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BRIEF COMMUNICATION

Evaluating chromosomal mosaicism by array comparative genomic hybridization in hematological malignancies: the proposal of a formula

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Array-based comparative genomic hybridization (aCGH) has proven indispensable to the study of unbalanced constitutional and acquired chromosomal anomalies, but its sensitivity for detecting mosaicism is still not well established. On the basis of the ADM2 algorithm used for microarray image analysis with one of the most widely used oligomer-based aCGH platforms [the whole genome 244K system by Agilent Technologies (Santa Clara, CA)] we suggest a formula to infer the percentage of cells bearing a chromosome imbalance in cases with constitutional or acquired mosaicism. Three examples of acquired mosaicism in which this formula was applied are reported together with parallel fluorescence in situ hybridization (FISH) to interphase nuclei with informative probes. Although some approximation affects both the results inferred from aCGH and FISH data, the proposed formula was successful in the three patients studied.

Keywords aCGH, chromosomal mosaicism

Microarray-based comparative genomic hybridization (aCGH) is becoming a conventional instrument to investigate both constitutional and acquired chromosome imbalances (1–3), but its sensitivity to detect chromosomal mosaicism is still not well established. The relevance of aCGH studies in detecting constitutional mosaicism was emphasized by Iourov et al. (4). Some data concerning sensitivity are available for bacterial artificial chromosome (BAC)-based aCGH platforms with different designs (5–7). In their effort to compare results that may be obtained by means of BAC and oligonucleotide-based aCGH, Neill et al. (8) concluded that mosaicism of 30% or greater may be easily detected with both methods, and that levels as low as 10% may also be detected, but only under optimal conditions. These authors claim that BAC-based arrays may be more sensitive for revealing mosaicism. In their study, three patients with a 10% level were detected with BAC-based platforms, while the lowest level of mosaicism detected with an oligomer-based array was 21%. The problem of sensitivity is even more relevant for acquired mosaicism in hematological malignancies, in particular with respect to two points. First, is the possible low proportion of abnormal cells that may be expected in patients affected by myelodysplastic syndrome (9), chronic myeloproliferative disorders, or non-malignant diseases associated with acquired clonal anomalies in the bone marrow (BM) with propensity to leukemia, such as Shwachman-Diamond syndrome (10). The second relates to monitoring the size of the abnormal clone during the disease course, which is often relevant in myelodysplastic syndrome and leukemia. In their paper on hematological malignancies, Maciejewski et al. (3) suggest a sensitivity of 25% abnormal cells for their single nucleotide polymorphism array system and a similar sensitivity for aCGH.

Materials and methods

We tried to establish a simple formula to infer the percentage of cells carrying a chromosome imbalance in patients with a constitutional or acquired mosaic clone. The ADM2 algorithm used for microarray image analysis for the whole-genome 244K system by Agilent Technologies (Santa Clara, CA), was the basis for extrapolating this formula, which was then applied to three examples of acquired mosaicism.

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Patient 1 was an 11-year-old boy with Shwachman-Diamond syndrome, with an acquired interstitial deletion of chromosome 20 in a small BM clone, monitored for the risk to develop myelodysplastic syndrome or acute myeloid leukemia. Patient 2 was a 10-year-old boy with thrombocytopenia and BM hypoplasia, with a large interstitial deletion of chromosome 13, in bands q13.3–q21.31, acquired in a small BM clone. Patient 3 was a 6-year-old girl with Shwachman-Diamond syndrome, with an acquired isochromosome for the long arm of chromosome 7, i(7)(q10), which causes monosomy of the short arm and trisomy of the long arm, and was present in her BM.

The DNA was extracted with the Qiagen Blood and Tissue kit (Qiagen, Hilden, Germany), and competitor DNA was purchased from Promega (Madison, WI). Slides were scanned with Agilent's microarray scanner G2565BA, and microarray images were analyzed by Agilent's Feature Extraction 9.5.1 software and by Agilent's Genomic Workbench software (5.0.14).

Fluorescence in situ hybridization (FISH) on interphase nuclei with informative probes, in relation to the unbalanced anomalies present, was performed in parallel with aCGH.

Results and discussion

Some preliminary considerations are necessary to discuss the use of aCGH results for inferring the percentage of abnormal cells with chromosomal imbalances. The ADM2 algorithm is considered the most robust and powerful tool to

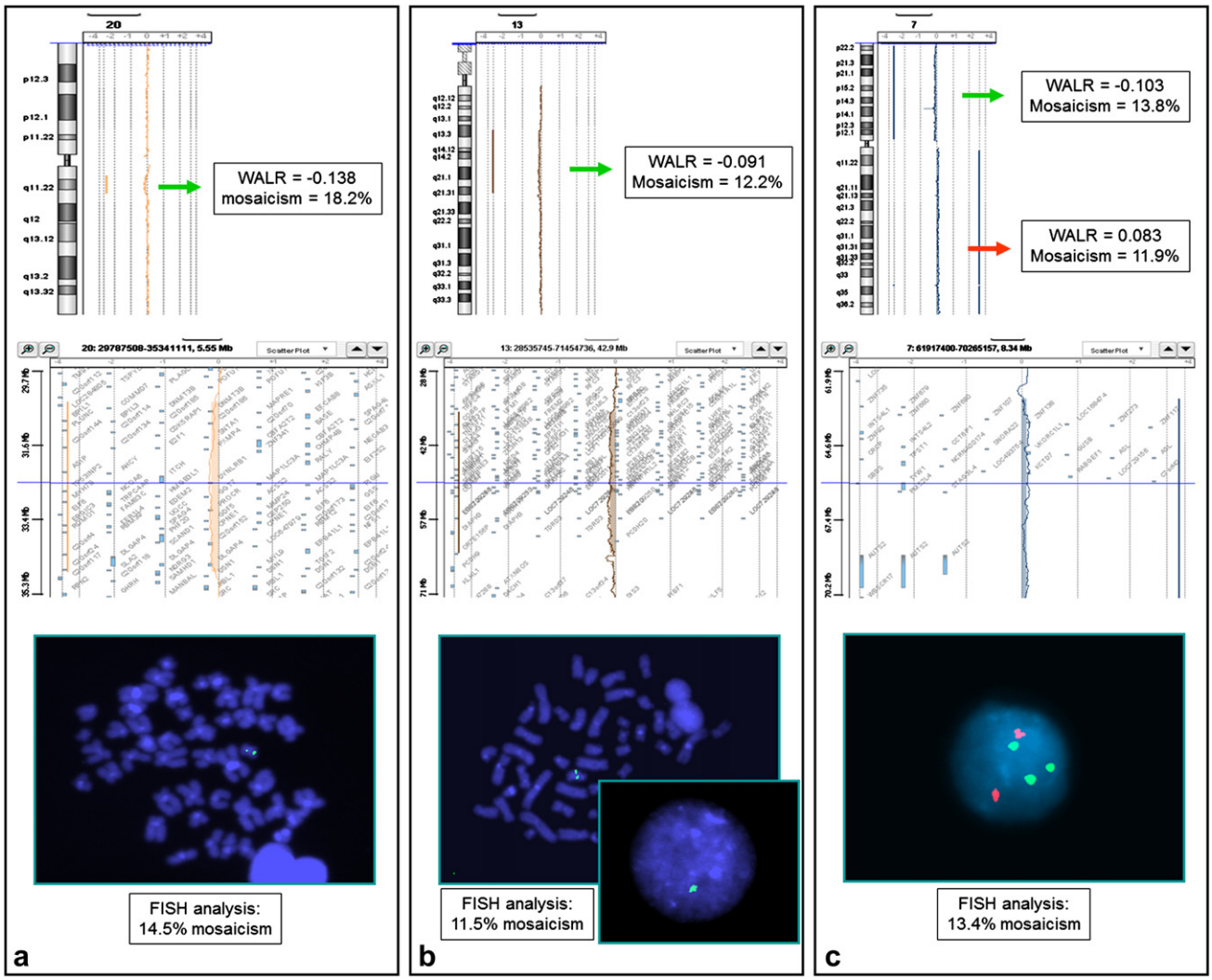

Figure 1 aCGH profiles (top, entire chromosome; center, magnification of the region of imbalance), with the results obtained with the formula proposed, and FISH results (bottom). WALR and mosaicism percentage evaluation by aCGH and by FISH are in evidence. (a) Patient 1. The deleted region of chromosome 20 (green arrow); FISH with BAC probe CTD-3092L7 identifying the deleted region, showing only one signal on the normal chromosome 20. (b) Patient 2. The deleted region of chromosome 13 (green arrow); FISH with BAC probe RP11-1001I7 identifying the deleted region, showing only one signal on the normal chromosome 13, in a mitosis and a nucleus. (c) Patient 3. The deletion of the entire short arm (green arrow) and duplication of the entire long arm (red arrow) of the chromosome 7; FISH with BAC probe CTD-3095D13 identifying a sequence on the duplicated long arm (green), and the alpha-satellite probe D7Z1 (red) used as control; it is shown in a nucleus with three green signals and two normal red signals. (A color figure can be found in the online version of this article.)

retrieve the aberrant regions in aCGH experiments, as suggested by Agilent, because it is based on weighted averages. Briefly, the algorithm identifies all the aberrant intervals that present significant deviation from the baseline of the weighted average $\log_2$ ratio (WALR) of fluorescence values for test and reference DNA. Thus WALR is more robust for statistical analysis than the pure mean log ratio, and every value is then shown by the "aberration text report" function. In the case of mosaicism, the WALR is obviously related to the percentage of abnormal cells. To evaluate this percentage, the first point to recall is that in a diploid genome, the comparative hybridization to a probe, in case of a deletion of one specific sequence in all cells of the DNA being tested, has double the fluorescence intensity of reference DNA compared to test DNA, while in a case of duplication of one sequence in all cells of the test DNA, it has a 1.5-fold increased fluorescence signal intensity compared to the reference DNA. So the fluorescence ratio would be $1/2 = 0.5$, or $3/2 = 1.5$, respectively, and the $\log_2$ for these ratio values are -1 and 0.584. In diploid cells carrying a deletion, the calculation would be: $0.5 \times 2 = 1$ sequence against $1 \times 2 = 2$ sequences; thus, 2 (normal sequences) $- 1$ (result of the calculation) $= 1$; that is, 100% of the cells harbor the deletion. The calculation would be similar in the case of a duplication. In this scenario, it is simple to figure out that when not all the cells carry the imbalance (constitutional or acquired mosaicism), the fluorescence log ratio value is between -1 and 0 for deletions, and between 0 and 0.584 for duplications. Thus, the formula to calculate the percentage of abnormal cells in mosaics in relation to a region found as aberrant by the ADM2 algorithm is

$$\%\ \text{of abnormal cells} = |2 - 2^{\text{WALR}} \times 2| \times 100.$$

The picture would be more complicated in the case of deletion of both copies of a sequence on the two homologous chromosomes, or number of copies above three (i.e., triplications, quadruplications). In such conditions—more frequently in solid tumors than in hematological malignancies—it would be important to have previous informative cytogenetic or molecular results (e.g., by FISH) clarifying the correct ploidy for the region of interest in order to permit a correct evaluation of the mosaic percentage. This evaluation would be possible by adjusting the formula as follows: % of abnormal cells $= (|2 - 2^{\text{WALR}} \times 2| \times 100) / |n - 2|$, where n is the total copy number of the sequences used to define the ploidy in the aberrant clone.

The results obtained applying the formula to our three mosaic patients are as follows. In patient 1, aCGH showed that the deletion was 4.116 Mb in band 20q11.22. The formula indicated that the size of the abnormal clone was 18.2% of BM cells (WALR $= -0.138$). On the same material, FISH with the BAC probe CTD-3092L7, that maps to the deleted region, showed the deletion in 68 nuclei out of 470 (14.5%) (Figure 1a). In patient 2, aCGH showed that the deletion encompassed a region of 26.3 Mb; the abnormal clone was expected to involve 12.2% of BM cells (WALR $= -0.091$). On the same BM sample, FISH with the BAC probe RP11-1001I7 indicated the deletion in 52 nuclei out of 452 (11.5%) (Figure 1b). In patient 3, the i(7)(q10) was also discernible with aCGH on material from peripheral blood. In peripheral blood DNA, the formula indicated a proportion of cells with an acquired monosomy of 13.8% (WALR $= -0.103$), and with an acquired trisomy of 11.9% (WALR $= 0.083$). Because cells with the isochromosome share both the monosomy and the trisomy, this difference gives a good evaluation of the extent of the precision of the method used. On the same peripheral blood sample, FISH with the BAC probe CTD-3095D13, recognizing a sequence on the long arm (7q21.3), showed the presence of the isochromosome in 27 out of 201 nuclei (13.4%) (Figure 1c).

Although some approximation affects both the results inferred from aCGH and FISH data, the proposed formula appears to perform well.

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