed consent. Conventional G-banding karyotype showed a possible deletion at band 21q22 in addition to the t(9;22) in all metaphases. Spectral karyotyping of these cells revealed a t(1;21) translocation in addition to the t(9;22) translocation (UPN 02-H056)(Fig. 1A and 1B). The t(1;21) translocation was not present in chronic phase cells isolated from this patient. Fluorescence in situ hybridization (FISH) experiments were performed on peripheral blood metaphases of the blast phase cells using bacterial artificial chromosomes (BACs) and P1-derived artificial chromosomes (PACs) clones obtained from the BACPAC Resource Center (Children's Hospital Oakland Research Institute in Oakland, California, <http://bacpac.chori.org/home.htm>). Clone physical positions and covered genes are available at the UCSC Human Genome Browser (<http://genome.ucsc.edu/>). The *RUNX1* gene located at band 21q22.12 was first studied using two selected BACs, RP11-771C10 (containing intron 6 to exon 8 and the 3' end of *RUNX1*) and RP11-299D9 (containing intron 1 to exon 8 and the 3' end of

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RUNX1). Split signals were observed with BAC RP11-299D9 (Fig. 1C). The candidate gene, *PRDM16*, located at band 1p36.32 was studied using two PACs, RP1-163G9 (containing the 5' end of *PRDM16*, exon 1 and intron 1) and RP4-785P20 (containing intron 3 to exon 17 of *PRDM16*) and the BAC RP11-659J6 (containing part of intron 1, exons 2 and 3, part of intron 3 of *PRDM16*). A split signal was detected using PAC RP1-163G9, indicating that the breakpoint was in intron 1 of *PRDM16* (Fig. 1D). On the basis of the results of the FISH studies, we designed PCR primers covering most exons of *RUNX1* in both directions (forward and reverse), and for *PRDM16* in exon 1 (forward), exons 2–4 (reverse) to assess the expression of *PRDM16/RUNX1* and *RUNX1/PRDM16* fusion products. RT-PCR analysis and sequencing of the amplicons revealed eight *RUNX1/PRDM16* transcripts joining *RUNX1* exons 5 or 6 and *PRDM16* exons 2 or 3. Two *RUNX1/PRDM16* cDNAs represented an open reading frame encoding exons 1–5 or exons 1–6 of the *RUNX1* gene containing the RUNT domain fused to exon 2 of *PRDM16* that contain the PR domain (Fig. 2). However, the reciprocal *PRDM16/RUNX1* fusion transcript was not detected. We analyzed the relative expression levels of *PRDM16* using real-time PCR (ABI Prism 7900HT Sequence Detection System) in our CML case at the time of chronic phase (UPN 00-H59) and blast crisis (UPN 02-H056), and we compared *PRDM16* transcript levels in this case to a series of 88 other myeloid malignancies without evident karyotypic abnormalities of chromosomal band 1p36.3 (including 4 CML-blast crisis derived cell lines, 6 CML blast crisis samples, 14 MDS and MDS transformed to AML samples, and 68 AML samples of different morphological and cytogenetic groups). The Ct (threshold cycle) values of *PRDM16* were normalized to an endogenous control gene (*GAPDH*) ($Ct = Ct_{PRDM16} - Ct_{GAPDH}$) and compared with a calibrator sample, using the Ct method ($Ct = Ct_{Sample} - Ct_{Calibrator}$). The human *GAPDH* predeveloped TaqMan® assay (PN4326317E) was used as the endogenous control. As the calibrator, we used the average Ct value in four normal bone marrow samples. *PRDM16* expression was quantified with an assay targeting exons 9 and 10 (Hs00922679_m1, ABI). An additional assay targeting exons 1 and 2 was performed in case UPN 02-H056 to discriminate between the level of expression of the normal *PRDM16* gene and the fused *PRDM16* (that does not contain exon 1 of *PRDM16*). High *PRDM16* expression was found in the CML blast crisis cells isolated from our patient when oligonucleotides specific for exons 9–10 were used (Fig. 3). In contrast, primers specific for cDNA sequences derived from exon 1 did not detect *PRDM16* expression although they could amplify material from control cells, indicating that the overexpression was specific to the fused *PRDM16* transcript and excluded up-regulation of the unrearranged allele (data not shown). Interestingly, a significant overexpression of *PRDM16* (mRNA levels more than 10-fold above value detected in normal bone marrow control cells) was detected in 17 other myeloid samples of different morphological types, including one CML in blastic phase (CML-BP), UPN 06-H006 (Fig. 3). The *RUNX1-PRDM16* fusion gene was not detected by FISH in these cases and experiments are currently being performed to detect other possible cryptic *PRDM16* rearrangements. The *PRDM16* gene was not expressed in the MC3 CML-BP cell line (kindly provided by Dr. C. Gambacorti-Passerini) that was shown to have an unbalanced t(1;21) translocation detected by M-FISH (Gribble et al., 2003). FISH studies also failed to reveal a *RUNX1-PRDM16* fusion in this cell line. In contrast, *PRDM16* expression was down-regulated not only in favorable-risk AML (t(8;21), t(15;17), and inv(16)), as previously reported (Barjesteh van

Waalwijk van Doorn-Khosrovani et al., 2003) but also in leukemias with *MLL* (11q23) translocations.

In this study, we describe a patient with CML who acquired a t(1;21)(p36;q22) translocation during the course of disease progression. Despite the frequent association of additional genetic abnormalities in the acute phase of CML, the molecular events leading to the transition from the chronic phase to the blast crisis remain poorly understood. Chromosomal translocations are infrequent secondary abnormalities in CML blast crisis. Indeed, the t(7;11)(p15;p15) has been described in less than 1% of myeloid blast crisis and the t(3;21)(q26;q22) in ~2%. Nevertheless, the chimeric genes associated with these translocations, *NUP98/HOXA9* (Mayotte et al., 2002) and *AML1/MDS1/EV11* (Cuenco and Ren, 2001), respectively, have been shown to be important in the pathogenesis of CML progression. The hematopoietic transcription factor *RUNX1* (*AML1*) is one of the most frequent target genes involved in leukemias. Numerous fusion partners of this gene have been identified in chromosomal translocations. In the blastic phase of CML, *RUNX1* has already been involved in rare cases of t(8;21)(q22;q22) and in t(3;21)(q26;q22). Here, we have identified *PRDM16* as another fusion partner of *RUNX1* in advanced CML. The *PRDM16* gene (also known as *MEL1*), encodes a zinc finger transcription factor highly similar to *MDS1/EV11*, a member of the PR-domain family involved in leukemogenesis. *PRDM16* was isolated as a gene transcriptionally activated by the t(1;3)(p36;q21) in cells from patients suffering of MDS and AML (Mochizuki et al., 2000). *PRDM16* belongs to a family of proteins containing a PR domain (PRDI-BF1-RIZ1 homologous region) that is related to the SET domain and may be involved in the regulation of chromatin-mediated gene expression as a protein-binding interface. As for some other PR domain members, two alternative splicing forms of *PRDM16* are expressed, although at a relatively low level in normal hematopoietic cells (Lahortiga et al., 2004). The longer form, *MEL1*, is similar to *MDS1/EV11*, an alternative splicing between *MDS1* and *EV11* containing the PR domain at the aminoterminal, whereas the shorter form, *EL1* or *MEL1S*, is comparable to *EV11* without the PR domain (Nishikata et al., 2003). Two opposite functions, depending on the presence or absence of the PR domain, have been suggested for some members of the PR domain family genes. The PR containing forms contribute to tumor suppression, while the PR-absent forms are oncogenic (Jiang and Huang, 2000). The *RUNX1/PRDM16* fusion in our case shares a high degree of sequence similarity with the *AML1/MDS1/EV11* (AME) fusion, including the presence of the RUNT domain of *RUNX1*, the PR domain, and the two zinc finger DNA binding domains of *PRDM16*, suggesting a similar molecular mechanism of leukemogenesis (Fig. 2). The abnormal *RUNX1* fusion proteins may mediate the aberrant recruitment of transcriptional corepressors leading to gene expression deregulation. Aberrant oligomerization may also contribute to the oncogenic activity of AME by the inappropriate recruitment of HDAC and CtBP corepressors, resulting in the transcriptional repression of *RUNX1* target genes and the repression of transforming growth factor- β -mediated growth inhibitory signaling (Nitta et al., 2005; Senyuk et al., 2005). The *RUNX1/PRDM16* fusion gene may also lead to the inappropriate expression of *PRDM16* driven by the *RUNX1* promoter contributing to the blastic transformation in our CML case. Interestingly, the translocation breakpoint in t(1;21) in the three patients published thus far (including one in this report), is in the same region as the retroviral integration site at mouse *Prdm16* locus in

immortalized immature myeloid progenitor cell lines. This retroviral integration promotes the expression of *Prdm16* lacking the PR-domain (Du et al., 2005). This recently published study identified *Prdm16* as a gene potentially involved in immortalization.

Our data suggested that the *RUNX1/PRDM16* fusion gene may play an important role in CML progression possibly contributing to immortalization of the leukemic stem cell. Since the t(1;21) translocation is difficult to detect by conventional cytogenetics, the *RUNX1/PRDM16* fusion gene may likely be underestimated in myeloid leukemia. Moreover, considering the significant proportion of myeloid malignancies with *PRDM16* overexpression in the absence of the *RUNX1-PRDM16* fusion gene in our study, the deregulation of *PRDM16* by other molecular mechanisms may play a larger role in myeloid leukemogenesis.

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Figure 1.

(A) Standard cytogenetic analysis of leukemic cells with the $t(9;22)(q34.1;q11.2)$ and $del(21)(q22)$ (UPN 02H056). (B) Spectral karyotype of leukemic cells with the $t(9;22)(q34.1;q11.2)$ and $t(1;21)(p36.32;q22)$ (UPN 02H056). (C) FISH on metaphases with the $t(1;21)(p36.32;q22)$ (UPN 02H056) using the *RUNX1* probe (BAC RP11-299D9) that shows one signal on the normal chromosome 21 and a split signal on the rearranged *der(1)* and *der(21)* (long arrows). (D) FISH on metaphases with the $t(1;21)(p36.32;q22)$ (UPN 02H056) using the *PRDM16* probe (PAC RP1-163G9) that shows one signal on the normal chromosome 1 (short arrow) and a split signal on the *der(1)* and *der(21)* (long arrows).

Figure 2. Schematic representation of RUNX1 and PRDM16 wild-type proteins and the putative chimeric protein RUNX1/PRDM16. Partial nucleotide and amino acid sequences of the two *RUNX1/PRDM16* in-frame fusions. Arrows indicate the t(1;21) breakpoints. RHD, runt homology domain; TA, transactivation domain; PRD, PR domain; ZnF, zinc finger domain; PRR, proline-rich domain; RD, repressor domain; AD, acidic domain.

Figure 3.

Expression analysis of *PRDM16* (exons 9 and 10) using real-time PCR in 105 samples: 4 normal bone marrow (BM) samples, CD34 isolated from normal bone marrow (CD34+), 5 normal peripheral blood (PB) samples, one colon carcinoma cell line (HCT-116), 4 CML-BP derived cell lines: K562, KU812, MC3, MEG01, 14 MDS and MDS/AML, 7 CML-BP (including present case with t(1;21), 02-H056), present CML case in chronic phase (CML-CP, 00-H59), 68 AML (FAB type: M0, M1, M2, M4, M5 of different cytogenetic groups and AML with t(11)(q23), t(15;17), and t(8;21) and with inv(16)). All samples were tested in duplicate and the average values were used for quantification. The graph shows ratios of 1/

Ct. The horizontal gray line indicates the average ratio in the four normal bone marrow samples (calibrator) and the horizontal dotted lines indicate an arbitrary 1/ Ct ratio of 0.21 and 0.09 for significant up or down-regulation respectively (or mRNA levels more than 10-fold above or under value detected in normal BM control cells). Ct values are: 04-H070: 3.1; 02-H056: 0.3; 06-H006: 3.4; 00-H59: 7.3; 02-H009: 0.2; 02-H075: 3.1; 03-H033: 1.9; 04-H120: 1.7; 04-H006: 0.5; 04-H054: -2.2; 03-H052: 1.1; Average normal BM: 8.0.