Deletion of sequences telomeric of the \textit{EVI1} gene in 3q26 associated with a novel pericentric inv(3)(p25q26) in a patient with acute myelogenous leukemia

In this report, we describe a novel acquired chromosome rearrangement affecting the \textit{EVI1} locus in band 3q26 in a patient with myeloid leukemia. The pericentric inversion, inv(3)(p25q26), was associated with a 750kb deletion, and led to \textit{EVI1} overexpression. Similar deletions were not found in five primary leukemia samples with the recurrent \textit{EVI1} rearrangements inv(3)(q21q26) and t(3;3)(q21;q26).

Several recurrent chromosome rearrangements affecting the \textit{EVI1} locus in chromosome band 3q26 have been described in myeloid leukemia. They lead either to overexpression of this oncogene, or to the formation of \textit{EVI1} fusion transcripts. We employed two separate split-signal interphase fluorescent in situ hybridization (FISH) assays that facilitate the detection of 3q26 and of 3q21 rearrangements\(^1\) (Figure 1A) to screen myeloid leukemia samples submitted to our laboratory for cytogenetic analysis. In the bone marrow of a 58 year old male patient suffering from acute myeloid leukemia (FAB subtype: AML M1; white blood cell count at presentation, 49.5×10^9/L with 84% blasts, hemoglobin 9.1g/dL, platelet count 53×10^9/L), we found aberrant signal patterns with the 3q26 assay, but not with the 3q21 assay. G-band analysis revealed monosomy 7 as the sole cytogenetic anomaly, and FISH with a whole chromosome paint did not give any indication of a chromosome 3 rearrangement. Analysis of metaphases hybridized with the 3q26 interphase FISH probe revealed that the red and the green signals, which are located centromeric and telomeric, respectively, of typical 3q26 breakpoints (Figure 1A) appeared at the opposite ends of the rearranged chromosome 3. This indicated the presence of a not previously described pericentric inversion of chromosome 3, inv(3)(p25q26) (see http://cgap.nci.nih.gov/Chromosomes/Mitelman). This inversion was somatically acquired, because it was not present in any of over 100 metaphases of PHA-stimulated peripheral blood leukocytes.

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**Figure 1.** Characterization of the inv(3)(p25q26) and the associated 3q26 deletion by FISH. **A)** Schematic representation of the 3q26 PAC clones used for FISH experiments. Appropriate PAC clones were identified using the University of California at Santa Cruz Genome Browser (http:// genome.ucsc.edu). They are from the RPCI11 library (9), and were obtained from the Resource Centre of the German Human Genome Project (RZPD) in Berlin. The relative positions of the PAC inserts were in part confirmed through hybridization of PAC end probes and cDNA probes onto membranes containing restriction digested PAC DNA. For the 3q26 interphase FISH assay, the PACs drawn in red and in green were labeled by nick translation with spectrum orange and spectrum green (Vysis, Downers Grove, IL, USA), respectively, and hybridized onto slides containing fixed, denatured cell nuclei. Separation of the normally co-localizing red and green signals indicates the presence of a 3q26 rearrangement. PAC clones deleted in the presented case are represented by broken lines. The positions of the \textit{EVI1} and MDS1 genes are indicated; boxes represent exons and lines introns. The introns in the \textit{EVI1} gene are too small to be discernible at this scale. **B)** Pericentric inversion, inv(3)(p25q26), in the leukemic cells of our AML patient. PAC 82C9, labeled with spectrum orange, and PAC 379K17, labeled with spectrum green, were hybridized onto slides containing sodium hydroxide-treated chromosome preparations of leukemic material. The red and the green signals appear close to the opposite ends of the inversion chromosome 3. **C, D)** A deletion is associated with the inv(3)(p25q26). C) PAC 362K14 was labeled with spectrum green and PAC 33A1 with spectrum orange. While clone 33A1 is present on both chromosomes 3, 362K14 is present on one, but absent from the other. D) PACs 368I23 and 33A1, located centromeric of the 3q26 breakpoint, as well as PAC 379K17, located telomeric of the breakpoint, were labeled with spectrum green, and PAC 115B16 was labeled with spectrum orange. On the normal chromosome 3, the red and green signals colocalized, while in the rearranged chromosome 3, the green signal was split and the red signal was missing.
Additional dual color FISH experiments using various PAC combinations corroborated the presence of the inv(3)(p25q26). Total RNA was isolated from the bone marrow of two healthy donors (C1, C2) and of the patient with AML (P), as well as from the cell line MDP (10) (+), which was used as a positive control, with Trizol reagent (Invitrogen, The Netherlands). For reverse transcription, the M-MLV enzyme and random hexamer primers (Invitrogen) were used. The cDNAs, as well as a DNA-free negative control (H2O) were subjected to PCR using primers hEVI1-5' (5'-AGCAACACTG-GAAATCAAGCGCCTTCAATGAT-3') and hEVI1-3' (5'-ACT-GACTGTAGAGCTCAGGGCTCAGGT-3') for the amplification of EVI1 (annealing at 62°C, 32 PCR cycles); hMDS1-51 (5'-GGGCGAGCTAGGAATATGGAC-3') and hMDS1/EVI1-EcoRV (5'-TGCCCCTTCTCGAAGATATCCG-3') for the amplification of MDS1/EVI1 (annealing at 58°C, 35 PCR cycles); and cyclo-fwd (5'-ATATGGAAAATGGGAGTTAAAGG-3') along with cyclo-rev (5'-TTGCCAGACGCTATTGTTTG-3') for the amplification of cyclophilin (annealing at 60°C, 35 PCR cycles), which was used to control for the abundance of cDNA in each sample. Equal amounts of each PCR reaction were separated on an ethidium bromide stained agarose gel. Molecular weight standard; sizes of relevant bands are indicated to the left.

Figure 2. EVI1, but not the splice variant MDS1/EVI1, is overexpressed in leukemic cells with the inv(3)(p25q26). Total RNA was isolated from the bone marrow of two healthy donors (C1, C2) and of the patient with AML (P), as well as from the cell line MDP (10) (+), which was used as a positive control, with Trizol reagent (Invitrogen, The Netherlands). For reverse transcription, the M-MLV enzyme and random hexamer primers (Invitrogen) were used. The cDNAs, as well as a DNA-free negative control (H2O) were subjected to PCR using primers hEVI1-5' (5'-AGCAACACTG-GAAATCAAGCGCCTTCAATGAT-3') and hEVI1-3' (5'-ACT-GACTGTAGAGCTCAGGGCTCAGGT-3') for the amplification of EVI1 (annealing at 62°C, 32 PCR cycles); hMDS1-51 (5'-GGGCGAGCTAGGAATATGGAC-3') and hMDS1/EVI1-EcoRV (5'-TGCCCCTTCTCGAAGATATCCG-3') for the amplification of MDS1/EVI1 (annealing at 58°C, 35 PCR cycles); and cyclo-fwd (5'-ATATGGAAAATGGGAGTTAAAGG-3') along with cyclo-rev (5'-TTGCCAGACGCTATTGTTTG-3') for the amplification of cyclophilin (annealing at 60°C, 35 PCR cycles), which was used to control for the abundance of cDNA in each sample. Equal amounts of each PCR reaction were separated on an ethidium bromide stained agarose gel. Molecular weight standard; sizes of relevant bands are indicated to the left.

References
Bone marrow and peripheral blood hematopoietic reserve in patients with B-cell chronic lymphocytic leukemia

We investigated the hematopoietic stem cell compartment of 46 patients with B-cell chronic lymphocytic leukemia (B-CLL). The results indicate that patients had fewer bone marrow stem cells and peripheral blood CFU-GM than did controls. Older patients have fewer committed progenitors in peripheral blood. Stem cells in BM show a more evident inverse relationship with the size of the B-CLL clone.

Autologous transplantation of bone marrow (BM) and/or peripheral blood (PB) stem cells is an increasingly used treatment for B-CLL, but little is yet known about the residual stem cell compartment and contradictory results have been published. In order to evaluate this hematopoietic reserve, we determined the number of colony-forming units (CFU) and burst-forming units (BFU) of myeloid (GM), erythroid (E) and myelomonocytic progenitors (M) in BM and PB using short-term in vitro culture tests, seeding 10^5 mononuclear cells (MNC) per mL. We analyzed samples from 46 B-CLL patients: 23 males, 23 females, median age 66 years; 16, 19, and 11 patients were in Rai stage 0, I+II, and III+IV, respectively. Four previously treated patients had been untreated for at least 4 months before sampling. BM and PB samples were simultaneously analyzed in 17 patients, BM alone in 25, and PB alone in four patients. The control group was formed of healthy, age and sex-matched individuals: 12 BM donors for allogeneic transplantation and 21 blood donors.

The median count of BM progenitors was lower in B-CLL patients: 11 CFU-GM and 19 BFU-E/10^5 MNC versus 53 CFU-GM and 32 BFU-E/10^5 MNC in controls (p < 0.001). The median CFU-GM/10^5 MNC in PB was 3 in B-CLL patients and 21 in controls (p = 0.022). There was no difference in BFU-E counts between B-CLL patients and controls (p = 0.907). The median BM CFU-GEMM count was lower in B-CLL patients than in controls, whereas the difference in PB CFU-GEMM was not statistically significant (Table 1).

Only the number of BM clonogenic cells correlated with evaluated parameters of disease (Table 2), the correlation being positive with preservation of normal hematopoiesis precursors (erythroid and myeloid in BM and CD34+ cells in both BM and PB) and negative with parameters indicating disease progression and tumor mass enlargement (PB leukocyte and lymphocyte counts, proportion of BM lymphocyte infiltration, advanced clinical stage according to the modified Rai classification and total tumor mass score – TTM). The proportion of BM CD34+ cells correlated with PB CFU-GM and BFU-E (p = 0.005). The correlation of lymphocyte proportion in PB leukocytes (%) was negative and significant only with BM CFU-GM.

The BM CFU-GM count was lower in patients with organomegaly (Rai I+II) and BM insufficiency (Rai III+IV) than in Rai stage 0 (8 in Rai I+II and 5 in Rai III+IV vs. 43.5 in Rai 0, p = 0.043). In contrast, BM BFU-E count was lower only in patients with Rai stage III+IV (27 in Rai 0 and 23 in Rai I+II vs. 9.5 in Rai III+IV, p = 0.037). These results suggest a difference between BM CFU-GM and BFU contents in association with clinical stage, the erythroid progenitors being better preserved till more advanced stages. In PB there was no difference in CFU-GM and BFU-E contents according to Rai stage. TTM was inversely correlated with BM CFU-GM (r = 0.54, p = 0.026) and BFU-E (r = 0.60, p = 0.011) in BM. The PB CFU-GM and BFU-E counts were significantly lower in patients over 60 years old (p < 0.05 for both).

Reports on the residual stem cell compartment in B-CLL are controversial. There are reports of a considerably increased CFU-GM at the time of diagnosis using clonogenic in vitro tests.