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Short communication

Chronic lymphocytic leukemia developing in a patient with chronic myeloid leukemia: evidence of distinct lineage-associated genomic events

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Abstract We describe the cytogenetic, fluorescence in situ hybridization (FISH), and molecular findings in a patient who developed a typical chronic lymphocytic leukemia (CLL) 20 months after the diagnosis of a Philadelphia (Ph)-positive chronic myeloid leukemia. Unstimulated bone marrow culture showed a 46,XX,t(9;22)(q34;q11) karyotype, and interphase FISH detected the presence of a *BCR/ABL* fusion signal in 13% of cells. On stimulated bone marrow culture, a normal karyotype and a 13q14 deletion by interphase FISH with D13S319 probe in 14% of the cells were found. Molecular studies detected the chimeric *BCR/ABL* messengers by nested reverse-transcriptase polymerase chain reaction. The B-cellular clone was documented by the presence of a clonal heavy chain inmunoglobulin rearrangement. The coexistence of these two hematologic malignancies leads to questions about their cell(s) of origin. We provide evidence that CLL arose in a Ph-negative clone. The implications of these findings are discussed.

1. Introduction

The coexistence of chronic lymphocytic leukemia (CLL) and chronic myeloid leukemia (CML) is a rare event that raises questions about whether the two disorders derive from the same or two different malignant clonal cells. Previous studies in the literature have described the simultaneous occurrence of both hematologic diseases [1–7] or the development of CML after exposure to an alkylating agent or radiation treatment for CLL [8–17]. Successive occurrence of CML and CLL has seldom been reported [18]. We report on a patient who developed a typical B-CLL 20 months after the diagnosis of a Ph-positive CML, whose two disorders originated from different clonal cells. Distinct genomic events in the myeloid and lymphoid cell populations are documented by cytogenetic, fluorescence in situ hybridization (FISH), and molecular studies.

2. Case report

In 1999, an 88-year-old white woman was referred to our center because of leukocytosis with left shift and thrombocytosis. Physical examination was unremarkable. Blood examination showed the following: hemoglobin (Hb) 14.3 g/dL, white blood cell (WBC) count 22×10^9/L (48% neutrophils, 5% eosinophils, 3% basophils, 10% lymphocytes, 7% monocytes, 1% promyelocytes, 18% myelocytes, and 8% band cells), and platelet count 940×10^9/L. Bone marrow (BM) aspiration was hypercellular, with myeloid and megakaryocytic hyperplasia. BM biopsy showed the same hypercellularity, without significant fibrosis. The cytogenetic analysis revealed a 46,XX,t(9;22)(q34;q11) karyotype, and the reverse-transcriptase polymerase chain reaction (RT-PCR) for *BCR/ABL* was positive. The diagnosis of CML in chronic phase was made and the patient was treated with hydroxyurea (500–1500 mg/day). Twenty months after CML onset, a progressive increase in mature-appearing lymphocyte count was noticed. In August 2001, the WBC was 66×10^9/L, with 29% neutrophils, 2% eosinophils, 1% basophils, 60% lymphocytes, 1% monocytes, 1% promyelocytes, 4% myelocytes, and 2% band cells, as well as Hb 14g/dL

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and platelet count 680×10^9/L. A BM aspirate showed the myeloid and megakaryocytic hyperplasia, but an infiltration of 30% mature lymphocytes was also present. The inmunophenotyping of the PB and BM lymphocytes revealed a CD5-, CD19-, CD20-, CD22-, and CD23-positive cell population with κ-light chain restriction. Two small lymph nodes were palpable in both axillary regions, and there was no hepato- or splenomegaly. The diagnosis of CLL during the course of the chronic phase of CML was made. The patient started with chlorambucil 10 mg/m^2/day for 15 days per month for 10 months. Her blood examination showed a WBC of 21×10^9/liter maintaining 50% of lymphocytes, with left shift, and a high platelet count (800×10^9/liter). The addition of hydroxyurea was needed to decrease the platelet counts. The patient is currently alive in a good general condition.

3. Cytogenetic and FISH analyses

At the time of CLL diagnosis, a new cytogenetic, FISH and molecular studies were performed. BM cells were cultured for 24 hours without mitogen and for 72–96 hours with phytohemagglutinin M, pokeweed, and lipopolysaccharide stimulation in F-10 medium with 15% of fetal calf serum. In addition, mononuclear cells (MNC) were obtained by centrifugation on Ficoll-Hypaque density gradient and the granulocyte (GNC) fraction was purified by dextran. Interphase FISH was performed with the following probes: LSI D13S319 SpectrumOrange (13q14), centromeric for chromosome 12 (CEP-12) SpectrumGreen, and LSI *BCR/ABL* extra signal (ES) dual-color (Vysis, Downers Grove, IL). Four-hundred interphase nuclei were analyzed for each probe. The cut-off for positive values (mean of normal controls + 3 SD) determined from samples of 10 normal donors was as follows: 3.5, 3, and 9.9% for trisomy 12, *BCR/ABL* probe, and monosomy of D13S319, respectively.

The cytogenetic study performed on unstimulated BM culture showed a 46,XX,t(9;22)(q34;q11) karyotype. On this material, interphase FISH detected the presence of a *BCR/ABL* fusion signal in 13% of cells. When the GNC slides were analyzed, the *BCR/ABL* fusion signal was observed in all myeloid elements (Fig. 1A), whereas the D13S319 and CEP 12 probes showed a normal pattern of signals. However, none of the lymphoid cells in the MNC slide showed the *BCR/ABL* rearrangement (Fig. 1B). A normal karyotype was found simultaneously on the stimulated BM cultures, and FISH analysis on interphase nuclei with the D13S319 probe detected a 13q14 deletion in 14% of cells (Fig. 1C). Neither *BCR/ABL* nor trisomy 12–positive nuclei were found.

4. Molecular studies

Total RNA was extracted from BM cells and RT-PCR for *BCR/ABL* was performed [19]. Genomic DNA was extracted to investigate the presence of a clonal heavy chain immunoglobulin gene rearrangement [20] and the methylation of $p15^{INK4b}$, $p16^{INK4a}$, and $p14^{ARF}$ genes by a methylation-specific PCR (MSP) [21,22]. Molecular analysis revealed the presence of a $p210^{BCR/ABL}$ chimeric transcript, and the B cellular clone was documented by immunoglobulin heavy chain–rearranged bands. MSP analysis did not show gene methylation (data not shown).

5. Discussion

In this study, we report on the second case of CLL that had developed almost two years after the diagnosis of CML

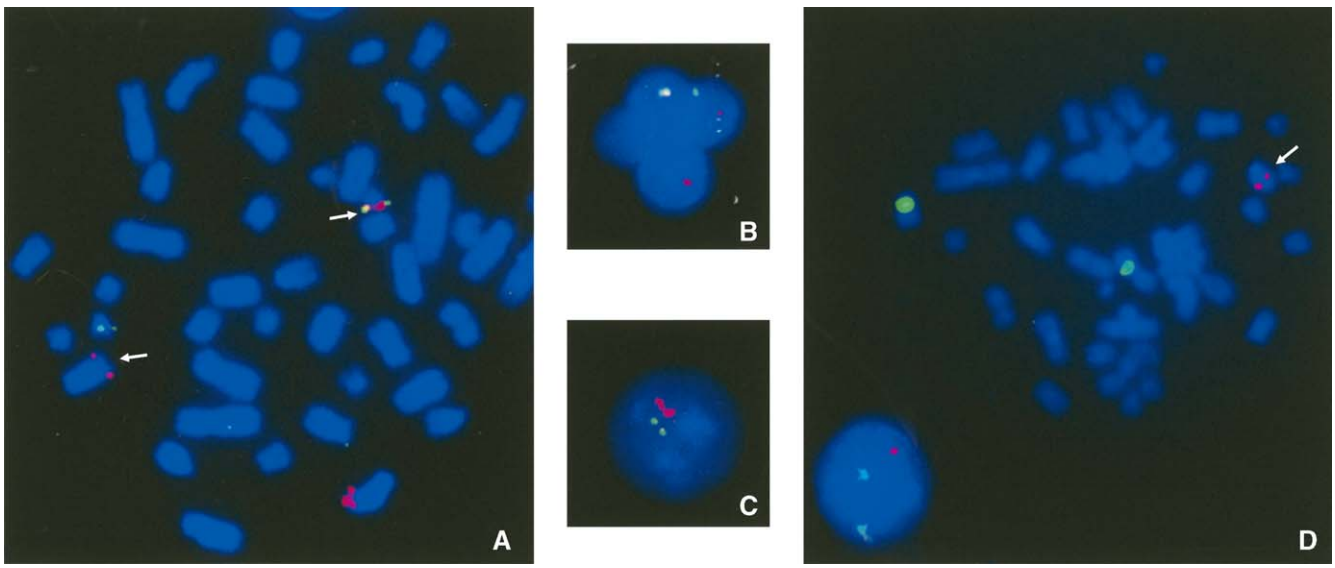

Fig. 1. FISH with *BCR/ABL* (green-red) double-color ES probe showing the following: (A) BM metaphase and interphase nuclei with the *BCR/ABL* fusion signal (yellow); (B) a polymorphonuclear cell showing the *BCR/ABL* fusion signal; (C) mononuclear cell with absence of *BCR/ABL* fusion signal; and (D) metaphase and interphase nuclei of BM-stimulated culture hybridized with D13S319 (red) and CEP 12 (green) probes showing two green signals and only one red, indicating the presence of a 13q14 deletion.

and in which the two disorders originated from different clonal cells. The B-CLL was characterized by typical CD5/CD19-positive mature lymphocytes, IgH rearrangement, and a 13q14 deletion detected by FISH, one of the most common aberrations in CLL [23]. In addition, the CML cells showed an immature morphology, a Ph-positive karyotype, and the presence of a $p210^{BCR/ABL}$ transcript. Thus, CLL developed in a cell different from the CML pluripotent progenitor cells.

This type of association between CML and CLL has been reported very rarely [18]. In most described patients, CLL preceded the development of CML [8–17], and in others the simultaneous occurrence of both disorders has been reported [1–7]. In some cases of CML occurring simultaneously with or after CLL, the presence of two independent clones was demonstrated [3–7,12,17,18]. In only one case, both myeloid and lymphoid cells were Ph-positive, suggesting a common origin [2]. A detailed review of the literature is shown in Table 1.

Patients with cancer have an increased risk of a second neoplasia that may be either an independent event or the effect of tumor therapy. Impaired immune surveillance in CLL patients might contribute to the increased risk of second malignancies [24]. They commonly appear several years after diagnosis and are usually nonhematologic neoplasms [25]. Recent studies, however, point to the possibility that alkylator-purine analog combination therapies may increase the risk of therapy-related myeloid malignancies [25]. In contrast, in CML, which has a natural evolution to a blastic phase [26], few patients with second hematologic malignancies [27–30] or secondary solid tumor have been reported [31–33].

In conclusion, during the chronic phase of CML, a mature, Ph-negative B-cell population originating in a different cell from the CML pluripotent stem cells expanded to produce the subsequent development of CLL. As far as we know, this is the first report in which cytogenetic, FISH, and molecular studies showed that the CLL developed during the course of CML, arising from a distinct Ph-positive stem cell.

Table 1
Patients previously reported with CLL and CML

Reference no.	Age/sex	Interval	Treatment
Simultaneous CML and CLL			
[1]	58/M	Simultaneous	
[2]	55/M	Simultaneous	
[3]	69/M	Simultaneous	
[4]	69/M	Simultaneous	H, Chl, ANA
[5]	71/F	Simultaneous	H
[6]	64/M	Simultaneous	H
[7]	68/M	Simultaneous	H, IFN-α
CML after CLL			
[8]	59/F	36 months	Chl, PDN
[9]	62/M	36 months	TBI
	74/M	24 months	None
[10]	55/M	61 months	Chl
[11]	83/M	2 months	Chl, BUS
[12]	55/M	84 months	Chl
[13]	47/F	72 months	Chl+PDN, Vin+Bleo, COP
[14]	82/F	60 months	None
[15]	66/M	72 months	Chl
[16]	43/M	73 months	TBI
[17]	76/F	12 months	None
CLL after CML			
[18]	54/F	36 months	H, IFN-α, IM
This study	88/F	20 months	Chl, H

Abbreviations: H, hydroxyurea; IFN-α, interferon-α; Chl, chlorambucil; ANA, anagrelide; TBI, total boby irradiation; IM, imatinib mesylate (Glivec); Vin, vincristine; Bleo, bleomycin; COP, cyclophosphamide + vincristine + prednisone; PDN, prednisone; BUS, busulfan.

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