Atlas of Genetics and Cytogenetics in Oncology and Haematology

OPEN ACCESS JOURNAL

INIST-CNRS

Case Report Section

Clinical Relevance of Normal Diploid Workflow in Microarray Analysis of Massively Aneuploid Genomes: Lessons from a Case of B-Lymphoblastic Leukemia

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Published in Atlas Database: July 2018
Online updated version : http://AtlasGeneticsOncology.org/Reports/AneuploidGenomePadeganehID100097.html
Printable original version : http://documents.irevues.inist.fr/bitstream/handle/2042/70485/07-2018-AneuploidGenomePadeganehID100097.pdf
DOI: 10.4267/2042/70485

Abstract

Case report on Clinical Relevance of Normal Diploid Workflow in Microarray Analysis of Massively Aneuploid Genomes: Lessons from a Case of B-Lymphoblastic Leukemia.

Clinics

Age and sex
29 years old male patient.

Previous history
No preleukemia; no previous malignancy; no inborn condition of note

Blood

WBC: Pancytopenia
Bone marrow: 75% blasts

Cyto-Pathology Classification

Phenotype
B-cell ALL

Immunophenotype
Flow cytometry confirmed the presence of an abnormal immature B-cell population that was positive for CD10, CD19, CD24, CD34, CD38, HLA-DR, and TdT.

Rearranged Ig Tcr N/A

Pathology
A bone marrow exam showed B-lymphoblastic leukemia involving a hypercellular marrow with 75% blasts and moderate reticulin fibrosis. Quantitative reverse transcriptase polymerase chain reaction (Q-RT-PCR) analysis of the bone marrow showed no evidence of BCR/ABL1 fusion transcripts. A bone marrow sample was submitted to the cytogenetics laboratory for evaluation and risk stratification.

Electron microscopy N/A

Diagnosis
B-lymphoblastic leukemia

Survival

Date of diagnosis
07-2017

Treatment
The patient was induced with a pediatric ALL regimen and achieved a minimal residual disease-negative remission by day 28 and continues on interim maintenance therapy.

Relapse: N/A

Status Alive

Last follow up
06-2018

Survival
11+months

Karyotype

Sample Bone Marrow

Culture time 24h

Banding GTG

Results
66,XY,+X,+1,+2,+5,+6,+8,+9,+10,+11,+12,+14,+16,+der(19)t(1;19)(q23;p13.3),+21,+21,+21,+21,+21,+21,+21[16/20]

Karyotype at Relapse N/A

Other molecular cytogenetics technics
FISH

Other molecular cytogenetics results
FISH studies confirmed trisomy for chromosomes 9, 10 and 11, nine copies of chromosome 21 and no evidence of the BCR/ABL1 translocation. However, the FISH probes targeting the TCF3/19p13.3 locus produced two normal signal patterns, ruling out involvement of TCF3 in the supernumerary der(19)t(1;19) or any deletion in this region (Figure 2B-C).

Other Molecular Studies

Technics:
Cytogenomic Microarray Analysis (CMA)

Results:
SNP-array analysis was conducted on genomic DNA isolated from the bone marrow using the Thermofisher CytoScan HD platform coupled with the Chromosome Analysis Suite (ChAS). Copy number calls based upon smooth signal and log2 ratio indicated apparent monosomies of chromosomes 3, 4, 7, 13, 15, 17, 18, 20 and 22 with concomitant gains of the X chromosome and 1q, as well as six copies of chromosome 21 (Figure 1A). Moreover, a ~7 Mb terminal deletion involving the TCF3 gene was detected on the short arm of chromosome 19. However, manual inspection of the SNP pattern and B-allele frequency (BAF) revealed discrepancies between copy number calls and SNP/BAF patterns (Figure 1A, middle and bottom panels). In the case of losses, three allele tracks were observed whereas, in disomic states, four allele tracks were seen with the heterozygous track splitting. An error in determination of the correct ploidy level and, therefore, erroneous normalization was suspected.

These confounding results prompted us to further investigate potential causes for such drastic differences between CMA and CC/FISH analyses. Upon review of the available materials, it was decided to perform the initial analysis of the data using an alternative method.

Therefore, the Normal Diploid Analysis (NDA) workflow was utilized in lieu of the routinely used Single Sample Analysis (SSA). Interestingly, conversion of the same data file using the NDA workflow resulted in remarkably concordant results that fully matched the CC and FISH with no discrepancies between copy number calls and SNP/BAF tracks, confirming a hyperdiploid pattern (Figure 1B).

Moreover, analysis of the SNP patterns enabled us to verify the presence of retained heterozygosity in the disomic chromosomes which, along with the absence of any tetrasomic chromosomes, ruled out any potential doubling event. Curiously, analysis of the TCF3 locus revealed a copy number of 2 with a corresponding ~7 Mb terminal region of copy neutral loss of heterozygosity.

Additionally, although present in 3 copies, PBX1 was located outside the breakpoint region on chromosome 1.

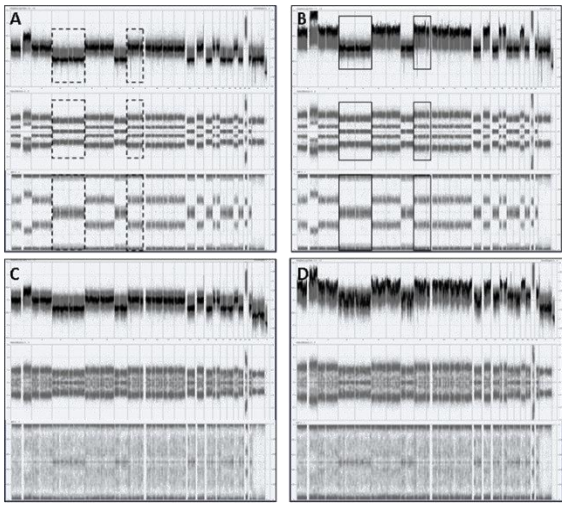

Figure 1: Whole genome-views of SSA and NDA workflows' assignment of copy number calls. The top, middle and bottom panels in A, B, C and D show log2 ratio/smooth signal, SNP patterns and BAF, respectively A. dashed boxes indicate examples of discrepancies between log2-based copy numbers and corresponding allele tracks in SSA. B. Solid boxes indicate concordance between log2 and allele patterns in NDA. C. Admixture of genomic DNA with an unrelated specimen to recapitulate a chimeric state, results in same copy number calls using SSA, with SNP and BAF patterns serving a less effective role in recognition of an error in normalization. D. Same experiment as in C with NDA of the data, resulting in correct normalization despite an overall noisy background due to SNP mismatch.

Other Findings

In an attempt to further assess the relevance of the NDA workflow, the specimen was mixed with a normal sample of genomic DNA derived from an unrelated patient to recapitulate a chimeric state. CMA analysis was done using both the SSA and NDA workflows. As anticipated, SSA produced copy number calls consistent with the previously-observed monosomies (Figure 1C). However, due to the admixture of the alleles, the SNP and BAF patterns were less informative in recognition of an error in copy number normalization. NDA, on the other hand, resulted in the correct copy number calls despite overtly noisy data as a consequence of allelic mismatch (Figure 1D). These results underscore the significance of using appropriate normalization parameters such as the NDA workflow in SNP-based genomic microarrays for tumor specimens in which massive genomic aberrations have been reported.

The constellation of peculiar findings such as the nonasomy of chromosome 21, absence of canonical trisomies in the context of hyperdiploidy, lack of TCF3 involvement in the der(19)t(1;19) and, more importantly, contrary results between SSA and NDA of the SNP-array, warranted more extensive investigation to better pinpoint accurate results. Excess chromosome 21 has been reported in hyperdiploid ALL (Berger R, 1997). However, to the best of our knowledge, this is the first report of an amplification-like event for the entire chromosome 21 in B-lymphoblastic leukemia. Review of the literature, did not provide any evidence supporting an iAMP21-like high risk phenotype for this finding. The t(1;19)(q23;p13) has been associated with clinical heterogeneity and, more recently, an intermediate prognosis (Moorman AV, 2016). Interestingly, the unbalanced form i.e. der(19)t(1;19) is more likely to be seen in the context of hyperdiploidy (Tirado CA et al, 2015), and the lack of the TCF3/PBX1 breakpoint involvement likely represents a unique subset without the anticipated phenotypic implications. The finding of losses of whole chromosomes 3, 4, 7, 13, 15, 17, 18, 20 and 22, as detected by CMA using the SSA workflow, suggested hypodploidy, which is associated with a high risk prognosis (Safavi and Paulsson K, 2017). However, review of the SNP/BAF patterns raised concern regarding the accuracy of the copy number calls. In monosomic chromosomes, the SNP/BAF patterns resembled those of disomic chromosomes with AA, AB and BB alleles whereas, for disomic chromosomes, the SNP/BAF resembled that of copy number gains with AAA, AAB, ABB and BBB alleles. The mosaic nature of the findings further hampered delineation of the correct copy number states. Use of the NDA workflow, however, resulted in entirely different sets of copy number calls with concordant SNP/BAF patterns. These results also had full concordance with the CC and FISH data, confirming the presence of true hyperdiploidy and a good prognosis.

In our hands, the SSA workflow had always yielded satisfactory results. However, this is the first case in which massive aneuploidy of the genome resulted in the failure of accurate normalization by this algorithm, thereby assigning wrong copy numbers to the chromosomes. The available materials from the manufacturer recommended the use of the NDA workflow for cases where >50% of the genome is aberrant. However, this information may not be readily available beforehand, particularly if CMA is the primary or only test performed by the laboratory. Under such circumstances, any discrepancy between copy number calls (smooth signal/log2) and SNP/BAF tracks must be diligently investigated and alternative analysis workflows such as NDA should be applied. However, as shown in the chimeric experiment, this may be further compromised in cases in which the patient has a history of allogenic stem cell transplantation, for the SNP/BAF patterns may no longer serve as a very relevant secondary means for verification of copy number calls. In this scenario, use of SSA may lead to incorrect ploidy determination, potentially resulting in an entirely different interpretation which might affect management decisions. Therefore, utilization of proper normalization parameters such as the NDA algorithm may be warranted as a primary workflow in neoplasms with massive genomic aberrations such as hyperdiploidy.

We explored upfront utilization of NDA using several other oncology specimens and side- by-side comparison using both the SSA and NDA workflows was performed (data not shown). The results indicated that the use of the NDA workflow produced identical results to SSA in the absence of massive chromosomal abnormalities. Consequently, use of NDA does not appear to adversely affect the results of tumor specimens if they are indeed negative for massive hypo- or hyperdiploidy. Dry-lab validation for implementation of NDA could be completed by each laboratory in a short amount of time using previously-tested specimens.

Comments

A challenging aspect in the cytogenetic workup ALL is due to a biological phenomenon reported in this condition whereby a near haploid or hypodiploid genome undergoes doubling without concomitant cell division, resulting in the generation of a pseudohyperdiploid clone.

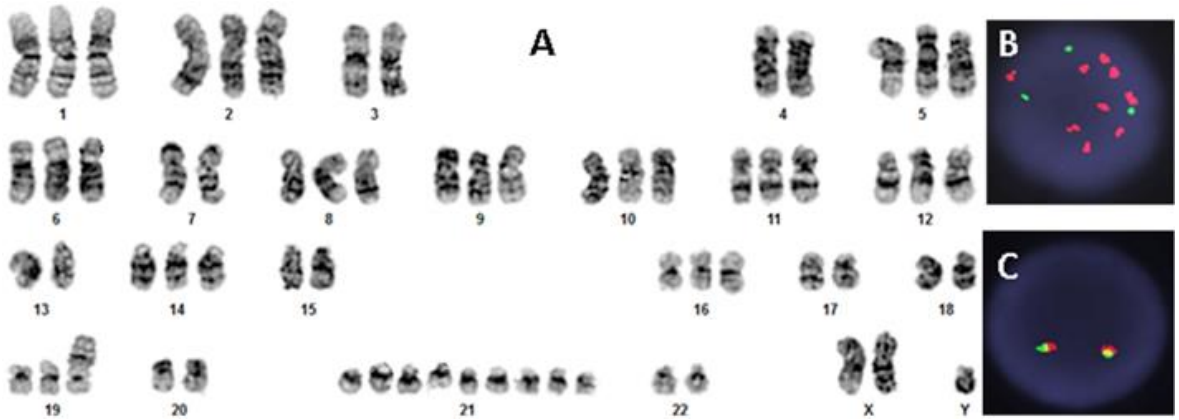

Figure 2: Conventional cytogenetics and FISH results consistent with atypical hyperdiploidy. A. Karyogram showing extra copies of chromosomes X, 1, 2, 5, 6, 8, 9, 10, 11, 12, 14, 16, a supernumerary der(19)t(1;19) and nine copies of chromosome 21. B. Interphase FISH showing three copies of ETV6 (green) at 12p13.2 and nine copies of RUNX1 (red) at 21q22.12. C. FISH probes targeting the TCF3 telomeric (green) and centromeric (red) sequences at 19p13.3 indicating an intact signal pattern

Distinction between pseudo- and true hyperdiploidy may therefore be a problem, particularly in cases with atypical patterns and result in uncertainty with regard to the prognostic implications.

By virtue of SNP probes and B-allele frequency (BAF), CMA can solve this problem. The retention or loss of heterozygous allelic loci on disomic chromosomes would indicate true- or pseudo-diploidy, respectively. However, as cautioned by ACMG guidelines (Cooley LD et al, 2013), overall ploidy status may affect the normalization of array data, in which case the resultant copy number gain and loss calls might be altered. This case underscores the clinical utility of rapid and low cost integration of NDA in cancer CMA analyses where the CytoScan HD platform is routinely used.

References

Berger R. Acute lymphoblastic leukemia and chromosome 21. Cancer Genet Cytogenet. 1997 Mar;94(1):8-12

Cooley LD, Lebo M, Li MM, Slovak ML, Wolff DJ. American College of Medical Genetics and Genomics technical standards and guidelines: microarray analysis for chromosome abnormalities in neoplastic disorders. Genet Med. 2013 Jun;15(6):484-94

Moorman AV. New and emerging prognostic and predictive genetic biomarkers in B-cell precursor acute lymphoblastic leukemia. Haematologica. 2016 Apr;101(4):407-16

Safavi S & Paulsson K. Near-haploid and low-hypodiploid acute lymphoblastic leukemia: two distinct subtypes with consistently poor prognosis Blood 2017; 129: 420-423

Tirado C A. et al.. A (1;19) translocation involving TCF3-PBX1 fusion within the context of a hyperdiploid karyotype in adult B-ALL: a case report and review of the literature Biomark Res 2015; 3: 4

This article should be referenced as such:

Padeganeh A, Song J, Aoun P, Mei M, Ali H, Weisenburger DD, Murata-Collins JL.. Clinical Relevance of Normal Diploid Workflow in Microarray Analysis of Massively Aneuploid Genomes: Lessons from a Case of B-Lymphoblastic Leukemia. Atlas Genet Cytogenet Oncol Haematol. 2019; 23(8):242-245.