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ORIGINAL PAPER

FISH analysis for *TET2* deletion in a cohort of 362 Brazilian myeloid malignancies: correlation with karyotype abnormalities

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Abstract We investigated the prevalence of *TET2* deletion by using a new FISH probe in a cohort of 362 Brazilian patients with myeloid neoplasms and their association with cytogenetic information (*G-banding* analysis). Normal karyotype was observed in 45.8 % of MDS ($n = 44$), 43.8 % of AML ($n = 39$) and 46.3 % of MPN ($n = 82$). Abnormalities of 4q24 (deletions, translocations or inversions) were associated with another chromosomal abnormality in four patients by *G-banding* analysis (2 MDS, 1 AML and 1 MPN). Interphase FISH analysis revealed deletion of *TET2* in 21 patients (6 patients with abnormal karyotype and in 15 patients with normal karyotype). arrayCGH analysis revealed a cryptic deletion of the region 4q24 in all eight patients selected with myeloid malignancies (3 MDS, 1 AML and 4 MPN). Considering the significantly high cost of determining the mutational status of *TET2* in patient samples by using conventional sequencing methods and sometimes the lack of regular use of SNP/aCGH array methodologies, FISH for the detection of *TET2* abnormalities may become a potentially useful clinical tool. The search for alterations in *TET2* gene may be important for the prediction of prognosis in normal/altered AML patients' karyotype or in the disease evolution of patients with MNP and MDS.

Keywords: *TET2* · MDS · AML · MPN · Cytogenetics · FISH · SKY · arrayCGH

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Introduction

TET2, a putative tumor suppressor gene, located at band 4q24 has been described as an important element in the pathogenesis of myeloid malignancies, including myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN) and acute myeloid leukemia (AML) [1, 2]. Recently, it has been demonstrated that the loss of *TET2* is associated with a progressive enlargement of the hematopoietic stem cell compartment and eventual myeloproliferation [3]; additionally, the loss of *TET2* leads to increased hematopoietic stem cell self-renewal and myeloid transformation in mice [3–5]. Somatic truncating mutations, including nonsense mutations and small insertions/deletions, and missense mutations in *TET2*, were described in patients with hematological malignancies [5]. These mutations are frequently acquired during progression of MPN or MDS to secondary AML [6–8] and have been associated with low overall survival (OS) rate in patients with AML [9–11].

Considering the significantly high cost of determining the mutational status of *TET2* in patient's samples by using conventional sequencing methods and the lack of regular use of SNP/aCGH array methodologies, FISH for detection of *TET2* abnormalities may become a potentially useful clinical tool. The search for alterations in *TET2* may be important for prediction of prognosis in patients with AML

and normal karyotype or intermediate risk cytogenetics or in the disease evolution of patients with MPN and MDS [6, 12].

We investigated the prevalence of *TET2* deletion by using a FISH probe developed by Agilent Technologies in patients diagnosed with MDS, AML and MPN (polycythemia vera, essential thrombocythemia and primary myelofibrosis) and their association with cytogenetic information (*G-banding* analysis). Also, we better characterized *TET2*-positive patients (normal karyotype and 4q24 abnormalities) by using spectral karyotype analysis (SKY). Additionally, we applied *arrayCGH* in order to confirm the loss of *TET2* on the samples studied. To the best of our knowledge, this is the first attempt to elucidate the frequency of *TET2* deletion by FISH and karyotype correlation in a cohort of Brazilian patients.

Material and methods

Patients

For this investigation, we performed FISH for *TET2* deletion in 362 bone marrow samples of hematological malignancies [MDS ($n = 96$), AML ($n = 89$), MPN ($n = 177$), polycythemia vera ($n = 54$), essential thrombocythemia ($n = 52$) and myelofibrosis ($n = 71$)]. The patients selected for this study had diagnosis confirmed by clinical and laboratory parameters, according to WHO classification [13]. Median age of the total cohort was 56 years (range 28–85 years; Table 1). Karyotype information assessed, by *G-banding* analysis and also FISH, for specific rearrangements was obtained in all patients at the moment of diagnosis. Bone marrow samples were collected between May 2003 and February 2012 from the Hematology Division, Medical School of Ribeirão Preto, University of São Paulo, Brazil; Hematology, Stem Cell Transplantation Unit, Pio XII Foundation, Barretos, Brazil; and Hematology Unit, Hospital of Base of São José do Rio Preto, São Paulo, Brazil. The study was approved by the University Hospital Ethics Committee (HCRP 1735/2011).

G-banding, fluorescence in situ hybridization (FISH) and spectral karyotyping analysis (SKY)

Cytogenetic analysis of hematopoietic cells was performed as described in the previous investigations [14]. Briefly, mononuclear cells were cultured for 72 h in RPMI 1640 (Invitrogen, Carlsbad, CA, USA), supplemented with 15 % heat-inactivated standard fetal bovine serum (HyClone, Logan, UT, USA), 2 mM L-glutamine, 100 U/mL penicillin and 100 mg/mL streptomycin. The karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN, 2009) [15]. The karyotype information was considered abnormal in the presence of at least two metaphases showing the same structural abnormality or chromosome gain in at least two metaphases, or loss of the same chromosome in at least three metaphase cells. The metaphase images were acquired by using an Axio Imager M2 microscope (Zeiss, Jena, Germany) equipped with the *BandView* software, version 5.5 (ASI, Carlsbad, CA, USA). In order to better classify the MDS group, we also applied a panel of "*MDS FISH probe set*" [inv(3q)(q21;q26.2), t(3;3)(q21;q26.2), -5/del(5q), -7/del (7q), +8 and del(20q)] on all samples ($n = 96$). The hybridization spots were evaluated using an Axio Imager M2 microscope (Zeiss, Jena, Germany) equipped with the *FISHView* software, version 5.5 (ASI, Carlsbad, CA, USA). The *cutoff* levels for t(3;3)/inv(3q) (>2.5 %), del(5q) (>3 %), del(7q) (>2.4 %), trisomy 8 (>2.5 %) and del(20q) (>2.0 %) were established according to the interphase FISH (iFISH) patterns observed in a group of 30 age- and sex-matched normal control peripheral blood samples studied with the same probes. The probes used in this study were purchased from Kreatech Diagnostics (Amsterdam, the Netherlands). For each sample three hundred interphase cells were viewed and counted.

For *TET2* analysis we applied a more sophisticated technology, as described below. From the same fixed cell suspension obtained at diagnosis and used for *G-banding*, we applied interphase FISH analysis, using SureFISH 4q24 TET2 probe (Agilent Technologies). At least 200 nuclei were scored per sample by using an epifluorescence microscope AxioImager M2 (Carl Zeiss, USA) coupled to the Metafer Slide Scanning System, allowing scan of eight slides (MetaSystems, USA). The low frequency of structural alterations in *TET2* by FISH in patients with hematological malignancies was decisive to perform an automatic FISH analysis. This tool is extremely valuable for diagnosis and offers a more precise result. Hybridization spots were obtained according to manufacturer's recommendations, and 200 nuclei were scored per sample. To establish the *cutoff*, 20 peripheral blood normal samples (12 male and 8 female) were used as controls. The *cutoff* value to consider one sample as positive was defined as average plus three standard deviations and was 2.66 % for deletions.

We also performed SKY analysis on samples with normal and altered karyotype, harboring *TET2* deletion by FISH ($n = 21$), in order to confirm abnormalities involving 4q24 and possibly detect cryptic abnormalities involving the same region. Chromosome labeling was performed with the SKY fluorescent labeling kit (Applied Spectral Imaging, Migdal HaEmek, Israel) according to the manufacturer's protocol. A minimum of twenty metaphases were analyzed using the SKYView 5.5 software (ASI, Carlsbad, CA, USA).

Table 1 Clinical characteristics and *TET2* deletion status in 362 patients with myeloid diseases

Characteristics	MDS $n = 96$	AML $n = 89$	MPN $n = 177$	Total cohort $n = 362$
TET2 deletions (FISH)	7/96 (7.3 %)	5/89 (5.6 %)	9/177 (5.0 %)	21/362 (5.8 %)
Males/females	42/54	49/40	92/85	183/179
Median age, years	54 (28–85)	58 (8–90)	69 (54–92)	69 (66–89)
Median hemoglobin, g/dL	8.6 (7.4–14.8)	12.6 (4.4–15.2)	11.2 (9.0–12.9)	10.8 (4.4–15.2)
Median platelet count, x109/L	77 (39–953)	74.5 (11–824)	497 (79–889)	216.1 (11–953)
Median WBC count, x109/L	5.6 (2.9–12.6)	52.2 (3.2–278)	17.4 (4.2–96.4)	25 (2.9–278)
Percentage bone marrow blasts	<5 %	56.2 % (27–89 %)	<5 %	–
Normal karyotype	52/96 (54.2 %)	29/89 (32.6 %)	82/177 (46.3 %)	163/362 (45 %)
Cytogenetic alterations	44/96 (45.8 %)	60/89 (67.4 %)	95/177 (53.7 %)	199/362 (55 %)
Abnormalities involving 4q24	2/44 (4.6 %)	1/60 (1.6 %)	3/95 (3.0 %)	5/199 (2.5 %)

arrayCGH

For *arrayCGH*, 44 k ($n = 8$), 60mer oligonucleotide microarrays were used (Human Genome CGH Microarray, Agilent Technologies, USA). Commercial male DNAs were obtained as a reference DNA with the same protocol (Promega, Madison, WI). The slides were scanned using a GenePix Professional 4200A scanner, and the results were interpreted by using the software CGH Analytics 4.0.85 (Agilent Technologies). Copy number changes were defined by intervals of two or more adjacent probes with $\log_2$ ratios suggestive of a deletion or duplication when compared with the $\log_2$ ratios of the adjacent probes, with a threshold of 5. We used the recommended default threshold for abnormalities detection module-2 algorithm, and the accuracy of aberration cells was confirmed on the basis of known FISH aberrations.

Statistical analysis

Comparisons between different groups were made using the Student's t test and the two-sided exact Fisher test (dichotomous variables). Statistical analyses were performed using $P < 5$ % was considered significant.

Results

Deletions of *TET2* were observed in 21 of 362 patients studied (6 %), in six patients with cytogenetically abnormal and in 15 patients with normal karyotype (Table 2). Abnormalities of 4q24 (translocations or inversion) were associated with another chromosomal abnormality in four patients by *G-banding* (2 MDS, 1 AML and 1 MPN). In MDS subgroup, the majority of patients were classified as refractory cytopenia with multilineage dysplasia (48/96; 50 %), followed by refractory anemia (22/96; 23 %), refractory anemia with excess blasts I (18/96; 19 %) and 5q syndrome (8/96; 8 %). Additionally, clonal chromosomal abnormalities were seen in 52 of 96 patients (54 %), and normal karyotype was observed in 44 patients (46 %). In this subgroup, with normal karyotype, FISH deletion of *TET2* was observed in five of 44 (11.5 %) patients (Table 2). Complex karyotype (i.e., presence of at least three chromosome aberrations) was observed in only one patient, in association with 4q24 rearrangement [t(4;12)(q24;q22)] (Fig. 1a, b). In another patient, we found loss of *TET2* associated with t(4;11)(q24;q13) and extra copy of chromosome 8 (Fig. 1c, d). Most of the MDS patients presented a single cytogenetic abnormality (39/96; 40 %). Numerical abnormalities were seen in 28 of 96 patients (30 %), being the extra copy of chromosome 8 the most common abnormality. On the other hand, structural abnormalities were also present in 37 of 96 patients (38 %).

In AML subgroup we stratified the patients according to the karyotype information. Normal karyotype was observed in 29 patients (36 %), in which FISH deletion of *TET2* was observed in four patients (4.5 %) (Table 2). Among the 60 patients with altered karyotype (68 %), nine harbored t(8;21)(q22;q22) (10 %), 26 t(15;17)(q22;q12) (29 %) and 11 inv(16)(p13q22)/t(16;16)(p13;q22) (12.5 %). Two groups of three patients presented isolated del(7)(q32) and trisomy 8, respectively. In two patients we found isolated trisomy 11 and one patient with the add(13)(q10). The remaining five patients were classified as having a complex karyotype, being one of them with abnormalities involving 4q24 [t(4;17)(q24;q25)] and loss of *TET2* (Fig. 2).

In the MPN subgroup, abnormal karyotype was observed in 95 of 177 patients (53 %) and normal karyotype was found in 82 of 177 (47 %). The polycythemia vera subgroup exhibited normal karyotype in 38 of 54 patients (70 %). In this subgroup we found FISH *TET2* deletion in three of 54 patients (5.5 %) (Table 2). Five patients displayed loss of chromosome Y (9 % of the male

Table 2 Hematological classification and karyotype information of 21 patients with 4q24 abnormalities and *TET2* deletion

Patients	Age/sex	Diagnosis	Karyotype (G-banding analysis)	Spectral karyotype (SKY)	FISH TET2-deletion
1	69/M	MDS-RA	46,XY[20]	46,XY[20]	189/200 (94.5 %)
2	77/M	MDS-RA	46,XY[20]	46,XY[20]	122/200 (61 %)
3	56/F	MDS-RAEB1	46,XX[20]	46,XX[20]	89/200 (44.5 %)
4	72/M	MDS-RAEB1	46,XY[20]	46,XY[20]	167/200 (83.5 %)
5	66/F	MDS-RA	46,XX[20]	46,XX[20]	129/200 (64.5 %)
6	59/F	MDS-RAEB1	46 ~ 47,XX,t(4;12)(q24;q22)[16], +19[4],del(20)(q11)[16]/46,XX[4]	46 ~ 47,XX,t(4;12)(q24;q22)[10], +19[2],del(20)(q11)[10]	176/200 (88 %)
7	78/M	MDS-RA	47,XY, +8,t(4;11)(q24;q13)[12]/46,XY[8]	47,XY, +8,t(4;11)(q24;q13)[14]/46,XY[6]	145/200 (72.5 %)
8	77/F	AML	46,XX[20]	46,XX[20]	92/200 (46 %)
9	64/M	AML	46,XY[20]	46,XY[20]	166/200 (83 %)
10	58/M	AML	46,XY[20]	46,XY[20]	194/200 (97 %)
11	53/F	AML	46,XX[20]	46,XX[20]	158/200 (79 %)
12	73/F	AML	48,XX,t(4;17)(q24;q25), +8, +9[16]/46,XX[4]	48,XX,t(4;17)(q24;q25), +8, +9[10]	188/200 (94 %)
13	67/M	MPN-PV	46,XY[20]	46,XY[20]	182/200 (91 %)
14	72/F	MPN-PV	46,XX[20]	46,XX[20]	169/200 (84.5 %)
15	56/F	MPN-PV	46,XX[20]	46,XX[20]	172/200 (86 %)
16	70/F	MPN-PM	46,XX[20]	46,XX[20]	171/200 (85.5 %)
17	68/M	MPN-PM	46,XY[20]	46,XY[20]	188/200 (94 %)
18	73/M	MPN-PM	46,XY[20]	46,XY[20]	196/200 (98 %)
19	59/F	MPN-PM	46,XX,t(4;15)(q24;q26)[20]	46,XX,t(4;15)(q24;q26)[10]	182/200 (91 %)
20	74/M	MPN-PM	47,XY,inv(4)(p16q28), +8[13]/46,XY[7]	47,XY,inv(4)(p16q28), +8[10]	176/200 (88 %)
21	82/F	MPN-PM	46,XX,t(4;6)(q24;p11)[17]/46,XX[3]	46,XX,t(4;6)(q24;p11)[10]	180/200 (80 %)

patients), and other chromosomal abnormalities were identified in 11 patients (20 %). Abnormalities included extra copy of chromosome 8 in six of 54 (11 %) patients, trisomy 9 in two patients (4 %) and del(20q) in three of 54 (5 %) patients. In essential thrombocythemia, 33 of 52 patients (64 %) showed normal karyotype and 19 of 52 (36 %) exhibited abnormal karyotype. Trisomy 8 and del(20q) were only sole abnormalities found in each case. No FISH *TET2* deletions were identified in essential thrombocythemia subgroup. On the other hand, among 71 cases of primary myelofibrosis ($n = 71$), abnormal karyotype was found in 60 of 71 patients (84 %) and only 11 of 71 presented normal karyotype. In three patients with normal karyotype, we found FISH *TET2* deletion. The most frequent abnormalities were del(20q), del(13q), +8 and +9. As previously presented, in three samples with apparently balanced abnormalities (4q24 rearrangements), we also identified FISH *TET2* deletion [t(4;15)(q24;q26);

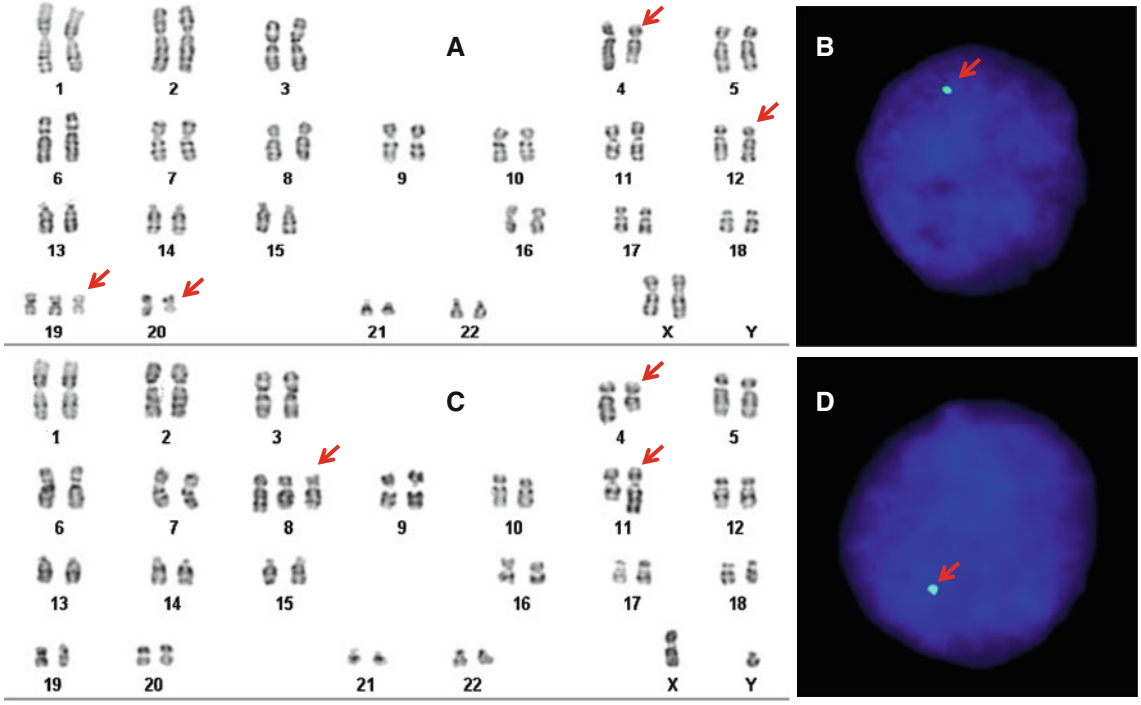

Fig. 1 Abnormalities involving 4q24 detected by *G-banding* analysis and confirmation of *TET2* loss by FISH on interphase nuclei. **a** Bone marrow sample of a MDS patient with a complex karyotype showing the t(4;12)(q24;q22), extra copy of chromosome 19 and del(20q) (*arrows*). **b** The same bone marrow patient's sample with *TET2* deletion, with a single green spot. **c** Another MDS patient with t(4;11)(q24;q13) and trisomy 8 (*arrows*). Additionally, detection of *TET2* loss in the same patients' sample by FISH (**d**)

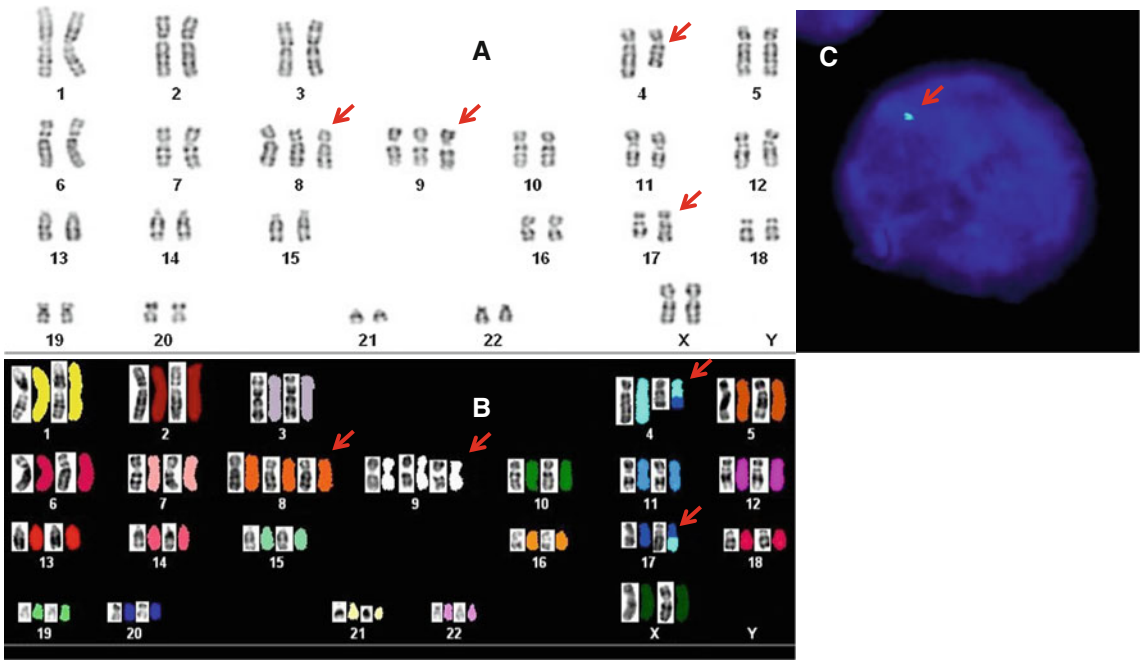

Fig. 2 Abnormalities involving 4q24 detected by *G-banding* and SKY analysis and confirmation of *TET2* loss by FISH on interphase nuclei, in an AML patient with complex karyotype. **a** Bone marrow sample of an AML patient with a complex karyotype showing the t(4;17)(q24;q25), extra copy of chromosomes 8 and 9 (*arrows*). **b** Confirmation of the same abnormalities previously seen by *G-banding*, by spectral karyotype analysis (SKY) (*arrows*). **c** Detection of *TET2* loss in the same patients' sample by FISH

inv(4)(p16q28) and t(4;6)(q24;p11)] (Table 2). Spectral karyotype (SKY) confirmed all 4q24 abnormalities detected by *G-banding* analysis (Table 2). However, SKY was not able to identify cryptic alterations involving 4q24, in patients with normal karyotype and *TET2* deletion.

Allele-specific, quantitative PCR analysis for $JAK2^{V617F}$ mutation status was performed in all 177 MPN patients using genomic DNA from bone marrow obtained at the time of the initial cytogenetic studies (diagnosis). $JAK2^{V617F}$ mutation was detected in 156 of the 177 (88 %) patients, without significant difference among the different cytogenetic profile. Additionally, all MPN patients with *TET2* deletion by FISH ($n = 9$) presented $JAK2^{V617F}$ mutation (*data not shown*).

ArrayCGH analysis of hematological disorders has become a valuable tool to identify cryptic alterations, define novel subtypes of disease and enhance prediction of OS [16]. We applied arrayCGH in eight samples, with normal karyotype in order to confirm the loss of *TET2* by FISH. The confirmation was obtained in three samples of MDS (RA, $n = 1$; RAEB1, $n = 2$), one of AML and four of MPN (polycythemia vera, $n = 1$; primary myelofibrosis, $n = 3$). Additionally, arrayCGH detected 42 abnormalities, including gains and losses (5.25 alterations per patient; range 3–7 changes) (Table 3; Fig. 3). The median size of copy number alterations was 0.1–34.6 Mb with a mean of 4.3 Mb. We also observed that the genomic amplifications displayed log2 ratios ($\sim$0.6) an indication of low copy number gains, which ranged in size from 0.1 to 34.6 Mb and deletions ranged in size from 0.1 to 22.6 Mb (Table 3; Fig. 3).

Discussion

We aimed to evaluate the frequency of *TET2* deletion by using a new FISH probe in a cohort of 362 Brazilian patients with clinical diagnosis of MDS, AML and MPN. Also, we correlated the frequency of *TET2* loss with the karyotypic information, and additionally, we performed arrayCGH analysis in order to confirm *TET2* deletion in a small group of patients. Currently, the most effective techniques used to identify *TET2* mutations are arrayCGH, SNP arrays and molecular techniques [17, 18]. However, considering the high cost of these methodologies, FISH may become an important tool for routine cytogenetic screening.

According to our findings, deletion of *TET2* was observed in 21 patients, being six with additional chromosomal abnormalities, including 4q24 rearrangements, and 15 with normal karyotype. The overall frequency was 6 %. The frequencies for each subgroup were 6.25 % in MDS, 5.6 % in AML and 5.8 % in MPN. In the latter subgroup we did not observe *TET2* loss in essential thrombocythemia. Another important finding in the present investigation confirms previous reports that *TET2* deletion is rarely seen in myeloid malignancies as it was detected in fewer than 6.5 % of cases in the present cohort [6, 17]. No relationship was noted between *TET2* deletion and karyotype information. In MDS ($n = 96$), for example, five patients with normal karyotype presented the deletion, and in only one patient with complex karyotype [46-47, XX,t(4;12)(q24;q22)[16], +19[4],del(20)(q11) [16]/46, XX[4]], *TET2* deletion was found. In AML patients we also detected *TET2* loss in a patient with complex karyotype [48,XX,t(4;17)(q24;q25), +8, +9[16]/46,XX[4]]. However, five of them with normal karyotype presented the deletion. In particular, patients with apparently balanced translocations, t(4;11)(q24;q13) t(4;12)(q24;q22), t(4;15)(q24;q26), t(4;6)(q24;p11) and t(4;17)(q24;q25), showed an absolutely unexpected loss of *TET2*.

Some previous studies suggested that additional genetic lesions may cooperate with *TET2* abnormalities in leukemogenesis [4, 19]. However, even if we consider the arrayCGH profile in attempting to detect cryptic alterations that may enhance prediction of OS in MDS group with normal karyotype and *TET2* deletion (RA, $n = 1$; RAEB1, $n = 2$), we found less than four abnormalities per patient, as presented in Table 3. *TET2* mutations are frequently acquired during progression of MPN or MDS to secondary AML [4, 19, 20], also associated with shorter OS in patients with AML [1, 21]. On the other hand, previous investigations confirmed that *TET2* mutations were either not significantly correlated with survival in patients with AML or associated with a decreased risk of progression of MDS to AML and longer OS [22]. In a recent study focused on *de novo* adult AML who had achieved complete remission with intensive chemotherapy, Nibourel and coworkers [23] observed that between patients with and without *TET2* alterations for most pretreatment characteristics, including gender, age, hemoglobin level, white blood cell count, platelet count, French-American-British subtype distribution, cytogenetics and *FLT3* or *CEBPA* alterations, no significant difference was seen. They also concluded that no significant difference was noted in OS or disease-free survival between patients with and without *TET2* mutations.

In MPN, the relationship between the presence of a *TET2* mutation and the prognosis for OS remains unclear [24]. According to previous investigations, mutational frequencies of *TET2* in polycythemia vera, essential thrombocythemia and primary myelofibrosis were 14, 8 and 20 %, respectively [25, 26]. In the majority of patients with MPN, only one copy of *TET2* is mutated. It argues in favor of a possible role of *TET2* haploinsufficiency in the pathogenesis of these diseases [20]. On the other hand, the

Table 3 arrayCGH analysis of eight patients with *TET2* deletion by FISH

Patients	Age/sex	Diagnosis	Karyotype (G-banding analysis)	Gain/loses	Chromosome band	Genes	Size, Mb
1	69/M	MDS-RA	46,XY[20]	L	*3q21.3*	*GATA2*	0.2
				L	***4q24****	***TET2****	0.4
				G	*5q35*	*NPM1*	0.1
				G	*11p15.4*	*HBD*	0.2
				L	*12q24*	*NOC4*	1.2
				L	*21q22.12*	*RUNX1*	0.4
2	56/F	MDS-RAEB1	46,XX[20]	L	***4q24****	***TET2****	5.2
				G	*6p22.1*	*Histone gene cluster*	0.4
				G	*15q26.1*	*IDH2*	0.6
				L	*20q11.21*	*ASXL1*	1.0
3	72/M	MDS-RAEB1	46,XY[20]	L	***4q24****	***TET2****	0.4
				G	*17p13.3*	*INPP5 K*	2.4
				G	*20p11.21*	*PYGB and others*	0.8
4	64/M	AML	46,XY[20]	G	*2q34*	*IDH1*	0.5
				G	*3q13.12*	*CD47*	12.4
				L	***4q24****	***TET2****	0.8
				L	*5q35*	*NPM1*	0.7
				L	*14q23.2*	*?*	11.6
5	67/M	MPN-PV	46,XY[20]	L	***4q24****	***TET2****	22.6
				G	*7q22.2*	*NAMPT*	0.6
				G	*8p11*	*MYST3*	34.6
				L	*9p24*	*JAK2*	0.4
				L	*11p15*	*NUP98*	0.8
				L	*13q12*	*FLT3*	2.8
6	70/F	MPN-PM	46,XX[20]	G	*1p34.2*	*MPLV*	13.5
				L	***4q24****	***TET2****	0.7
				L	*7q32.3*	*MIR29A*	1.1
				L	*19p13.3*	*ELANE*	0.6
				L	*16q22*	*CBFB*	0.9
				G	*17q25.3*	*THOC4*	1.7
				G	*19q13.32*	*ZNF296*	4.6
7	68/M	MPN-PM	46,XY[20]	G	*3q13.13*	*KIAA1524*	5.4
				L	***4q24****	***TET2****	0.6
				L	*6q21*	*NKAIN2*	0.1
				L	*10q22*	*TET1*	1.6
				G	*19q13.1*	*RASGRP4*	10.4
8	73/M	MPN-PM	46,XY[20]	L	*2q33.3*	*IDH1*	8.6
				L	*3p22*	*MIRN128-2*	0.2
				G	*3q21*	*MCM2*	0.9
				L	***4q24****	***TET2****	22.4
				L	*9p24*	*JAK2*	6.4
				L	*15q26.1*	*BLM*	0.4

relationship between $JAK2^{V617F}$ mutation and molecular mechanisms of MPN progression is also not clear. However, some studies have demonstrated that a $JAK2^{V617F}$ mutation is an unfavorable prognostic factor based on hematologic, clinical and cytogenetic parameters; only a low number of studies showed different observations [19, 27]. The reported overall frequency of chromosomal abnormalities in MPN varies between 3 and 40 % [27]. Our cohort showed an overall rate of chromosomal abnormalities of 53 %, which is at the higher end of what has been reported. However, this percentage may reflect the criteria of selection used in this investigation that firstly considered

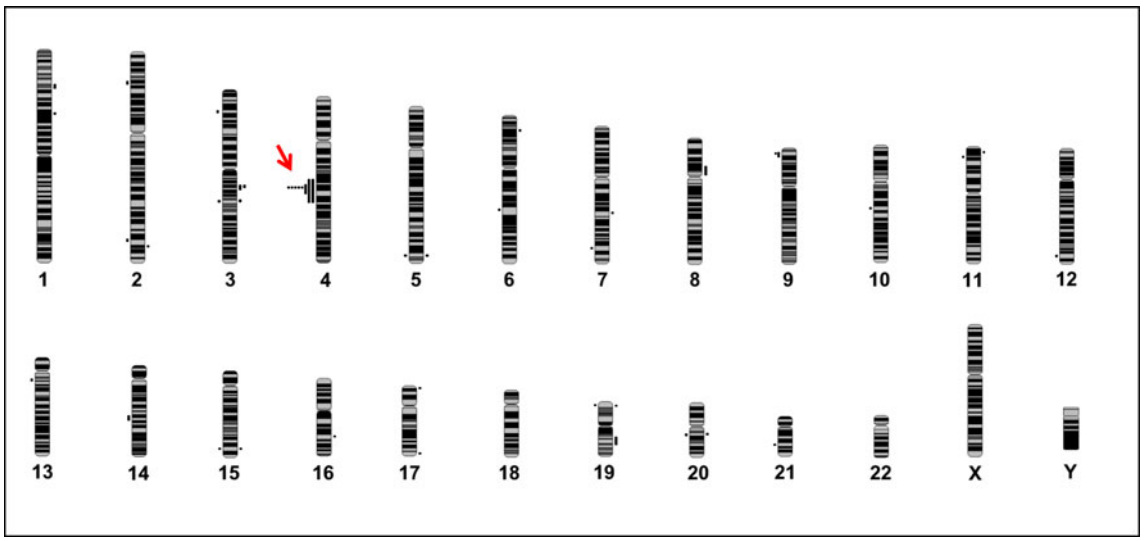

Fig. 3 arrayCGH profile of cryptic abnormalities in MDS, AML and MPN patients, with TET2 deletion identified by FISH (*arrow*). Genome frequency distribution of chromosomal imbalances in eight patients as detected by aCGH and visualized by SeeGH software. Lines to the *right* indicate gains of genomic material and to the *left* of the chromosome indicate loss of genomic material

patients positives for $JAK2^{V617F}$ mutation, to date 156 of the 177 (88 %) patients. No relationship was observed between patients with normal/altered karyotype and *TET2* deletion. In primary myelofibrosis, we identified three patients with normal karyotype and also more than three patients with balanced 4q24 abnormalities, both groups harboring *TET2* loss and $JAK2^{V617F}$ mutation.

We also observed that patients with *TET2* deletion ($n = 21$) presented older age (average 67.7 years, range 53–82 years) compared with patients without deletion ($P < 0.05$). According to the previous studies, the prevalence of *TET2* mutations gradually increased with age, from 7 % in adults younger than 30 years of age to 32 % in patients aged 70 years or older [9]. Increased age is an indicator of poor prognostic in MDS [16]. Our finding suggests that poor outcome with increasing age may be related to greater disruption of the genome of hematopoietic stem/progenitor cells of older patients. Alternatively, these findings may represent an epigenetic phenomenon resulting from cumulative genotoxic exposure over time in older patients in general. Also, *TET2*-mutated patients presented with a higher WBC and were more likely to be female than *TET2* wild-type patients [16]. However, in our cohort we did not find a correlation with a higher WBC or sex distribution ($P > 0.05$).

Although arrayCGH is considered a high-cost strategy for a routine diagnostic laboratory, our data suggest that this approach will be a useful diagnostic and prognostic tool for evaluation of patients with myeloid diseases, clustered into the low-risk group (normal karyotype). Additionally, we observed that more extensive genome imbalances may be a feature key of older patients. In our analysis, we did not take into consideration the consequences of specific genomic alterations in the prognosis or management of the patients studied. As mentioned before, arrayCGH findings were important to confirm the loss of *TET2* by FISH among the samples studied. However, copy number alterations in our analysis involving genes, such as *GATA2, NPM1, RUNX1, IDH2* and *ASXL1* (Table 3; Fig. 3), are consistent with the features of MDS and propensity to develop AML [28]. The only AML sample investigated by arrayCGH showed gain of *IDH1* and loss of *NPM1*. The first one is associated with a more MDS advanced disease and progression to AML [28]. On the other hand, mutations in *NPM1* gene are the most frequent molecular alteration in AML with normal karyotype (approximately 60 % of the patients) [29]. As a consequence, the NPM1 mutant intracellular traffic is altered leading to its aberrant accumulation in the cytoplasm of leukemic cells and generally characterized by good response to induction chemotherapy and favorable prognosis [29]. The average of genomic imbalances in MPN was higher than MDS (6 vs. 4.3, respectively) (Table 3). Loss of *JAK2, NUP98, FLT3, MIR29A, CBFB, TET1 and IDH1* was also observed in MPN subgroup. In two MPN patients, being one polycythemia vera and the other one a case of primary myelofibrosis, we identified loss of *JAK2* gene by arrayCGH and mutation of the present allele, $JAK2^{V617F}$.

Our study is the first Brazilian attempt to combine the role of FISH test for *TET2* deletion in a routine karyotype analysis for monitoring disease progression and stratifying risk in MDS, AML and MPN patients. We conclude that although 4q24 structural abnormalities are predictive of *TET2* deletion, it would be reasonable to expect that FISH could be useful in routine clinical practice to analyze the status of *TET2* deletion, especially in patients with normal karyotype. However, it is important to consider that *TET2*

is more frequently affected by loss-of-function mutations than deletions, as demonstrated by an extensive review of the literature [18–27]. Although FISH may represent a cheap way to stratify the patients according to *TET2* deletion status, it may underestimate the percentage of cases with abnormalities involving *TET2*. Thus, the relative prognostic importance of this new test should not only be considered in multivariable models but also needs to be investigated in the context of accepted classification systems and multicenter studies which are important to develop a molecular-cytogenetic-based risk stratification system for malignant disease management.

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Conflict of interest All the authors reported no potential conflicts of interest.

References

1. Abdel-Wahab O, Mullally A, Hedvat C, Garcia-Manero G, et al. Genetic characterization of TET1, TET2, and TET3 alterations in myeloid malignancies. Blood. 2009;114(1):144–7.
2. Delhommeau F, Dupont S, Della Valle V, et al. Mutation in TET2 in myeloid cancers. N Engl J Med. 2009;360(22):2289–301.
3. Moran-Crusio K, Reavie L, Shih A, et al. TET2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. Cancer Cell. 2011;20(1):11–24.
4. Abdel-Wahab O, Manshouri T, Patel J, et al. Genetic analysis of transforming events that convert chronic myeloproliferative neoplasms to leukemias. Cancer Res. 2010;70(2):447–52.
5. Quivoron C, Couronné L, Della Valle V, et al. TET2 inactivation results in pleiotropic hematopoietic abnormalities in mouse and is a recurrent event during human lymphomagenesis. Cancer Cell. 2011;20(1):25–38.
6. La Starza R, Crescenzi B, Nofrini V, et al. FISH analysis reveals frequent co-occurrence of 4q24/TET2 and 5q and/or 7q deletions. Leuk Res. 2012;36(1):37–41.
7. Pronier E, Delhommeau F. Role of TET2 mutations in myeloproliferative neoplasms. Curr Hematol Malig Rep. 2012;7(1):57–64.
8. Tripodi J, Hoffman R, Najfeld V, Weinberg R. Frequency of heterozygous TET2 deletions in myeloproliferative neoplasms. Cancer Manag Res. 2010;2:219–23.
9. Metzeler KH, Maharry K, Radmacher MD, et al. TET2 mutations improve the new European LeukemiaNet risk classification of acute myeloid leukemia: a cancer and leukemia group b study. J Clin Oncol. 2011;29(10):1373–81.
10. Makishima H, Jankowska AM, McDevitt MA, et al. CBL, CBLB, TET2, ASXL1, and IDH1/2 mutations and additional chromosomal aberrations constitute molecular events in chronic myelogenous leukemia. Blood. 2011;117(21):198–206.
11. Roche-Lestienne C, Marceau A, Labis E, et al. Mutation analysis of TET2, IDH1, IDH2 and ASXL1 in chronic myeloid leukemia. Leukemia. 2011;25(10):1661–4.
12. Pignataro DS, Abdel-Wahab O. FISHing for TET2: utility of FISH for TET2 deletions detection in clinical samples. Leuk Res. 2012;36(1):25–6.
13. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood. 2002;100(7):2292–302.
14. Morato de Oliveira F, Lucena-Araujo AR, Favarin MD, et al. Differential expression of AURKA and AURKB genes in bone marrow stromal mesenchymal cells of myelodisplastic syndrome: correlation with G-banding analysis and FISH. Exp Hematol. 2012. doi:10.1016/j.exphem.2012.10.009 (Epub ahead of print).
15. Shaffer LG, Slovak ML, Campbell LJ, editors. ISCN 2009: an international system for human cytogenetic nomenclature 2009. Basel: S. Karger; 2009.
16. Starczynowski DT, Vercauteren S, Telenius A, et al. High-resolution whole genome tiling path array CGH analysis of CD34 + cells from patients with low-risk myelodysplastic syndromes reveals cryptic copy number alterations and predicts overall and leukemia-free survival. Blood. 2008;112(8):3412–24.
17. Bacher U, Weissmann S, Kohlmann A, et al. TET2 deletions are a recurrent but rare phenomenon in myeloid malignancies and are frequently accompanied by TET2 mutations on the remaining allele. Br J Haematol. 2012;156(1):67–75.
18. Kohlmann A, Grossmann V, Klein HU, et al. Next-generation sequencing technology reveals a characteristic pattern of molecular mutations in 72.8% of chronic myelomonocytic leukemia by detecting frequent alterations in TET2, CBL, RAS, and RUNX1. J Clin Oncol. 2010;28(24):3858–65.
19. Schaub FX, Looser R, Li S, et al. Clonal analysis of TET2 and JAK2 mutations suggests that TET2 can be a late event in the progression of myeloproliferative neoplasms. Blood. 2010;115(10):2003–7.
20. Saint-Martin C, Leroy G, Delhommeau F, et al. Analysis of the ten-eleven translocation 2 (TET2) gene in familial myeloproliferative neoplasms. Blood. 2009;114(8):1628–32.
21. Kosmider O, Gelsi-Boyer V, Ciudad M, et al. TET2 gene mutation is a frequent and adverse event in chronic myelomonocytic leukemia. Haematologica. 2009;94(12):1676–81.
22. Kosmider O, Gelsi-Boyer V, Cheok M, et al. TET2 mutation is an independent favorable prognostic factor in myelodysplastic syndromes (MDSs). Blood. 2009;114(15):3285–91.
23. Nibourel O, Kosmider O, Cheok M, et al. Incidence and prognostic value of TET2 alterations in de novo acute myeloid leukemia achieving complete remission. Blood. 2010;116(7):1132–5.
24. Brecqueville M, Rey J, Bertucci F, et al. Mutation analysis of ASXL1, CBL, DNMT3A, IDH1, IDH2, JAK2, MPL, NF1, SF3B1, SUZ12, and TET2 in myeloproliferative neoplasms. Genes Chromosom Cancer. 2012;51:743–55.
25. Tefferi A, Lim KH, Abdel-Wahab O, et al. Detection of mutant TET2 in myeloid malignancies other than myeloproliferative neoplasms: CMML, MDS MDS/MPN and AML. Leukemia. 2009;23(7):1343–5.
26. Tefferi A, Pardanani A, Lim KH, et al. TET2 mutations and their clinical correlates in polycythemia vera, essential thrombocythemia and myelofibrosis. Leukemia. 2009;23(5):905–11.
27. Zamora L, Xandri M, Garcia O, et al. Association of JAK2 mutation status and cytogenetic abnormalities at diagnosis in myeloproliferative neoplasms and myelodysplastic/myeloproliferative neoplasms. Am J Clin Pathol. 2012;137(4):677–8.
28. Bejar R, Levine R, Ebert BL. Unraveling the molecular pathophysiology of myelodysplastic syndromes. J Clin Oncol. 2011;29(5):504–15.
29. Falini B, Martelli MP. NPM1-mutated AML: targeting by disassembling. Blood. 2011;118(11):2936–8.