Different boundaries characterize isolated and non-isolated 5q deletions in myelodysplastic syndromes and acute myeloid leukemias

Chromosome 5 long arm deletions, del(5q), are heterogeneous cytogenetic changes. An isolated 5q interstitial deletion is typically associated with erythroid hypoplasia, hypolobated micromegakaryocytes, less than 5% bone marrow blasts, macrocytic anemia, mild leukopenia, normal or increased thrombocytes, female preponderance and a relatively good prognosis.[1] In contrast, a 5q loss in a complex karyotype is associated with high-risk myelodysplastic syndromes (MDS) and acute myeloid leukemias (AML), including cases arising after radio- and/or chemotherapy.[2] Much emphasis has been given to common deleted regions (CDRs) and genes.[3] We recently showed that telomeric breakpoints were significantly different in cases with isolated and non-isolated del(5q). Non-isolated del(5q) showed *NPM1*/5q35.1 monoallelic loss in 38 of 84 cases (45.2%) *versus* one of 43 (2.3%) of isolated del(5q) (Fisher's exact test $P<0.001$)[4]. Moreover, gross chromosomal anomalies and monosomies, as seen in high-risk MDS/AML phenotype, were significantly related to *NPM1* haploinsufficiency.[4]

In the present study, we extended our deletion mapping analysis to 170 cases, including a new prospective series of 64 patients with MDS/AML; namely 24 cases with isolated del(5q) and 40 cases with non-isolated del(5q). In the last group, FISH with clone RP11-117L6/*NPM1* (5q35.1) detected *NPM1* monoallelic deletion in 15 of 40 (37.5%) cases *versus* one of 24 (4.1%) cases with isolated del(5q) (Fisher's exact test $P=0.002$).

Twenty-four cases with complex karyotypes and monosomy 5 were also included after FISH correction (Vysis LSI EGR1/D5S23,D5S721 Dual Color Probe and/or LSI CSF1R/D5S23,D5S721; Chromosome 1, 5, 19, α-satellite, Qbiogene) which demonstrated two copies of 5p15.2 and/or of chromosome 5 centromere, thus indicating that only 5q genomic material was missing.

Centromeric breakpoints were narrowed with 24 genomic clones mapping from 5q11 to q14 (Table 1). 5q11: RP11-648C7, RP11-317O24, RP11-97O9, RP11-640L3, RP11-20I7, RP11-528L24, RP11-175M2, RP11-479O16, RP11-266N13. 5q12: RP11-489L13, RP11-298P6, RP11-79C20. 5q13: RP11-170N5, RP11-195G20, RP11-195E2, RP11-771B3, RP11-79P5, RP11-229C3, RP11-469J18, RP11-996M9. 5q14: RP11-168A11, RP11-80K5, RP11-1089B2, RP11-885P10. Telomeric breakpoints distally to *NPM1* were investigated with the following 3 genomic clones: 5q35.2-q35.3: RP11-286C20, RP11-549A4. 5q35.3: RP1-240G13 (subtelomeric probe) (Table 1). DNA clones were selected from *http://www.ncbi.nlm.nih.gov*, *http://genome.ucsc.edu/* and *http://projects.tcag.ca/variation/* and were kindly provided by Mariano Rocchi (Department of Genetics and Microbiology, University of Bari, Italy). Furthermore, SNP-aCGH (Affymetrix Cytogenetics Whole-Genome 2.7M Array) data were available in 2 cases.

On the centromeric side, the RP11-80K5 clone emerged

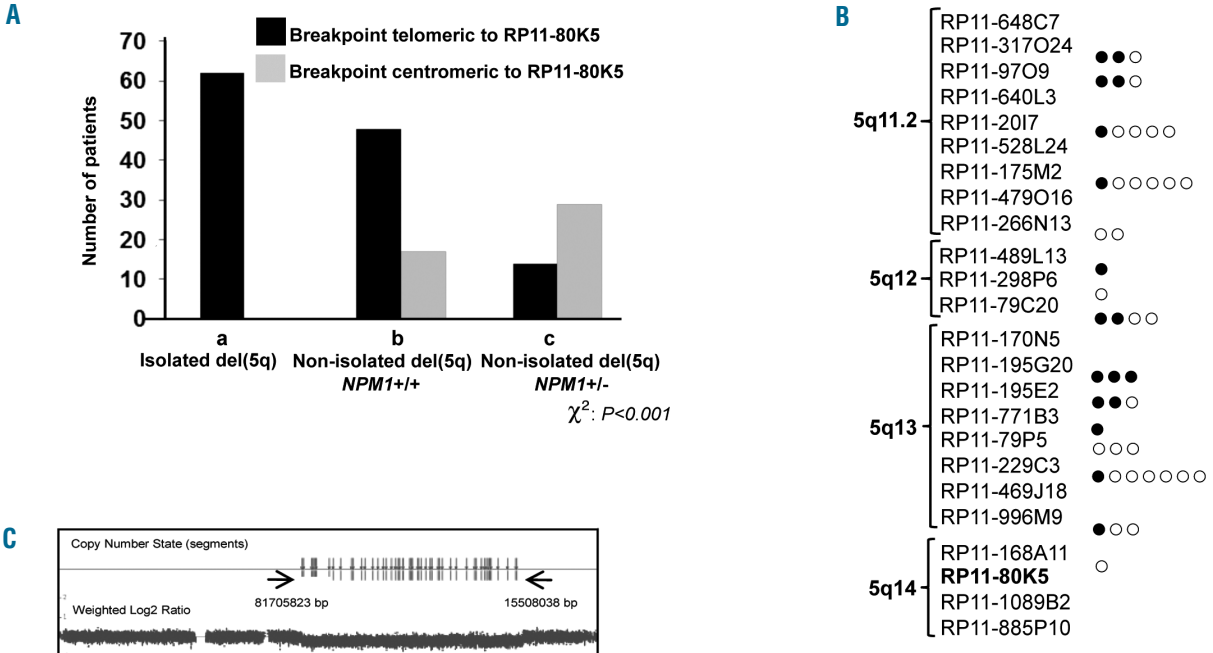

Figure 1. Characterization of centromeric breakpoints in MDS/AML with del(5q). (A) A proximal breakpoint centromeric to RP11-80K5 was found in none of the 62 patients with isolated del(5q) (a), in 17 of 65 (26.1%) cases of non-isolated del(5q) *NPM1*+/+ (b) and in 29 of 43 (67.4%) cases of non-isolated del(5q) *NPM1*+/- (c). (B) Distribution of breakpoints centromeric to RP11-80K5 (indicated in bold) in cases maintaining (●) or losing (○) the *NPM1* gene. (C) SNP-aCGH (Affymetrix Cytogenetics Whole-Genome 2.7M Array) analysis in 2 patients with isolated del(5q). Arrows indicate start (→) and end (←) of deletions.

Table 1. Mapping of FISH clones used to characterize centromeric and telomeric boundaries of 5q deletions.

Clone	Map	Start (bp)	End (bp)	Size (kb)	Source
RP11-648C7	5q11.1	49620320	49682523	62	DGV Build 36 Mar.2006 hg18
RP11-317O24	5q11.1	50064745	50257680	193	UCSC Feb.2009 (GRCh37/hg19)
RP11-97O9	5q11.1-q11.2	50621202	50781894	161	UCSC Feb.2009 (GRCh37/hg19)
RP11-640L3	5q11.2	52119148	52284408	165	DGV Build 36 Mar.2006 hg18
RP11-2O17	5q11.2	52251522	52407776	156	UCSC Feb.2009 (GRCh37/hg19)
RP11-528L24	5q11.2	54345225	54528725	183	UCSC Feb.2009 (GRCh37/hg19)
RP11-175M2	5q11.2	55004189	55181373	177	DGV Build 36 Mar.2006 hg18
RP11-479O16	5q11.2	57894750	58084125	189	UCSC Feb.2009 (GRCh37/hg19)
RP11-266N13	5q11.2	58252640	58432917	180	DGV Build 36 Mar.2006 hg18
RP11-489L13	5q12.1	60190094	60370893	181	DGV Build 36 Mar.2006 hg18
RP11-298P6	5q12.3	64070008	64258158	188	DGV Build 36 Mar.2006 hg18
RP11-79C20	5q12.3	64627185	64813120	186	UCSC Feb.2009 (GRCh37/hg19)
RP11-170N5	5q13.1	67551829	67703015	151	UCSC Feb.2009 (GRCh37/hg19)
RP11-195G20	5q13.2	68404119	68544376	140	UCSC Feb.2009 (GRCh37/hg19)
RP11-195E2	5q13.2	70198842	70389712	191	UCSC Feb.2009 (GRCh37/hg19)
RP11-771B3	5q13.2	71490559	71661653	171	UCSC Feb.2009 (GRCh37/hg19)
RP11-79P5	5q13.2	72642971	72815978	173	UCSC Feb.2009 (GRCh37/hg19)
RP11-229C3	5q13.3	74286604	74469980	183	DGV Build 36 Mar.2006 hg18
RP11-469J18	5q13.3	75877437	76051493	174	UCSC Feb.2009 (GRCh37/hg19)
RP11-996M9	5q13.3	76077125	76285214	208	UCSC Feb.2009 (GRCh37/hg19)
RP11-168A11	5q14.1	79174857	79348166	173	UCSC Feb.2009 (GRCh37/hg19)
RP11-80K5	5q14.1	80283279	80446040	163	UCSC Feb.2009 (GRCh37/hg19)
RP11-1089B2	5q14.2	82347847	82547014	199	UCSC Feb.2009 (GRCh37/hg19)
RP11-885P10	5q14.2	82478397	82622687	144	UCSC Feb.2009 (GRCh37/hg19)
RP11-117L6	5q35.1	170746923	170922033	175	UCSC Feb.2009 (GRCh37/hg19)
CTC-286C20	5q35.2-q35.3	176391374	176542028	151	DGV Build 36 Mar.2006 hg18
CTC-549A4	5q35.2-q35.3	176483021	176610399	127	DGV Build 36 Mar.2006 hg18
RP1-240G13	5q35.3	180512362	180626032	114	DGV Build 36 Mar.2006 hg18

as an interesting tool to differentiate simple and complex karyotypes by FISH. In fact, breakpoints fell distally to RP11-80K5 (band 5q14.1) in all the 62 cases with isolated del(5q) (24 from the prospective study and 38 from the previous series; Figure 1A, a) including the 2 cases with loss of the *NPM1* gene. In this study, centromeric breakpoints were evaluated in 26 cases with isolated del(5q). Results showed a cluster in a ~1.9 megabases region between RP11-80K5 and RP11-1089B2 in 6 of 26 (23%). Within this region, SNPs further refined the molecular heterogeneity (Figure 1C). All the remaining breakpoints (76%) were located distal to RP11-885P10. Interestingly, in SNP studies, Gondek *et al.* showed similar results in 9 cases with isolated del(5q) at karyotype.[5] As far as we know, only one case with isolated del(5q) and a centromeric breakpoint above RP11-80K5, at 5q12-q13, has been reported by Royer-Pokora and co-workers.[6]

In contrast, in non-isolated del(5q), centromeric breakpoints were significantly more frequently proximal to RP11-80K5 (46 of 108, 42.5%, *vs*. no cases in isolated del(5q) group; Fisher's exact test: $P<0.001$). However, depending on concomitant loss of *NPM1* at the telomeric side, they spread differently. Breaks were upstream RP11-80K5 in 17 of 65 (26.1%) *NPM1*+/+ and in 29 of 43 (67.4%) *NPM*+/- cases (Fisher's exact test $P<0.001$) (Figure 1A, b and c). Notably, from band q11.2 to q14.1, four clusters of breakpoints (each found in ≥4 cases) emerged: two at band 5q11.2 (between RP11-20I7 and RP11-528L24, ~1.9 megabases and between RP11-175M2 and RP11-479O16, ~2.7 megabases), one at band 5q12-q13 (between RP11-79C20 and RP11-170N5 ~2.7 megabases), and one within band 5q13 (between RP11-229C3 and RP11-469J18, ~1.4 megabases) (Figure 1B).

The most telomeric boundaries were defined in 31 of 43 *NPM1*+/- cases. In 5 of 31 (16.1%) cases, breakpoints fell between RP11-117L6 (*NPM1*/5q35.1) and RP11-286C20/5q35.2-q35.3 (~5.7 megabases), while subtelomeric sequences were lost in 26 of 31 (83.8%) cases suggesting that, in the large majority of cases, *NPM1* deletion may reflect unbalanced 5q rearrangements.

In conclusion, we demonstrated that, in MDS/AML, the del(5q) boundaries are strongly correlated to karyotype complexity. Indeed, in our cohort of patients, and in previously published studies,[5] large 5q deletions involving both the RP11-80K5 clone and the *NPM1* gene have been found only in non-isolated 5q deletions (approx. 26% of cases in this series). Altogether these results suggest that the identification of the number and type of haploinsufficient genes outside the CDRs might contribute to our understanding of the clinical phenotype associated with different 5q deletions in good or poor risk MDS/AML.

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