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A cryptic deletion in 5q31.2 provides further evidence for a minimally deleted region in myelodysplastic syndromes

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Recurrent deletions of 5q in myeloid malignancies encompass two separate regions: deletion of 5q33, which is associated with the 5q− syndrome and haploinsufficiency of *RPS14,* and deletion of a more proximal locus at 5q31. We present a case with a cryptic 1.3 Mb deletion in 5q31.2 identified by array comparative genomic hybridization that places the proximal boundary of the deletion proximal and close to the candidate *EGR1* gene. The patient was diagnosed initially with a myelodysplastic syndrome, with a del(20)(q11.2q13.3) as the sole abnormality identified by karyotyping. The patient progressed to acute myeloid leukemia with no change to the G-banded karyotype. The 1.3 Mb deletion on the long arm of one chromosome 5 was confirmed to have been present both at presentation with myelodysplastic syndrome and at transformation. This is an interesting case because there are few array studies identifying cryptic 5q deletions, and the study of these small deletions helps to refine the common deleted region. This case, together with previously published studies, suggests that the proximal boundary of the common deleted region may lie within the *KDM3B* gene.

Keywords Myelodysplastic syndromes, *EGR1*, chromosome 5, deletion, common deleted region

Chromosome abnormalities are observed in the bone marrow aspirates of approximately 40–50% of patients with myelodysplastic syndrome (MDS) (1,2). According to the International Prognostic Scoring System (IPSS), a normal karyotype or loss of the Y chromosome, deletion of 5q, and deletion of 20q as sole abnormalities help define a good prognosis subset of MDS (1).

Deletion of 5q in MDS is thought to encompass two separate regions, with deletion of the distal region defining the entity known as MDS associated with isolated del(5q), or 5q− syndrome, according to the World Health Organization Classification of Tumors (3–5). Recently, the ribosomal subunit protein *RPS14* gene at 5q33 has been proposed as the critical target gene deleted in the 5q− syndrome, as loss of expression of RPS14 phenocopied the erythroid differentiation defect characteristic of the 5q− syndrome. Restored RPS14 expression rescued erythroid differentiation in primary cells from patients with the 5q− syndrome (6). *RPS14* may not be the target gene in MDS patients with 5q deletions who do not have the distinctive 5q− phenotype. In other cases, when gene mapping studies are limited to those with small deletions, common deleted regions (CDRs) have been shown to recur in the vicinity of the *EGR1* gene, which is one of the favored candidate genes.

Cytogenetic analysis is a low resolution whole genome test. Deletions of a megabase (Mb) or more may be overlooked in the karyotype of a leukemia cell if the chromosome preparation does not produce chromosomes of sufficient length and banding resolution, and so more sensitive techniques such as those based on arrays may provide more exact prognostic information. A patient studied serially at the Victorian Cancer Cytogenetics Service provided us with some insight into cryptic abnormalities in MDS.

Case report

A 67-year-old man was diagnosed with MDS in February 2005. He was pancytopenic with a hemoglobin of 91 g/L,

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white cell count of 3.0 $\times$ 10^9/L, neutrophil count of 1.8 $\times$ 10^9/L, and platelet count of 103 $\times$ 10^9/L. A bone marrow biopsy was performed and revealed a hypercellular marrow with 1% blast cells and dyserythropoiesis in over 10% of erythroid cells with 30% ring sideroblasts. A diagnosis of MDS with refractory anemia with ring sideroblasts (RARS) was made. In May 2007, a repeat bone marrow biopsy confirmed disease progression. The bone marrow contained 15% blast cells; and the diagnosis was then refractory anemia with excess blasts type 2 (RAEB-2).

In February 2008, a full blood examination showed that the peripheral blood blast count was 24.4 $\times$ 10^9/L and the platelet count was 3 $\times$ 10^9/L. A bone marrow biopsy was performed, and the diagnosis was acute myeloid leukemia (AML). The patient died 2 weeks later.

Materials and methods

Cytogenetics

Cytogenetic studies were performed sequentially at different disease stages. Bone marrow cells were cultured in RPMI with 10% fetal calf serum overnight or synchronized with fluorodeoxyuridine and released with bromodeoxyuridine after 2–3 days in culture (7,8). Cells were subjected to hypotonic treatment, fixed in methanol:acetic acid (3:1), dropped onto clean microscope slides (9), and G-banded (10). Karyotypes were reported using the ISCN (2009) (11) (Table 1).

Array comparative genomic hybridization

Test DNA was extracted from the MDS/RAEB-2 bone marrow specimen (May 2007) and from fixed cytogenetic cell suspensions (12) of the AML specimen from February 2008 with a DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD) and hybridized to two different Agilent custom arrays according to the manufacturer's instructions (Agilent Technologies, Santa Clara, CA). Dual dye swap experiments were performed with the MDS/RAEB-2 specimen with a 44K custom array, and a single experiment was performed with the AML specimen with a 105K custom array. Test DNA and control DNA (Promega [Madison, WI] pooled Human Genomic DNA of the opposite sex) were labeled with cyanine 3-dUTP and cyanine 5-dUTP with an Agilent Genomic DNA Labeling Kit PLUS (Agilent, Santa Clara, CA). DNA from the MDS/RAEB-2 specimen was also sent for service array on an Agilent catalog array (design 021924, Agilent Technologies, Santa Clara, CA) by Pacific Laboratory Products (Blackburn, Australia) and on a Roche NimbleGen 12x135K Whole Genome Tiling array by Roche NimbleGen (Reykjavik, Iceland). Agilent data were analyzed and visualized by Genomics Workbench (version 5.0.14, Agilent Technologies), and Roche NimbleGen data were analyzed by the SignalMap software (version 1.9, Roche NimbleGen). All nucleotide positions given refer to the Ensembl human genome browser, version 54, hg18 (13).

Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) studies were undertaken on residual cytogenetic cell suspension from the February 2005 (MDS/RARS) and February 2008 (AML) specimens with the following commercial probes: LSI EGR1(5q31) SpectrumOrange/D5S23, D5S721 SpectrumGreen probe (Abbott, Downers Grove, IL), LSI D20S108 (20q12) SpectrumOrange probe (Abbott), and WC20 Whole Chromosome 20 Paint, Aqua (Kreatech, Amsterdam).

The EGR1 probe was first chosen to validate the array comparative genetic hybridization (CGH) result because it was readily available and array CGH studies suggested that the *EGR1* gene was fully deleted. According to the manufacturers, the probe is about 200 kb long, but no information was available about its precise positioning. The bacterial artificial chromosome (BAC) clone RP11-26J10, labeled with Alexa Fluor 488, was also used, and purchased from BlueGnome (Cambridge, UK). It encompasses the position chr5:137,879,653–138,033,032 at 5q31.2.

FISH was carried out according to the Abbott co-denaturation protocol as previously published (14). A hybridization mixture was prepared containing Abbott and BlueGnome probes each diluted 1:9 with the supplied Abbott LSI/WCP hybridization buffer and Cot-1 DNA (Invitrogen, Carlsbad, CA) to a final concentration of 100 ng/μL. The premixed Kreatech probe was diluted 1:4 with this hybridization mixture, and the recommended Kreatech pretreatment (2 $\times$ SSC, pH7.0, 37°C for 2 minutes followed by ethanol dehydration and air drying) was used when the Kreatech probe was included (15). Co-denaturation was at 73–74°C for 1.5–2 minutes.

This study has received ethics approval from the Human Research Ethics Committee, St Vincent's Hospital (Melbourne, Australia).

Results

Conventional G-band karyotype analysis was performed on five bone marrow aspirates drawn sequentially throughout the patient's disease course. A del(20)(q11.2q13.3) was the sole abnormality at presentation in February 2005 and at each subsequent analysis (Table 1, Figure 1).

Retrospectively, we analyzed specimens taken in May 2007 (MDS/RAEB-2) and February 2008 (AML) by array CGH. FISH was then used to confirm our findings in the AML specimen. FISH was also performed on the diagnosis (MDS/RARS) specimen procured in February 2005 (Table 1) for which there was insufficient material to perform array studies.

Consistent with the cytogenetic findings, a 23 Mb deletion from the long arm of chromosome 20 was confirmed by array CGH data in both samples tested, with flanking oligonucleotides at chr20:33,844,063–33,844,123 and chr20:56,698,692–56,698,752 (Table 1). A 20q deletion was also confirmed by FISH showing loss of one *D20S108* signal (Table 1). In addition, a 1.3 Mb deletion at 5q31.2 was identified by array CGH with the Agilent platform in both the MDS/RAEB-2 and AML samples, with flanking oligonucleotides at chr5:137,736,007–137,736,066 and chr5:139,017,982–139,