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Monosomy 7 in myeloid malignancies: parental origin and monitoring by real-time quantitative PCR

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Monosomy 7 is one of the most frequent chromosome changes observed in patients with myelodysplastic syndromes (MDS) and acute myeloid leukaemia (AML), and it may also be found superimposed to the Philadelphia chromosome in chronic myelocytic leukaemia (CML) in accelerated or blastic phase. It is even more common in secondary MDS/AML, and is associated with a variety of Mendelian and non-Mendelian predisposing disorders or in the so-called monosomy-7 families, where it recurs in subjects developing MDS/AML.[1] Also partial monosomy 7 due to structural rearrangements (which thus has a different mechanism of origin as compared to monosomy, that is chromosome breakage vs non-disjunction) is frequently found in association with all the disorders mentioned above, but a single common region of deletion has not yet been identified. Familial cases indicated as unlikely the action of an oncosuppressor gene in the pathogenesis of MDS/AML associated with monosomy 7,[1] and its role is thought to be mediated by gene dosage effects, recently investigated by microarray analysis.[2] The presence of monosomy 7 portends a poor clinical outcome, both in MDS[3] and in AML,[4] and hence the clinical relevance of accurate monitoring of the abnormal clone during the course of the disease. We present here data concerning the methods to monitor the consistency of the monosomic clone and the parental origin of the chromosome 7 loss. As to the first point, the monitoring results obtained with cytogenetic techniques were compared with those of a newly developed method based on real-time quantitative polymerase chain reaction (RQ-PCR)[5,] which we suggest is more reliable. As to the parental origin of the lacking chromosome 7, some data are already available in the literature,[6] and our aim was to further contribute to clarifying the issue.

Table 1 lists the clinical and cytogenetic characteristics of the 18 monosomy 7 patients included in this study. Chromosome changes additional to monosomy 7 were present in 11 of them, either as a constitutional or as a clonal anomaly in the bone marrow (BM). The cohort includes 10 men and 8 women, with an age range between 3 and 42 years. Patients 1 and 3 were affected with Down syndrome, and an underlying Mendelian disease was present in patients 9, 10, 11 and 14 (familial platelet disorder with propensity to acute myelogenous leukaemia, and Kostmann disease). Patients 1, 8, 9, 10, 11, 17 and 18 were reported in detail. Chromosome analyses were part of the routine work made for clinical purposes, and were performed on BM direct preparations and 24–48 h cultures, and on peripheral blood (PB) unstimulated 24–48 h cultures with routine techniques. QFQ-banding technique was applied. The entire cohort of patients was used to investigate the parental origin of the missing chromosome 7. DNA was extracted with routine techniques from the same material drawn for the chromosome analyses showing the monosomy 7, and from the peripheral blood of both parents of each patient; it was genotyped selecting at least five short tandem repeats, scattered along the entire chromosome 7. All methods were as described previously.[1] Fluorescence *in situ* hybridization (FISH) on interphase nuclei was carried out on BM and PB preparations by standard procedures with a chromosome 7-specific alphoid centromeric probe (courtesy of Professor M Rocchi, Cytogenetic Unit, University of Bari, Italy – Resources for Molecular Cytogenetics) to quantify cells with monosomy 7.

Ten DNA samples from BM and PB of patients 1, 5, 8, 9, 10 and 12 were used to assess the proportion of cells with monosomy 7 by our newly developed RQ-PCR method. The DNA was from the same cells used for chromosome and FISH analyses. Four samples of patient 1 were drawn in different phases of the disease (CML). All these six patients were constitutionally heterozygous for the

Table 1 Characteristics of the patients included in the study and parental origin of the missing chromosome 7

Case no.	*Age/sex*	*Diagnosis*[a]	*Anomalies additional to monosomy 7*	*Parental origin of the missing 7*
1	11/M[b]	CML-BP	t(9;22)(q34;q11),+21c	Maternal
2	7/F	RAEB	+X	Paternal
3	7/M[b]	MDS, sec. (AML-M6)	+21c	Paternal
4	9/M	JMML	−19	Maternal
5	12/F	AML-M2	+21	Paternal
6	8/M	AML	–	Maternal
7	3/M	JMML	–	Maternal
8	12/F	RA	+21/+19,+21	Paternal
9	42/M	RA	+8 (in a different clone)	Paternal
10	8/M	RA > RAEB-t > AML-M6	i(17)(q10),+22	Maternal
11	12/F	RA	–	Maternal
12	4/F	RAEB	–	Maternal
13	13/M	RAEB-t	+8	Paternal
14	8/F	MDS > AML-M1 or M2	–	Maternal
15	3/M	MDS	–	Maternal
16	6/M	MDS	–	Maternal
17	17/F	RA > AML-M0	+8	Paternal
18	12/F	RA	add(2)(q?),add (13)(q?)	Maternal

[a]AML, acute myeloid leukaemia; M6, M2, M1, M0, FAB-types; CML-BP, chronic myelocytic leukaemia in blastic phase; JMML, juvenile myelomonocytic leukaemia; MDS sec, secondary myelodysplastic syndrome; RAEB, refractory anaemia with excess of blasts; RA, refractory anaemia; RAEB-t, refractory anaemia with excess of blasts in transformation.
[b]Affected with Down syndrome.

Table 2 Results of chromosome analyses, FISH on interphase nuclei (nuc ish) and RQ-PCR on patients 1, 5, 8, 9, 10 and 12

Case no.	*Material*[a]	Date	*Metaphase cytogenetics*	*nuc ish*[b] *(%)*	*RQ-PCR*[c] *(95% confidence interval)*
1	BM	13.10.94	46,XY,−7,t(9;22)(q34;q11),+21c [3]/47,XY, t(9;22)(q34;q11),+21c [1]	−7 in 443/581 (76%)	91.7% (90.4–92.9)
	BM	12.01.95	46,XY,−7,t(9;22)(q34;q11),+21c [13]	Not available	94.6% (94–95.2)
	BM	01.02.95	46,XY,−7,t(9;22)(q34;q11),+21c [1]/47,XY,+21c [41]	−7 in 314/1277 (25%)	27.5% (16.6–37)
	PB	21.06.95	46,XY,−7,t(9;22)(q34;q11),+21c [15]	Not available	88.5% (87.1–89.8)
5	BM	27.01.98	46,XX,−7,+21 [23]	Not available	95.9% (94.9–96.8)
8	PB	04.09.00	46,XX,−7,+21 [1]/47,XX,−7,+19,+21 [2]	Not available	22.1% (13.5–29.8)
9	PB	04.06.01	45,XY,−7 [2]/46,XY [1]	−7 in 64/267 (24%)	30.2% (22–37.5)
10	PB	01.06.01	45,XY,−7 [1]/46,XY,-7,i(17)(q10), +22 [3]/46,XY [6]	Not available	89.6% (88.4–90.7)
12	BM	01.12.04	45,XY,−7 [18]/46,XY [2]	−7 in 275/462 (59%)	77.8% (77,1–78.6)
	PB	01.12.04	45,XY,−7 [2]/46,XY [3]	−7 in 300/472 (63%)	70.9% (70.3–71.4)

Abbreviations: BM, bone marrow; FISH, fluorescence *in situ* hybridization; PB, peripheral blood; RQ-PCR, real-time quantitative polymerase chain reaction.
[a]BM, bone marrow direct preparations and 24–48 h cultures; PB, peripheral blood unstimulated 24–48 h cultures.
[b]Number of monosomic/total scored nuclei.
[c]Percentage of monosomic cells.

diallelic insertion/deletion polymorphism MID1064,[7] which maps in 7q35, and which we used in preliminary experiments to set up and validate the method.[5] Briefly, we demonstrated in pooled DNA of disomic and monosomic cells that the percentage of the monosomic ones could be extrapolated accurately and precisely from the ratio of the two alleles measured by means of an allele-specific RQ-PCR assay, which is performed by targeting the two alleles with allele-specific forward primers.[5] Each quantification was performed on 100 ng DNA, and was repeated nine times. A calibrator (DNA from a healthy heterozygous subject) was used to normalize the results. Nine repetitions were sufficient for statistical analysis of the data; the imprecision in the measurements was expressed as 95% confidence interval. The formula used then to infer the proportion of monosomic cells is

$$100\times(1-2^{-(\Delta C_t[\text{sample}]-\Delta C_t[\text{blank}])})$$

where ΔC_t is the difference between the threshold cycles of the two alternative MID 1064 alleles, as obtained in the DNA under study (sample) and in the healthy heterozygous subject (blank).

The parental origin of the missing chromosome 7 in our 18 patients is reported in Table 1. Table 2 gives the results of the chromosome analyses, FISH on interphase nuclei (nuc ish) and RQ-PCR used to monitor the proportion of monosomic cells in 10 BM and PB samples from patients 1, 5, 8, 9, 10 and 12.

The possibility that the chromosome 7 lost in myeloid dysplastic and proliferative diseases in childhood had a non-random parental origin has been debated in the literature. Reported data are available for a total of 29 cases, taking into account both sporadic and familial cases;[6] the origin of the missing 7 was paternal in 16 cases and maternal in 13. Our results refer to 18 patients, and in these the missing 7 was of paternal origin in 7 cases, and maternal in 11 (Table 1). Thus, overall, the origin of the monosomy 7 is paternal in 23 cases and maternal in 24. The distribution of paternal and maternal origin was not significantly different for any of the variables examined: total or partial monosomy, predisposing conditions (Mendelian,

MDS/AML familiarity, trisomy 21), presence or absence of numerical or structural chromosome changes in addition to monosomy 7. As to unpublished cases, a large number of samples were collected by O A Haas from many different laboratories (the collection includes seven of our 18 patients also); the parental origin of the missing chromosome 7 was investigated with a different method, and approximately half of more than 130 informative cases had either lost the paternal or maternal homologue (O A Haas, personal communication, November 2006). Therefore, we can definitely confirm that there is no preferential parental origin of the missing 7, thus ruling out pathogenetic hypotheses based on genomic imprinting phenomena.

The monosomy 7 in course of MDS/AML is usually monitored by chromosome analysis and FISH on interphase nuclei. Our approach through the use of RQ-PCR proved to be more precise and of considerable practical interest. In this regard, it is worth noting that the results obtained by RQ-PCR refer to 900 ng of DNA, which corresponds to the content of about 128 000 cells, according to the available evaluations of human genome size.[8]

The comparison of chromosome analysis, nuc ish, and RQ-PCR results (Table 2) led to the following observations.

In patient 1, the proportion of monosomic cells evaluated on the BM taken on 13 October 1994 was different using the nuc ish approach and with RQ-PCR (76 vs 90%), while chromosome analysis showed three monosomic mitoses out of four. In the material drawn on 12 January 1995 and on 21 June 1995, RQ-PCR showed a portion of normal cells, not found at chromosome analysis, as the percentage of monosomic cells evaluated were 94 and 88, respectively. In the material obtained after the patient had received chemotherapy for CML blast phase (1 February 1995), monosomic cells seemed to be very few at chromosome analysis (1 out of 42), while both nuc ish and RQ-PCR showed that they still represented about 25% of BM cells.

In patient 5, RQ-PCR showed the presence of a small proportion (around 5%) of normal non-monosomic cells, not found at chromosome analysis.

In patient 8, only three cells all with monosomy 7 were available for chromosome analysis, while RQ-PCR showed that the majority of the BM cells were normal, the percentage of monosomic cells being 25–30.

In patient 9, again very few mitoses were available for chromosome analysis (two being monosomic out of three); both nuc ish and RQ-PCR consistently showed that the monosomic cells represented around 25% of BM cells.

In patient 10, the results were highly discordant as only four mitoses out of the 10 available were monosomic at chromosome analysis, but RQ-PCR showed that the monosomy involved around 90% of BM cells.

The results were also discordant in patient 12, in whom the majority of BM cells were monosomic at chromosome analysis (18/20), whereas their proportion was evaluated to be 59% by nuc ish and around 78% by RQ-PCR, respectively. In this case, the amount of normal cells was shown by RQ-PCR and by nuc ish to be higher than expected, with a slight difference between these two results: this was also observed in PB.

The number of mitoses that were possible to be analyzed with conventional chromosome analyses of our patients was between 3 and 43, and the number of nuclei scored by FISH ranged between 267 and 1277 (Table 2). It is therefore obvious that the results obtained by RQ-PCR are more reliable because they are representative of larger samples, that is of the order of 10^5 cells. This method is confirmed to be efficient and accurate in the detection of the percentage of cells with monosomy 7. The comparison of the results obtained by chromosome analysis, FISH on nuclei, and RQ-PCR showed similar results in some BM and PB samples, and differences in others. Little significance may be given to the differences of RQ-PCR evaluations compared to chromosome analysis, both due to the fact that the latter often refer to very limited number of mitoses and to the fact that chromosome analysis evaluates only cells which are in mitotic division. More significant are the differences with the results of FISH on nuclei, and the evaluations by RQ-PCR are invariably higher than those by nuc ish (Table 2). Altogether, our results indicate the superiority of the RQ-PCR method, which is time-saving and cost-effective, both for scientific speculations and for clinical use.

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