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Submicroscopic deletions in 5q- associated malignancies

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A B S T R A C T

Background and Objectives. The deletion of the long arm of chromosome 5 is common in myelodysplastic syndromes (MDS) but is not limited to the 5q- syndrome as it is also seen in acute myeloid leukemia (AML), where it is often associated with other karyotypic aberrations. The aim of this study was to investigate whether deletions of known suppressor sequences occur in myeloid malignancies associated with 5q- .

Design and Methods. Thirty patients with MDS or AML were selected for the presence of a 5q karyotypic deletion, either isolated (19 cases) or associated with other chromosome changes (11 cases). Multiple fluorescent *in situ* hybridization (FISH) in interphase nuclei was applied in all cases using a panel of eleven probes for known suppressor genes or loci deleted in MDS/AML. Metaphase FISH was also performed to clarify discrepancies between conventional and molecular cytogenetics.

Results. No additional deletions were found in nineteen cases with an isolated 5q-. Mono-allelic deletions where found in 9/11 cases, 3 of which were related to monosomies by conventional cytogenetics. Interphase-FISH showed p53, AML1, D13S25, NF1, or Ikaros in six out of nine (66%) patients with 5q- and additional karyotypic changes. Metaphase FISH was helpful in assigning some of these cryptic events to non-proliferating cells.

Interpretation and Conclusions. Our study emphasizes that isolated 5q- is the marker of a highly stable clone in both MDS and AML. AML with isolated 5q- are molecularly closer to 5q- syndrome than to AML with complex changes. Interphase-FISH data strongly support a mutator phenotype underlying complex karyotypes with a 5q deletion.

Key words: 5q-, complex karyotype, microdeletions

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An interstitial deletion of the long arm of chromosome 5, the only cytogenetic marker of the 5q- syndrome, remains isolated during this long-lasting disease.[1] On the other hand, isolated 5q- or 5q- combined with other karyotypic changes may be associated with acute myeloid leukemia (AML), frequently arising after radio-chemotherapy.[2,3] In the myelodysplastic syndrome (MDS)/AML with 5q- within complex karyotypes, gross chromosomal changes are mainly deletions involving 3p, 7, 12p, and 17p.[4] Mutations of the p53 gene are also frequent in this cytogenetic subgroup.[5]

Although cytogenetic analysis shows that band 5q31 is always involved in 5q- associated malignancies, molecular studies have not definitively identified critical suppressor gene(s). In this interphase fluorescent *in situ* hybridization (FISH) study we investigated whether putative suppressor sequences other than those at 5q are lost in myeloid malignancies associated with a 5q. The results add strong evidence that MDS/AML with only 5q- and those with 5q- in complex karyotypes are the products of different genomic backgrounds and possibly of different microenvironmental influences.

Design and Methods

Patients

Thirty patients with a diagnosis of MDS or AML and an isolated 5q- (19 cases) or a 5q- plus other karyotypic changes (11 cases) were selected from the files of the Hematology and Bone Marrow Trasplantation Unit of the University of Perugia, Italy, of the Hospital Sant Pau in Barcelona, Spain and of the Hematology Clinic of the University of Florence, Italy. Morphologic diagnoses had been made according to the FAB classification in all cases.

Table 1. Genomic probes used in the multiple I-FISH study.

Clone	*Genes/loci*	*Chromosome localization*	*Standard of monosomy (%)*
B88J3	FHIT	3p14.2	5
Bikaros	ikaros	7p13	6.1
LSI D7S486 SO/CEP 7 SG	D7Z1	7q31	1.96
B380G5	PTEN	10q23.3	4.5
PP74J1	WT1	11p13	4.8
B241D13	ATM	11q23	5.7
LSI D13S25 SO	D13S25	13q14	4.3
B199F11	TP53	17p13	4.7
P926B9/1002G3	5'NFI/3'NF1	17q11	3.14
B748M14	MADR2	18q21	6.1
PDJ1107L6	AML-1	21q22	4.7

P: PAC; B: BAC.

Conventional cytogenetics and painting

Cytogenetic analysis was performed at time of diagnosis on bone marrow metaphases after short-term cultures. Karyotypes were examined after G-banding with Wright's stain. Whole chromosome painting (WCP) for chromosomes 7, 13, 17 and 21 was used to analyze complex karyotypes (Appligene Oncor, Resnova, Italy) in four cases for which material was available.

FISH

Panel probes. Eleven genomic probes for regions containing known suppressor genes and regions involved in deletions of MDS/AML were selected (Table 1). FISH probes for 13q14 and 7q31 were bought from Vysis (Vysis, Downers Grove, IL, USA); the Ikaros gene (7p13) probe was kindly provided by Y. Hosokawa (Cancer Center of Tokyo, Japan); the AML1 (21q22) gene probe was kindly provided by M. Rocchi (University of Bari, Italy); the PAC 144G9 (5q31) probe was kindly provided by F. Birg (Institute Paoli Calmettes, Marseilles, France); the cosmid probe for PDGFRβ gene (5q33) was kindly provided by S. Morris (Department of Experimental Oncology, Memphis, USA); all other probes were kindly provided by P. Marynen (Catholic University of Leuven, Belgium).

I-FISH. Interphase-FISH on naked nuclei was performed as previously described.[6] Each probe was digoxigenin- or biotin-labeled, and validated for chromosomal assignment on normal metaphases. Digoxigenin- and biotin-labeled probes were both used in double color experiments. A normal bone marrow sample was added as a control in each experiment. Four hundred nuclei were evaluated in specimens from each of the 30 patients and from each of the experiment controls. The cut-off for monosomy was taken to be the upper limit in a total of 5,200 control nuclei evaluated for each probe.

M-FISH. Metaphase FISH was done as previously described[7] in all 9 cases with complex karyotypes and evidence of deletions in interphase experiments. At least four metaphases were evaluated for each probe.

Results

Patients, conventional cytogenetics, and painting

In the 19 patients with isolated 5q deletion refractory anemia (RA) was diagnosed in eleven (2M/9F; age range: 46-84 years), refractory anemia with excess blasts (RAEB) in two (2F; aged 55 and 75 years), and AML in six (2M/4F; age range: 32-68 years). Conventional cytogenetics showed a typical interstitial 5q deletion, from q13 to q33 in all cases (*data not shown*).

In the eleven patients with 5q- associated with other karyotypic abnormalities, *de novo* AML was diagnosed in five (2M/3F; age range 56-76 years), secondary AML in three (1M/2F; aged 76,63,79 years), MDS-RA in two (1M/1F; aged 56 and 67 years), and MDS-RAEB in one (M; 76 years old). Cytogenetic and painting results are summarized in Table 2.

The size of the 5q deletion appeared in our resolution banding to be the same in all cases, from q13 to q33. Cases #4 and #11 had additional aberrations, i.e., a monosomy 17 or an additional marker in two independent clones in case #4, and trisomy 8 in case #11.The other nine cases showed a so-called complex karyotype with four or more additional structural and/or numerical aberrations.

In case #2 WCP for chromosome 21 labeled all copies of karyotypic markers. WCP 17 labeled the normal 17 and the der(21) from the t(17;21). In case #5 WCP 17 labeled normal 17 and also partially labeled the add(17p), confirming the presence of extramaterial; WCP 13 labeled normal 13 and the two markers. In case #6 WCP13 labeled two normal chromosomes 13. In case#8 WCP 7 labeled the normal 7 and partially the add(7p).

I-FISH and M-FISH

The upper limits for monosomies, established by analyzing 5,200 nuclei from 13 healthy controls, were: FHIT 5%, Ikaros 6.1%, D7Z1 1.96%, PTEN 4.5%, WT1 4.8%, ATM 5.7%, D13S25 4.3%, p53 4.7%, NF1 3.14%, MADR2 6.1%, AML1 4.7% (Table 1).

Interphase FISH deletions were not detected in the 19 patients with isolated 5q-. Results for each probe were below normal standards of disomy. Table 2 shows that interphase FISH deletions were detected in 9/11 patients with 5q- and other changes. Cryptic deletions involved the p53 gene in three cases (#1, 3 and 5), all with an add(17p) in the karyotype. In two (cases #1

Table 2. Cytogenetic, whole chromosome painting, and I-FISH results in 11 patients with 5q- associated with other karyotypic abnormalities.

	Diagnosis	*Sex/Age*	*Cytogenetics*	*Painting*	*I-FISH deletions (% nuclei)*
1	MDS-RA	F/56	46,XX [2/11] 45,XX,del(5)(q13q33),del(7)(q31q34),add(17)(p13),der(17p),-20 [9/11]	nd	**P53 (50)** D7Z1 (50)
2	SAML	M/76	47-53,XY,del(3)(p14p25),del(5)(q13q33),der(21),t(17;21)(q11;q22),-18,+21,-22, +2~8 mar, [cp8] 47-53,XY, idem,i(8)(q10), [cp5]	Ish +2-8 markers (wcp 21+)	**AML1(37)** P53/**NF1** (86/65) FHIT (84)
3	AML	M/70	46,XY [8/10] 46,XY,t(3;6)(q12;q27),del(4)(q),del(5)(q13q33),-9,-10,-10,-13,-14,-15,add(17)(p13),-19,+mar1,+mar2,+mar3,+mar4, [2/10]	nd	**P53 (21)**
4	AML	F/?	46,XX [1/12] 46XX,del(5)(q13q33) [4/12] 45,XX,del(5)(q31q33),-17 [4/12] 47,XX,del(5)(q31q33), +mar [3/12]	nd	P53/NF1 (86/65)
5	MDS-RAEB	M/76	46,XY [6/13] 39-41,XY,-2,-7,-9,der(11)add(11)(p?),add(11)(q24),-13,-13,add(15)(p11),der(16)add(16)(p13)del(16)(q),del(5)(q13q33),add(17)(p13),-21,-22,-22,+mar1,+mar2 [cp7]	Ish add(17)(p13)(wcp17+) Ish markers (wcp13+)	**P53 (89)** D7Z1 (86) D13S25 (82)
6	SAML	F/69	43,XX,del(5)(q13q33),add(10)(p),-12,add(14)(p),-16,-17 [4/10] 44,XX,add(1)(p36),del(5)(q13q33),i(6)(q11),add(10)(p),-11,-12 [6/10]	Ish nl(13)(wcp13)	P53/NF1 (87/90) **D13S25 (23)***
7	MDS-RA	M/67	46-50,X,-Y,t(1;19;13),(p13;p13;q11),del(5)(q13q33),+add(19)(p13),-20,add(21)(p11),+mar1,+mar2x2,+mar3 [cp14]	nd	No Deletions
8	AML	F/79	52-57,XX,+der(1),+2,+3,del(5)(q13q33),add(7p15),+8,+8,+9,+10,der(11),+i(11)(q11),+13,14,-17,+21,+22,+ mar1,+mar2,+mar3 [cp13]	Ish add(7)(p15)(wcp 7+)	P53/NF1 (88/86) **Ikaros (32)***
9	AML	F/58	42-43,XX,del(5)(q13q33),-7,-11,add(12p13),-16,-18,+mar1,+mar2,+mar3 [cp10]	nd	D7Z1 (71)
10	SAML	F/63	46,XX [4/14] 45,XX,-4,del(5)(q13q33),-17,-18,-del(21)(q),+mar1x2 [10/14]	nd	P53/NF1 (27/33) AML1 (67) MADR2 (20)
11	AML	F/61	47,XX, del(5)(q13q33),+8 [15/15]	nd	No Deletions

*In bold: cryptic deletions without cytogenetic evidence of loss of material; *in these two cases deletions were not found in M-FISH but only in interphase nuclei. S: secondary; MDS: myelodysplastic syndromes; AML: acute myeloid leukemia; nd: not done.*

and 5) metaphase FISH assigned the monoallelic deletion to the 17p derivative. Material was not available for metaphase FISH experiments in the third case.

In case #2 (Table 2) there was a cryptic deletion of AML1 despite 2 to 8 copies of a 21-like chromosome marker. Metaphase FISH with the AML1 probe showed only one signal in the normal 21. In the same case a cryptic loss of NF1 was identified as a breakpoint associated deletion at the 17q11 involved in the 17;21 translocation.

In case #6 the absence of chromosome 17 in the karyotype corresponded with the loss of both p53 and NF1 in interphase FISH. An unexpected further deletion was detected by the probe for the D13S25 locus. As both alleles were labeled after metaphase FISH, the deletion clearly occurred in a non-proliferating subset of cells (Figures 1A and B).

Further experiments with double color FISH using the CosB probe for the PDGFβR gene at 5q33 and the D13S25 probe for the 13q14 region showed two independent clones: one in 80% of nuclei missing only cosB, and one in 20% of nuclei missing only D13S25. The same results were obtained using PAC 144G9(5q31) and D13S25.

Similar findings emerged in case #8 with cryptic monoallelic loss of Ikaros gene in interphase nuclei, while both normal 7 and the add(7p) were labeled in metaphases (Figures 1C and D). Further investigation with double color interphase FISH, using PAC 144G9 for 5q31 region and Ikaros for 7p13, showed two inde-

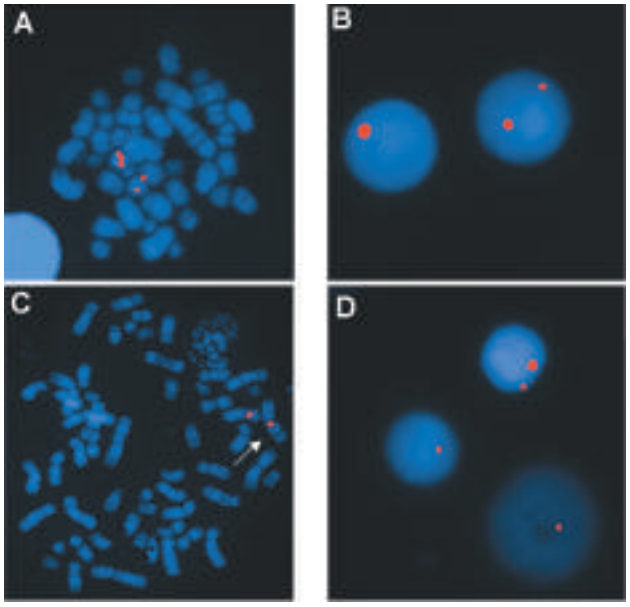

Figure 1. Case #6, a, Metaphase FISH with D13S25/13q14 probe showing normal labeling of both chromosomes 13; b, D13S25 in interphase nuclei detecting both monosomic and disomic populations. Case #8, c, Metaphase FISH with the probe for Ikaros gene/7p13 shows signals in both normal 7p and 7p with extra material (arrow); d, Interphase FISH identifying disomy and monosomy for Ikaros gene.

pendent clones: one with 30% of nuclei with loss of Ikaros and the other with 58% of nuclei with loss of PAC 144G9.

In case #9, although one copy of chromosome 18 was lost in the karyotype, two signals corresponding to the MADR2 gene were present in interphase. Metaphase FISH assigned one of these spots to an unclassifiable marker observed at cytogenetic analysis. Only expected deletions were found in cases #4 and 10. No interphase FISH deletions were found in cases # 7 and #11.

Discussion

This study with multiple I-FISH was designed to investigate whether cryptic genomic microdeletion(s) occur in the multistep process underlying 5q- associated malignancies. No deletions were observed in the nineteen cases with isolated 5q- , while 9/11 of cases with 5q- plus other changes had a high degree of genetic instability, not only at the chromosomal level but also at the molecular level. In six out of the eleven cases (54%) cryptic deletions emerged only after I-FISH. Monoallelic loss of p53 was the most frequent event, being present in eight patients . In five cases (# 2, 4, 6, 8 and 10) it was the counterpart of monosomy 17 found at karyotyping and was associated with monoallelic loss of NF1. In the other three (#1, 3 and 5) it occurred as an unexpected event in the presence of one normal chromosome 17 and one rearranged 17p, with extra material instead of deletion. Preudhomme *et al.*[8] have reported similar observations in AML with unbalanced translocations at 17p.

NF1 was missing not only because of monosomy 17, but also because of a cryptic deletion at the breakpoint region of the 17;21 translocation in case #2. This is similar to the cryptic deletions accompanying more frequent leukemic rearrangements such as t(9;22) in chronic myeloid leukemia[9] and t(8;21) in acute myeloid leukemia.[10] NF1 has only sporadically been found to be involved in acute myeloid leukemia by mutation analysis.[11,12,13] AML1 was lost twice; in case #10 because of a concomitant karyotypic deletion, and in case #2 as a cryptic event accompanying multiple karyotypic rearrangements of chromosome 21. Haploinsufficiency due to monoallelic AML1 mutations has been found in familial thrombocytopenia predisposing to acute leukemia.[14] In this study for the first time we found haploinsufficiency because of deletion in two cases of secondary AML. Interestingly these two cases identify a hitherto unknown subgroup in the series lacking p53.

Loss of D13S25 was found twice, associated with monosomy 13 in case #5 and as a cryptic event in case #6. In the latter we demonstrated that non-proliferating cells without 5q- were affected, suggesting the clone without D13S25 proliferates poorly. Ketterling *et al.*[15] described a similar finding in a case of RAEB with a normal karyotype and a cryptic clone with a 13q deletion that was only detected by FISH .

Another cryptic event involving non-proliferating cells was present in case #8 with deletion of the Ikaros gene. This is the first observation of Ikaros gene deletion in a human myeloid malignancy. The Ikaros gene is necessary for development of lymphoid lineages and is also important in the early steps of neutrophil differentiation.[16]

Unfortunately due to lack of material we could not assign the anomaly to specific cell lineage(s). The heterogeneity of both chromosomal changes and genomic deletions as well as demonstration of different deletions in dividing and non-dividing cells strongly suggest that clonal expansion in malignancies associated with 5q- and complex changes occurs through selection of mutations favoring growth.[17] I-FISH in eleven cases of RA (typical 5q- syndrome) provided strikingly different results as no genomic losses were detected. These observations add new insights to the concept that the 5q- syndrome is characterized by a very stable clone. Similarly deletions were absent in all 8 other cases with isolated 5q-, including two cases of RAEB and six cases of AML. Interestingly AML with isolated 5q- is molecularly closer to the 5q- syndrome than to AML with 5q- and complex karyotypes.

The genetic characteristics of the malignant clone with isolated 5q- seem to be insufficient in themselves to account for either a long-lasting chronic disease, as is the 5q- syndrome, or acute myeloid leukemia. Microenvironmental factors, such as stromal cell interactions, interleukin balance, and immunological reactions, might be critical in the growth of the malignant clone with isolated 5q-. Recently the immunological background has been demonstrated to play a specific role in the control of abnormal bone marrow clones by isolation of host T lymphocytes with selective toxic activities against bone marrow cells with trisomy 8.[18]

In conclusion our FISH study adds to the understanding of the genomic differences between cases of AML/MDS with isolated 5q- and those with 5q- plus other changes. In this latter group, stability gene mutations are probably the first step towards multiple chromosomal and molecular aberrations during clonal selection. In addition to p53, haploinsufficiency for AML1, NF1, D13S25, and the Ikaros gene was shown in malignancies with 5q- and complex karyotypes.

BC was the principal investigator who provided cytogenetic and FISH data and wrote the paper. RLS performed the metaphase FISH experiments. SR, DB, and GB were involved in cell culturing, karyotyping, and interphase FISH; AA, SC, and CN provided clinical and cytogenetic data of patients from their Centers; PM was involved in the selection of the genomic probes; MFM was involved in diagnosis and management of patients; CM was responsible for the conception and supervision of the study and of the paper. The author wish to thank Dr. Geraldine Anne Boyd for assistance in the preparation of the manuscript. The authors reported no potential conflicts of interest.

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