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BRIEF COMMUNICATION

Trisomy 8 in Myelodysplasia and Acute Leukemia Is Constitutional in 15–20% of Cases

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The trisomy 8 found in malignancies may derive from a constitutional trisomy 8 mosaicism (CT8M), and in these cases the trisomy itself may be regarded as the first mutation in a multistep carcinogenetic process. To assess the frequency of CT8M in hematological dysplastic and neoplastic disorders with trisomy 8, an informative sample of 14 patients was collected. The data ascertained included chromosome analyses of fibroblast cultures and of PHA-stimulated blood cultures in patients with normal blood differential count, as well as possible CT8M clinical signs. One patient showed trisomy 8 in all cell types analyzed and undoubtedly has a CT8M; a second patient consistently showed trisomy 8 in PHA-stimulated blood cultures when no immature myeloid cells were present in blood and should be considered as having CT8M; a third patient, with Philadelphia-positive chronic myelocytic leukemia, was more difficult to interpret, but the possibility that she had CT8M is likely. A few clinical signs of CT8M were also present in these three patients. Our data indicate that the frequency of CT8M in hematological dysplastic and neoplastic disorders with trisomy 8 is approximately 15–20%.

In 1996, we postulated that trisomy 8 found in neoplasms, particularly in hematological malignancies, may be derived from a constitutional trisomy 8 mosaicism (CT8M) (Seghezzi et al., 1996). Trisomy 8 was the first mutation of the multistep carcinogenesis in the three patients reported and, most likely, in another eight patients reviewed from the literature. We noted that eight out of the 12 documented cases of CT8M developing a malignancy had myeloproliferative disorders (Danesino et al., 1998), which is not unexpected, as trisomy 8 is particularly frequent in these malignancies (Mitelman et al., 2001).

We have collected from our laboratory files all the patients with myelodysplastic syndromes (MDS), acute leukemia (AL), or myeloproliferative disorder (MD) with trisomy 8 in order to establish how frequently they arise in CT8M. We tried to obtain three types of data which might be able to demonstrate CT8M: 1) results of chromosome analyses of fibroblasts; 2) results of chromosome analyses of PHA-stimulated blood cultures in patients with a normal blood differential count; and 3) possible clinical signs of CT8M, which we searched for personally, based on the most reliable available list (Schinzel, 1994). In total, 14 patients were retrieved, of which one (case 7) has been reported previously. The clinical and cytogenetic findings are summarized in Table 1.

Chromosome analyses of fibroblasts provided evidence for CT8M in case 11, whereas PHA-stimulated blood cultures suggested CT8M in case 8. The combined results of chromosome analyses of bone marrow (BM) and peripheral blood (PB) of case 7 indicated the possibility that this patient also had a CT8M. Table 2 gives the details of relevant chromosome analyses performed in these three patients.

A search for clinical signs of CT8M was performed in 13 of the 14 patients, excluding case 4, who was affected with Down syndrome. We did not find any significant features of CT8M in any of the patients, although some minor signs were often

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TABLE 1. Patients Included in the Study

Patient	Age/Sex	Diagnosis[a]	Significant +8 karyotype—Material[b]	Available data on PB-PHA[b] cytogenetics	Available data on CT8M[c] phenotype	Available data on FB[b] cytogenetics
1—SMG	79/F	MDS	48,XX,+8,+9[15]—BM	−	+	+
2—SA	54/M	AML-M4	47,XY,+8[19]/46,XY[1]—BM	+	+	+
3—MB	66/M	AML-M2	47,XY,+8,inv(9)[24]—BM	+	+	+
4—CD[d]	1/M	ALL	49,XY,+8,+21"c",+21[20]/47,XY,+21"c"[5]—BM 49,XY,+8,+21"c",+21[8]/48,XY,+8,+21"c" [2]—PB	+	+	+
5—RT	69/F	MDS-S	47,XX,+8[8]/46,XX[9]—BM	+	+	+
6—MI	24/M	RA	47,XY,+8[3]/46,XY[37]—BM	+	+	+
7—TM	61/F	CML	47,XX,+8,t(9;22)[3]/47,XX,+8[3]/46,XX,t(9;22)[7]/ 46,XX[9]—BM	+	+	−
8—FF	3/M	RA	47,XY,+8[20]—BM	+	+	+
9—ME	58/F	CML-BP	47,XX,+8,t(9;22)[12]/46,XX[1]—PB	+	−	+
10—MA	72/F	MDS	47,XX,+8[13]—BM	+	+	+
11—BCI	25/F	RA	47,XX,+8[24]/46,XX[1]—BM	+	+	+
12—BC	76/F	PV	47,XX,+8[3]/46,XX[40]—BM	+	−	+
13—CV	77/M	MDS	46,X,−Y,+8[7]/45,X,−Y[19]—BM	−	−	+
14—FA	12/M	AML-M2	47,XY,+8[22]—BM	+	+	+

[a]MDS = myelodysplastic syndrome; AML = acute myeloblastic leukemia; M4, M2 = FAB type; ALL = acute lymphoblastic leukemia; MDS-S = secondary MDS; RA = refractory anemia; CML = chronic myelocytic leukemia; BP = blastic phase; PV = polycythemia vera.
[b]BM = bone marrow direct preparations and 24–48 hr cultures; PB = peripheral blood unstimulated cultures; PB-PHA = PHA-stimulated blood cultures; FB = fibroblasts from skin or other tissue biopsy.
[c]CT8M = constitutional trisomy 8 mosaicism.
[d]Affected with Down syndrome.

present. It has been known since 1980, however, that CT8M may be associated with a near-normal phenotype and a normal IQ (Chandley et al., 1980). The wide variation of phenotypes in CT8M has been extensively discussed (Seghezzi et al., 1996; Danesino et al., 1998; Habecker-Green et al., 1998), and we searched for CT8M signs only to obtain possible ancillary evidence.

Below, data favoring CT8M in patients 7, 8, and 11 are summarized.

Patient 7

We previously reported this patient, affected with chronic myelocytic leukemia (CML), due to her peculiar cytogenetic pattern, with cells with the Philadelphia chromosome (Ph), cells with the Ph and trisomy 8, and cells with trisomy 8 without the Ph (Casali et al., 1992). Three similar cases, with Ph-negative trisomic 8 clones in patients with Ph-positive CML, have been reported since then (Bilhou-Nabera et al., 1993; Ariyama et al., 1995; Shepherd et al., 1996). In these cases, however, no cells were present with the trisomy 8 superimposed on the Ph, as in our patient (Table 2), a finding associated with accelerated/blastic phase (BP) of CML. Ariyama et al. (1995) also provided evidence that in their case the trisomic 8 clone showed no *BCR/ABL* rearrangement.

In our previous article (Casali et al., 1992), we suggested several explanations for this cytogenetic feature. One, which we deem very unlikely, is a retromutation to correct the Ph translocation in a subclone derived from the one with Ph and trisomy 8. We favored the possibility of a secondary MDS in a clone independent from CML, which by chance had acquired the same anomaly. Bilhou-Nabera et al. (1993) agreed with this explanation. We must admit, however, that no signs of MDS were ever noted in the bone marrow of our patient, or documented in the other three mentioned above, and that our patient entered a BP only in July 1997, 11 years after CML diagnosis and 7 years after the finding of the additional trisomy 8. Again, secondary MDS is infrequent in the course of CML, and the severe pancytopenic phase of our patient (Casali et al., 1992) may well be explained by prolonged α-interferon (IFN) therapy. CT8M is a third possible explanation for these observations, and it is certainly compatible with a normal or near-normal phenotype, as was the case in our patient: the only argument against CT8M would be that the two Ph-positive clones, with and without trisomy 8, should imply an origin of CML in at least two different marrow cells, constitutionally with two and three chromosomes 8. CML is strictly considered monoclonal in origin, and even in sub-

TABLE 2. Results of Chromosome Analyses in Patients 7, 8, and 11

Date	Material[a]	Karyotype
		Case7—TM
18.06.90	BM	46,XX,t(9;22)(q34;q11)[7]/47,XX,t(9;22),+8[3]/47,XX,+8[3]/46,XX[9]
16.07.90	BM	46,XX,t(9;22)(q34;q11)[17]/47,XX,t(9;22),+8[6]/46,XX[5]
	PB-PHA	46,XX,t(9;22)(q34;q11)[1]/47,XX,+8[2]/46,XX[32]
15.05.96	PB	46,XX,t(9;22)(q34;q11)[14]
	PB-PHA	46,XX,t(9;22)(q34;q11)[20]/46,XX[40]
		Case 8—FF
12.10.92	PB-PHA	47,XY,+8[5]/46,XY[62]
12.01.93	PB-PHA	47,XY,+8[7]/46,XY[93]
	PB-DEB	47,XY,+8[1]/46,XY[50]
16.04.93	BM	47,XY,+8[5]/46,XY[8]
07.06.93	BM	47,XY,+8[20]
30.06.93[b]	BM	46,XX[30]
14.12.93	PB-PHA	46,XX[30]
	SF[c]	46,XY[50]
	SF[d]	46,XY[50]
		Case 11—BC
02.12.98	BM	47,XX,+8[24]/46,XX[1]
24.02.99	BM	47,XX,+8[21]/46,XX[5]
02.03.99	PB-PHA	47,XX,+8[14]/46,XX[6]. nuc ish 8cen(PZ8.4×2)[290]/(PZ8.4×3)[210]
12.05.99	PB-PHA	47,XX,+8[15]/46,XX[56]
12.07.99	PB-PHA	47,XX,+8[8]/46,XX[12]
	PB-LPS	47,XX,+8[1]/46,XX[2]
	SF	47,XX,+8[80]/46,XX[40]

[a]BM = bone marrow direct preparations and 24–48 hr cultures; PB-PHA = PHA-stimulated peripheral blood cultures; PB = unstimulated peripheral blood cultures; SF = skin fibroblast culture; PB-DEB = PHA-stimulated peripheral blood cultures with diepoxybutane (0.01 g/ml) added; PB-LPS = peripheral blood cultures stimulated with *E. coli* lipopolysaccharide.
[b]After allogeneic bone marrow transplantation from a female donor.
[c]First growth halo.
[d]Second growth halo.

TABLE 3. Reported Cases With Chromosome Changes (CC) in Ph-negative Cells in the Course of Ph-positive CML After α-Interferon Therapy

Patient	CC in Ph − cells	Presence of the same CC in Ph + cells	Evidence of MDS	Reference
Casali et al., 1992—present case n. 7	+8	+	−	5
Bilhou-Nabera et al., 1993	+8	−	−	6
Ariyama et al., 1995	+8	−	−	7
Seghezzi et al., 1996—pt. 3	−7	−	−	8
Seghezzi et al., 1996—pt. 4	+8	−	−	8
Seghezzi et al., 1996—pt. 5	5q-	−	−	8
Fayad et al., 1997—pt. 1	del(5)(q13q34), clonal evolution	−	+	11
Fayad et al., 1997—pt. 2	18p+, −6	−	−	11
Fayad et al., 1997—pt. 3	del(11)(q21q23)	−	−	11

jects with a constitutional chromosome mosaicism it was demonstrated that it was restricted to one cell population (Chaganti et al., 1982; Oguma et al., 1989).

Five patients with Ph-positive CML who developed Ph-negative clones with abnormalities different from trisomy 8 have been reported (Shepherd et al., 1996; Fayad et al., 1997). Thus, a total of nine Ph-positive CML cases are known in which a Ph-negative clone with different chromosome anomalies was found, and in all cases after IFN therapy (Table 3). A specific effect of IFN was therefore suspected, with the possible development of MDS, but although IFN in CML seems to lead to Ph-positive clones with aberrant chromosomal evolution patterns (Johansson et al., 1996), Fayad et al. (1997) concluded that it is not possible to attribute the origin of abnormal Ph-negative clones to IFN treatment. It is noteworthy that MDS was documented only in one of these patients (Table 3). A

Ph-positive clone with the same anomaly found in Ph-negative cells was present only in our patient (Table 3).

Two efforts to demonstrate CT8M in our patient by means of chromosome analysis of fibroblasts cultured from a skin biopsy and from a biopsy of uterine mucosa unfortunately failed. The most plausible explanation for the results obtained is that our patient had a CT8M, that the Ph arose in a trisomic bone marrow cell at the onset of the disease, and that the trisomy was then corrected to disomy in a subclone. If this is true, the trisomy 8 would not indicate a progression towards BP.

In order to explain cases such as those listed in Table 3, Fayad et al. (1997) suggested the possibility that, since CML is a stem-cell disorder, the selective Ph suppression obtained with IFN therapy may allow the expansion of other clones originating from "abnormal Ph-negative stem cells" (Shepherd et al., 1997), which are usually masked by proliferating Ph-positive cells. According to our interpretation of CT8M, the stem-cell abnormality is likely to be the constitutional trisomy 8.

Patient 8

This 3-year-old child was investigated due to anemia, and a trisomy 8 was found in PHA-stimulated blood cultures, performed also with diepoxibutane (DEB) to search for the chromosome breakage syndrome Fanconi's anemia. A few months later, refractory anemia (RA) was diagnosed, with subsequent evolution into RA with excess of blasts in transformation (RAEB-T). He underwent an allogeneic bone marrow transplantation. Chromosome analysis of fibroblasts cultured from a skin biopsy did not reveal any trisomic 8 cells (Table 2). While the finding of trisomy 8 in these cells would be conclusive for CT8M, its absence does not exclude mosaicism because the distribution of the different cell lines in tissues of a subject with a chromosome mosaicism is unpredictable.

The phenotype of this child was evaluated twice, when he was 4 and 5 years old, and judged normal, as was mental development, with only a few possible CT8M signs.

Our conclusion of CT8M relies on the consistent presence of trisomic cells at the analyses made on PHA-stimulated blood cultures (also in the presence of DEB) at onset on two different occasions before RA diagnosis (Table 2), when differential blood counts did not include any immature cells. Two hematologists independently reviewed the original blood smears of the same dates of chromosome analyses, and they confirmed the normal results.

Patient 11

Slightly reduced levels of neutrophils and erythrocytes in peripheral blood, but absence of any other symptoms, were noted in this patient, who presented at the age of 9 years. RA was diagnosed at 25 years of age when chromosome analyses of bone marrow cells revealed a trisomy 8 (Table 2). Cells with trisomy 8 were found in all tissues examined: PHA-stimulated lymphocytes, LPS-stimulated peripheral blood culture, and fibroblasts from a skin biopsy (Table 2). A fluorescence in situ hybridization (FISH) analysis on interphase nuclei, with a centromere-specific chromosome 8 probe, was also done to ascertain better the proportion of trisomic cells. The patient was a university student, and her clinical phenotype was normal, with only some possible CT8M signs. The cytogenetic and FISH results obtained are summarized in Table 2 and are compatible with CT8M.

Thus, 3 out of 14 cases of MDS, AL, and MD characterized by trisomy 8 originated from a CT8M—a frequency of 21%. If we exclude patient 7, whom we believe to be CT8M, but with less solid evidence than for patients 8 and 11, we would conclude that the frequency was 2/14 (14%). Obviously, our sample is too small to draw any firm conclusions, but we may postulate that the proportion of MDS/AL/MD which harbor a supposed acquired trisomy 8 and are in fact CT8M is 15–20%. In these cases, as in the ones previously reported by us (Seghezzi et al., 1996), the constitutional trisomy 8 would be the first mutation of multistep carcinogenesis.

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