Detection of Trisomy 8 in Philadelphia Chromosome–Positive CML Patients Using Conventional Cytogenetic and Interphase Fluorescence in situ Hybridization Techniques and its Relation to c-*myc* Involvement

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Abstract. Trisomy 8 (+8) is a common clonal evolution marker for progression in chronic myelogenous leukemia. The relationship of +8 to various stages of t(9;22) leukemias is not firmly established. To explore this association we examined bone marrow (BM) cells from 10 Philadelphia chromosome positive (Ph+) chronic myeloid leukemia (CML) patients in different stages of the disease, using conventional cytogenetic technique(CCT) and interphase fluorescence in situ hybridization (FISH). FISH detection of chromosome 8 was accomplished using the D8Z2 (Oncor) probe specific for the centrometric region of chromosome 8. Five hundred interphase nuclei were counted for each patient. Three of the 10 patients were selected for detection of c-*myc* gene locus located in the 8q24.2-24.3 region using the LSI c-*myc* probe (Vysis). One hundred interphase nuclei were counted for the presence of the c-*myc* gene for each patient. We found that the percentage of interphase nuclei showing 3 hybridization spots indicative of trisomy 8 was significantly lower by FISH than by CCT (metaphase analysis) in patients with chronic phase (CP) CML (11% versus 24%), nearly similar in patients with accelerated phase (AP) CML (13% versus 10%), and significantly higher in patients with myeloid blast crisis (mBC) (81% versus 33%). Hybridization spots for the c-*myc* locus were consistent with the chromosome 8 interphase FISH results in each of the patients tested. It is hypothesized that cells with supernumerary chromosome 8 may have a cell cycle time that differs from that of the disomic cells according to the stage of the disease. The c-*myc* locus expression in Ph+ CML patients with trisomy 8 is related to an increased copy number of the gene. *(received 17 August 2000; accepted 25 September 2000)*

Keywords: chronic myeloid leukemia, CML, c-*myc*. trisomy 8, Philadelphia chromosome, FISH

Introduction

Chronic myeloid leukemia (CML), a clonal disorder of pluripotent hematopoietic stem cells, has the cytogenetic hallmark of the Philadelphia chromosome [t(9;22)(q34;q11)] [1,2]. Upon progression to accelerated phase or blastic crisis, many CML patients develop additional cytogenetic abnormalities. Trisomy 8 is the most common among these, but isochromosome 17q, two Philadelphia chromosomes, and other abnormalities have also been observed [3,4]. Trisomy 8 is one of the most common chromosome abnormalities detectable in Ph+ CML associated with disease evolution. Early detection of cells containing an extra chromosome 8 is important for diagnostic, therapeutic, and prognostic reasons [5]. Trisomy 8 in Ph+ CML patients is frequently observed as a secondary chromosome change and is considered one of the non-random abnormalities associated with the blastic phase of the disease [6,7].

The trisomy for chromosome 8 is usually detected by CCT on metaphases obtained from bone marrow or peripheral blood cells. These techniques sometimes cannot provide complete information on the karyotypic abnormalities present in bone marrow samples

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because of technical limitations [8,9]. FISH is a useful technique for the detection of chromosome abnormalities because it enables the analysis of interphase cells (depending on the probe), the characterization of marker chromosomes, the screening of a large number of cells within a short period of time, and the ability to study samples with few or poorly assessable metaphases, especially when α-satellite or locus-specific probes are used.

This study analyzes 10 Ph+ CML patients in different stages of the disease using a α-satellite probe specific for chromosome 8 and the locus specific probe for c-*myc*. FISH is complementary to conventional karyotyping in that the percentage of interphase nuclei with trisomy 8 can differ from that obtained in metaphase. These changes may reflect increased trisomy 8 according to the stage of the disease. FISH using a locus-specific probe for c-*myc* can identify increased copy number of c-*myc* gene in Ph+ CML patients with trisomy 8.

Materials and Methods

Patients. Ten Ph+ patients at the University of Texas M.D. Anderson Cancer Center with the clinical and mophologic diagnosis of CML who were known to have trisomy 8 were examined. The patients ranged in age from 22 to 70 yr. Seven men and 3 women composed the study group. Of these, 5 patients were in the chronic phase of the disease, 2 patients in the accelerated phase, and 2 patients in myeloid blast crisis. One patient was studied twice, once while in the chronic phase and again 3 mo later while in lymphoid blast crisis. In each patient, an extra chromosome 8 was detectable at diagnosis or during the course of the disease in 1 or more cytogenetic evaluations.

Control specimens. Bone marrow cells from 10 untreated leukemia subjects that were diploid for chromosome 8 by conventional cytogenetics were used as controls for the D8Z2 probe (Oncor, Inc., Gaithersburg, MD). Cells of controls and patients were cultured and harvested using the same procedures. For FISH analysis, 2 observers, using areas of the slides on which cells were well spread, scored 500 interphase nuclei. Bone marrow cells from 2 healthy subjects known to be diploid for chromosome 8 by conventional cytogenetics were used as controls for the LSI c-*myc* probe, with 2 observers scoring 100 interphase nuclei using areas of the slides on which cells were well spread.

Cytogenetic analysis. Conventional cytogenetic and FISH analysis were performed on mononucleated cells from bone marrow samples. Bone marrow specimens were cultured for 24 hr. The chromosomes were harvested and the metaphase spreads were obtained from cultured bone marrow cells using the methanol acetic acid fixation method. Cell suspensions were dropped on air-dried clean slides. Conventional cytogenetic analysis and FISH were carried out on matched samples. The G-banding technique was used for the CCT interpretation.

FISH. For detection of trisomy 8, the α-satellite chromosome specific DNA centromeric probe (D8Z2) was used to determine the number of chromosome centromeres in interphase nuclei. The locus-specific, LSI c-*myc* probe was used to detect the gene locus located in the 8q24.2-24.3 region of chromosome 8.

Slide pretreatment and denaturation. Slides were treated with 2x standard saline citrate (SSC) for 30 min at 37°C, dehydrated in 70%, 85%, and 100% ethanol for 2 min each; denatured in 70% formamide, 2 x SSC [pH 7.0] at 72°C for 2 min; immediately transferred into cold 70%, 85%, and 100% ethanol for 2 min each; and then air-dried.

Probe denaturation and hybridization. An α-satellite (D8Z2) probe was mixed well with Hybrisol VI and denatured at 72°C for 5 min. A LSI c-*myc* probe was mixed well with hybrid buffer and H_2O in the dark and denatured at 72°C for 5 min. Each probe was immediately cooled on ice and then placed onto the examined slides. The slides were covered with a 22 x 22 mm cover slip, sealed with rubber cement, and incubated overnight at 37°C in a humidified chamber.

Probe detection and microscopy. After incubation, slides that were hybridized with the α-satellite probe were treated in 0.25 x SSC for 5 min at 72°C; the signal was detected by anti-digoxigenin conjugated with fluorescent isothiocyanate (FITC). Slides were then washed in phosphate buffer with detergent (PBD)

3 times for 2 min each. The slides were examined using a Zeiss fluorescent microscope; a dual wavelength filter was used to view FITC and propidium iodide simultaneously. Slides hybridized with the LSI-c-*myc* probe were washed in 0.4 x SCC/0.3% NP-40 at 72°C for 2 min, followed by a 2 x SCC/0.1% NP-40 wash for 1 min. They were then air-dried and counterstained with DAPI. The slides were examined using a Zeiss fluorescent microscope; a dual wavelength filter was used to detect spectrum orange (SO) and DAPI.

Results

Controls. Diploid karyotypes were present in all examined metaphases of the controls. FISH data were obtained with the centromeric probe for chromosome 8, and results were interpreted following enumeration of the signals in 500 interphase nuclei. A mean of 93.9% (SD ±1.5%) diploid cells was determined for chromosome 8. These data are consistent with those previously reported from our laboratory [10]. For the LSI c-*myc* probe controls, FISH data were obtained and results were interpreted following enumeration of the signals in 100 interphase nuclei. Two signals for c-*myc* were found in 98 interphase nuclei in control 1 and in 97 interphase nuclei in control 2.

Patients. The clinical data and the metaphase karyotypes of the 10 Ph+ CML patients in different stages of the disease at the time of specimen collection are shown in Table 1 (on page 71). At diagnosis, some patients showed the Ph chromosome as the sole chromosome abnormality and trisomy 8 appeared as a karyotype evolution later on. Others showed the Ph chromosome and trisomy 8 at diagnosis. One patient showed karyotype evolution in addition to the Ph chromosome and +8 (case 10). The percentage of trisomic metaphases ranged from 5% to 40%. Table 2 (on page 71) summarizes the FISH and G-banding metaphase data on bone marrow cells for trisomy 8 for each patient.

Figure 1(on page 72) shows 7 bone marrow interphase nuclei with 2, 3, 4, and 5 signals for chromosome 8 after FISH, demonstrating the centrometric regions of chromosome 8 from diploid to quintosomy. FISH data from 100 interphase nuclei observed in 2 diploid controls and 3 of the 10 patients (cases 1, 9, and 10), using the LSI c-*myc* probe are shown in Table 3 (below). Figure 2 (on page 72) shows 7 nuclei with 2, 3, 4, and 5 signals indicative of diploid to multiple c-*myc* gene locus 8q24.2-24.3 regions, suggesting normal to abnormal multiples of the gene.

Comparing the percentages obtained by G-banding metaphase with those obtained by interphase FISH for detection of +8, we found that the percentages of +8 cells detected by interphase FISH were lower in patients with the chronic phase of the disease (11% versus 24% in cases 1-5), similar in patients with accelerated phase (13% versus 10% in cases 6 and 7), the same in 1 patient with lymphoid blast crisis (5% in case 8), and higher in 2 patients with myeloid blast crisis (81% versus 33% in cases 9 and 10). The percentage of interphase FISH nuclei showing 3 signals of the c-*myc* locus was very similar to the results obtained by interphase FISH for +8 for 3 selected patients (5%, 78%, 90% versus 2%, 77%, 83% in cases 1, 9, and 10, respectively).

Discussion

The FISH technique using specific DNA sequences probes for the centromeric region of human chromosomes can be applied to interphase nuclei to detect numerical chromosomal aberrations [11]. An important advantage of FISH is that the number of cells studied is larger (500 nuclei) than that which can be examined by CCT (20 metaphases), providing better statistical information. Using CCT and

Table 3. FISH data for the c-*myc* gene locus in controls and selected patients.

	Hybridization spots per nucleus				
	0	1	2	3	>4
Control					
1	0	1	98	0	1
2	0	1	97	1	1
Case					
1	0	1	94	5	0
9	0	0	21	78	1
10	0	0	9	90	1

Table 1. Patients' characteristics at time of specimen collection.

Case	Age/Sex	Diagnosis	Blast %	Karyotype
1	27/M	CP	1	46,XY,t(9;22)(q34;q11)[11]/47,XY,+8,t(9;22)(q34;q11)[3]/46,XY[6]
2	28/M	CP	2	47,XY,+8,t(9;22)(q34;q11)[2]/46,XY[18]
3	35/M	CP	1	46,XY,t(9;22)(q34;q11)[12]/47,XY,+8,t(9;22)(q34;q11)[8]
4	22/F	CP	1	46,XX,t(9;22)(q34;q11)[15]/47,XX,+8,t(9;22)(q34;q11)[5]
5	41/F	CP	1	47,XX,+8,t(9;22)(q34;q11)[5]/46,XX[12]
6	38/M	AP *	4	46,XY,t(9;22)(q34;q11)[16]/47,XY,+8,t(9;22)(q34;q11)[3]
7	55/M	AP	22	46,XY,t(9;22)(q34;q11)[19]/47,XY,+8,t(9;22)(q34,q11),del (11)(q22)[1]
8	27/M	LBC	77	46,XY,t(9;22)(q34;q11)[18]/47,XY,+8,t(9;22)(q34;q11)[1]/46,XY[1]
9	27/F	MBC	30	46,XX,t(9;22)(q34;q11)[15]/47,XX,+8,t(9;22)(q34;q11)[5]
10	70/M	MBC	60	46,XY,+8,t(9;22)(q34;q11),-13[2]/46,idem,del(11)(p12)[7] 49,idem,del(11)(p12),+del(11)(p12),+12,+der(22)t(9;22)[4]/ 47,X,-Y,+8x2,t(9;22)(q34;q11),der(17;21)(p11.1;p13),+der (22)t(9;22)[2]/ 45,XY,+8,t(9;22)(q34;q11),add(13)(q34), -15,der(17;21)(p11.1;p13)[2]/47,XY,+8,t(9;22)(q34;q11), del(11)(p12),-13,+der(22)t(9;22),+mar[cp2]/46,XY[1]

AP Accelerated Phase, CP Chronic Phase, LBC Lymphoid Blast Crisis, MBC Myeloid Blast Crisis
* Basophilia (21%)

Table 2. FISH and G-banding metaphase data on bone marrow cells for trisomy 8 on 10 Ph+ CML patients with +8.

Case	Hybridization spots per nucleus for chromosome 8				FISH +8/500 Interphases Examined (%)	G-Banding +8/Metaphases Examined (%)
	1	2	3	4		
1	3	487	10	0.0	2	15
2	3	475	20	2	4	10
3	13	439	48	0.0	9.6	40
4	2	408	89	1	17.8	25
5	15	366	117	2	23.4	29.4
6	16	379	101	4	20.2	15.7
7	2	471	25	2	5	5
8	3	472	25	0.0	5	5
9	2	104	387	7	77.4	25
10	2	45	420	33	84	40

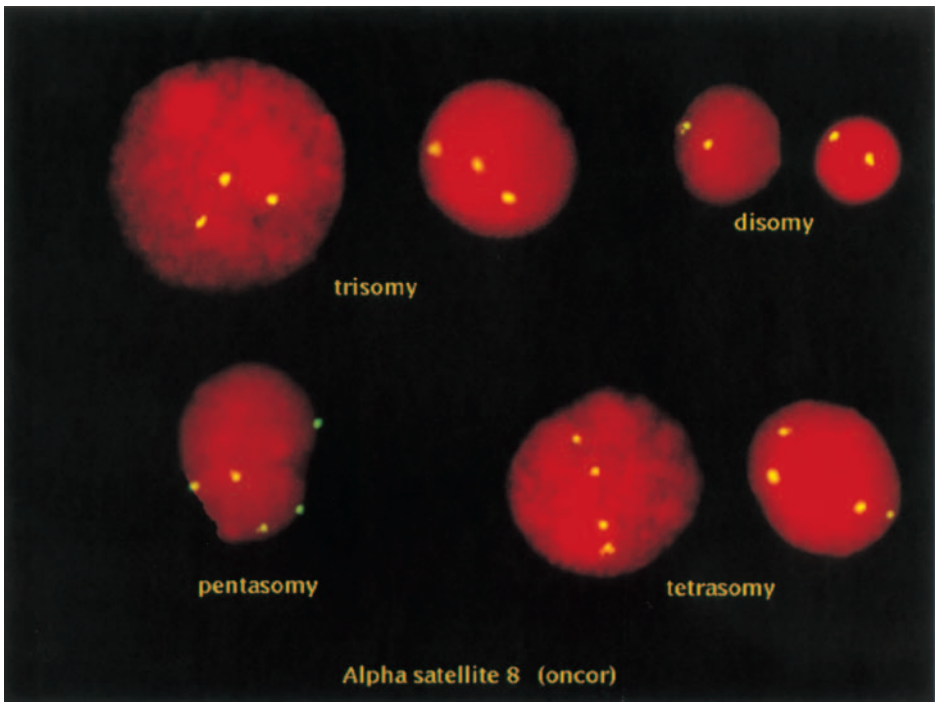

Fig. 1. Seven bone marrow interphase nuclei showing 2, 3, 4, and 5 signals for chromosome 8.

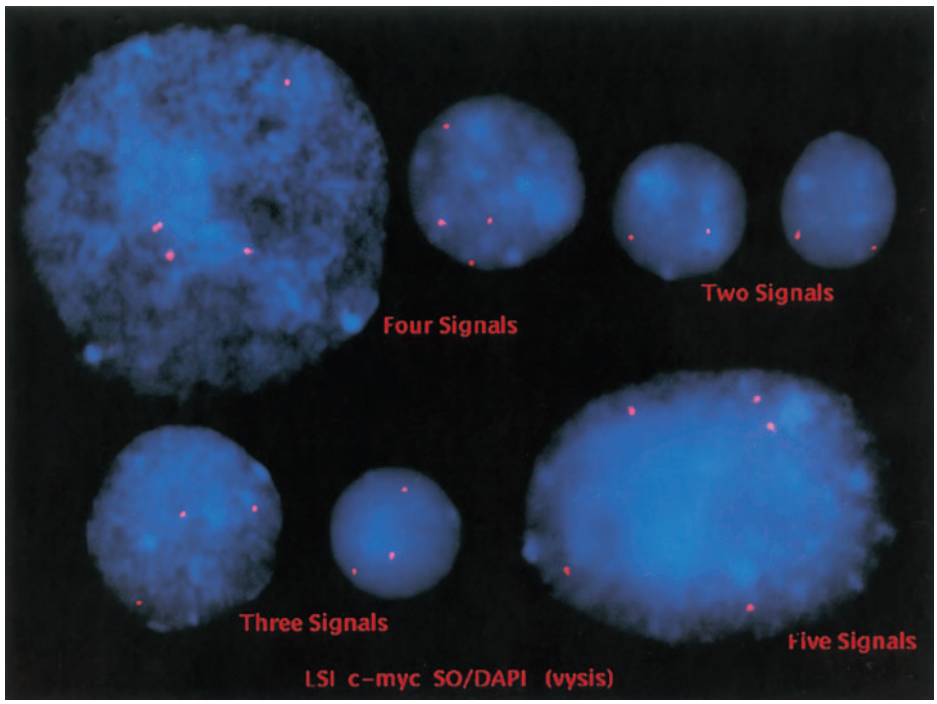

Fig. 2. Seven bone marrow interphase nuclei showing 2, 3, 4, and 5 signals for the c-*myc* gene locus 8q24.2-24.3 region.

interphase FISH, we analyzed bone marrow cells from 10 Ph+ CML patients in different stages of the disease in which +8 had been detected with G-banding. A Ph chromosome was present at initial diagnosis in some or all metaphases for each patient.

Trisomy 8 was present in 5-40% of metaphases. Trisomy 8 was detected at a lower percentage by FISH than by metaphase analysis in patients in the chronic phase of the disease. Similar results have been reported [12]. This finding might be explained by the fact that trisomic cells may have a variable replication time when compared to disomic cells during the chronic phase of the disease. Similar percentages of trisomy 8 in interphase and metaphase nuclei were obtained in patients with the accelerated phase of the disease, suggesting an approximate synchronous cell cycling and a transformation of cells in interphase.

In the patient with lymphoid blast crisis evolving from Ph+ CML, there were similar trisomy 8 percentages determined by metaphase analysis and interphase FISH (5%). This raises the question of whether lymphoid transformation might be independent of +8, since the association of +8 and lymphoid blast transformation is rarely found and is only present in an estimated 1-2% of patients with acute lymphoblastic leukemia [13]. The percentage of trisomy 8 is increased in the interphase FISH studies compared to the studies of metaphase kayotypes in myeloid blast crisis, suggesting that genetic activities localized on chromosome 8 may play an important role in myeloid transformation.

One patient in the chronic phase of the disease (case 1) and 2 in myeloid blast crisis (cases 9 and 10) were analyzed with the LSI c-*myc* probe for the c-*myc* gene locus located in the 8q24.2-24.3 region. Where trisomy 8 was detected by interphase FISH, there were three signals of c-*myc* gene locus found by FISH. These findings are expected since the gene is located in chromosome 8. Several studies have shown that c-*myc* expression is elevated in the progression of CML and in association with trisomy 8 in blast crisis of CML [14-17].

In summary, this study compares conventional cytogenetic analysis and FISH for detection of trisomy 8 in Ph+ CML patients and examines the c-*myc* gene copy number in select trisomy 8 cases. Lower percentages of interphase cells were found to have trisomy 8 (as detected by FISH), in comparison to metaphase cells (conventional cytogenetics) in cells from patients in chronic phase CML. The opposite pattern was observed in 2 specimens from patients in myeloid blast crisis. Three copies of the c-*myc* gene were detected in 3 cases with trisomy 8.

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