Near-haploid B lymphoblastic leukemia with an apparent hyperdiploid karyotype: the critical role of SNP analysis in establishing proper diagnosis

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Abstract Molecular genetic analysis is indispensable in both classification and prognostication of hematopoietic malignancies. The evolution of genetic analysis from conventional cytogenetics to novel genomic technologies has increased our ability to detect subtle and nonconventional mutations in leukemia and lymphoma. Near haploidy, a subtype of hypodiploidy, is a rare entity that carries a particularly poor prognosis in B lymphoblastic leukemia (B-ALL). Near-haploid B-ALL usually quickly evolves by doubling of the chromosome number, creating confusion with the prognostically favorable hyperdiploid B-ALL subtype. Here, we describe a case of B-ALL in which single-nucleotide polymorphism (SNP) microarray analysis was used to identify a hyperdiploid clone that had evolved from a presumptive, near-haploid clone. By conventional methods, this clone may have been easily misinterpreted as a common hyperdiploid clone. Given the extreme prognostic differences of the two clones, this distinction is especially critical to accurately guide therapy. In this case report and brief review, we discuss the role of SNP microarray analysis in the diagnosis and proper subclassification of B-ALL as well as its potential in the identification of genetic therapeutic targets.

Keywords SNP array · Hypodiploid · B lymphoblastic leukemia · Haploid · ALL

Introduction

B lymphoblastic leukemia (B-ALL) is the most common neoplasm in children, and despite modern advances in therapy that have vastly improved overall prognosis, approximately 20 % of patients experience disease recurrence [1]. Numerous recurrent cytogenetic abnormalities have been described in B-ALL, many of which have been established as independent risk factors for survival in both children and adults [2–13]. Included among these is chromosome number, which provides important prognostic information that frequently influences management of pediatric B-ALL. Those cases with high hyperdiploidy (51–65 chromosomes), especially trisomy of chromosomes 4, 10, and 17, have a favorable prognosis in children [13]. In contrast, hypodiploidy (<46 chromosomes), which is seen in 5–8 % of B-ALL patients, affords an extremely unfavorable prognosis [2, 4–6, 13, 14]. Hypodiploid B-ALL can be further subdivided based on degree of aneuploidy into near-haploid (23–29 chromosomes), low-hypodiploid (33–39 chromosomes), and high-hypodiploid (40–45 chromosomes) groups [14–16].

B-ALL with near-haploid or low-hypodiploid karyotypes have a propensity to undergo duplication of their entire chromosome complement, resulting in a chromosome number of 50–78 and thus complicating the distinction from a hyperdiploid karyotype [17]. Given the prognostic and therapeutic differences between hyperdiploid and hypodiploid B-ALL, awareness of this phenomenon, and the ability to distinguish these two scenarios, is essential.

While conventional chromosome analysis and fluorescence in situ hybridization (FISH) have been the traditional

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standards for defining chromosome number and identifying recurrent translocations in leukemic cells, the introduction of newer molecular technologies such as whole genome single-nucleotide polymorphism (SNP) microarray analysis has led to a greater understanding of the genetics of these neoplasms [7, 13, 17, 18]. High-density SNP analysis, which simultaneously measures allele-specific copy number at approximately one million unique loci, is particularly useful for analyzing neoplastic lesions in an unbiased, comprehensive manner. Importantly, SNP arrays can detect both copy number changes as well as copy number neutral loss of heterozygosity, which cannot be detected using traditional karyotyping [7, 12, 18–20]. Recent studies using this technology in cases of ALL have identified several new cryptic abnormalities that have not been detected using standard methodology [7, 12, 19, 21]. By correlating these abnormalities with clinically relevant information, these studies demonstrate that SNP analysis can be used in identifying clinically significant patient subgroups as well as in identifying possible therapeutic targets [7, 19, 21, 22].

Here, we describe a case of pediatric B lymphoblastic leukemia with a hyperdiploid karyotype that has evolved from a near-haploid clone that was not detectable by conventional cytogenetics. This case illustrates the limitations and possible pitfalls of conventional cytogenetic methods and highlights one role for SNP array analysis in establishing accurate genetic subclassification of this neoplasm.

Materials and methods

Pathologic evaluation and bone marrow studies

Bone marrow aspirates were prepared and stained with a Wright–Giemsa stain. Flow cytometric analysis was performed on bone marrow samples by CPA Laboratories (Louisville, KY) per their protocol using antibodies to CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11c, CD13, CD14, CD19, CD20, CD23, CD25, CD33, CD34, CD38, CD45, CD56, CD117, CD138, HLA-DR, kappa surface light chain, and lambda surface light chain.

Cytogenetic and FISH analysis

Karyotyping was performed by Integrated Oncology, Laboratory Corporation of America Holdings (Brentwood, TN) on bone marrow aspirates using standard protocols. Interphase FISH analysis on bone marrow aspirates was performed by Integrated Oncology, Laboratory Corporation of America Holdings (Brentwood, TN) per their protocols using the following probes: CEN 4, 10, and 17; ETV6(TEL)/RUNX1(AML1); MLL; BCR/ABL; p16(CDKN2A); and TCF3(E2A).

SNP microarray analysis

SNP microarray analysis was performed by Integrated Oncology, Laboratory Corporation of America Holdings (Brentwood, TN). Briefly, SNP microarray analysis was performed using the Affymetrix CytoScan HD platform. A total of 250 ng of genomic DNA was extracted, digested with NspI, ligated to NSPI adaptors, and amplified using Titanium Taq with a GeneAmp PCR System 9700. PCR products were purified using AMPure beads and quantified using NANOdrop 8000. Purified DNA was fragmented, biotin-labeled, and hybridized to the Affymetrix CytoScan HD GeneChip. Data were analyzed using Chromosome Analysis Suite and were based on the GRCh37/hg19 assembly.

Clinical history and results

Clinical history, bone marrow, and flow cytometry results

The patient is a 10-year-old, previously healthy boy who presented to his physician complaining of fatigue and bone pain in September 2012. There was no reported family history of childhood cancer. A complete blood count was performed and demonstrated a WBC count of 17,500 cells/ μ L, 60 % of which were blasts, with concomitant anemia and thrombocytopenia. A bone marrow study was then performed, demonstrating a hypercellular marrow with greater than 90 % blasts (Fig. 1). Flow cytometry revealed the blasts to be of B cell origin with the following immunophenotype: positive for CD19, CD10, CD22, CD34, CD38, HLA-DR, and TdT; negative for CD3, CD20, sIgM, cIgM, MPO, kappa, lambda, and other myeloid and T cell antigens. These combined findings were diagnostic of B lymphoblastic leukemia. There was no evidence of central nervous system or testicular involvement.

Fig. 1 Bone marrow aspirate demonstrates numerous small- to medium-sized lymphoblasts. Wright–Giemsa stain, $\times 1,000$

Cytogenetic and FISH analysis

Conventional metaphase analysis revealed the following karyotype: 52,XY,+X,+Y,+14,+14,+21,+21[cp20]. The gain of the Y chromosome as well as tetrasomy for chromosomes 14 and 21 was suggestive of duplicated near-haploid state, although no such metaphases were identified (Fig. 2).

FISH studies demonstrated that 95 % of nuclei possessed four copies of the *RUNX1* locus on chromosome 21q22 (Fig. 3). No *ETV6/RUNX1*, *MLL*, *BCR/ABL*, or *TCF3* gene rearrangements were detected. No deletion of *p16* was seen. Centromeric probes for chromosomes 4, 10, and 17 revealed two copies of each in 100 % of cells. Consistent with the metaphase karyotype, single-copy signals consistent with near haploidy were not detected.

SNP microarray analysis demonstrates near haploidy

Consistent with karyotype analysis, whole-genome SNP microarray analysis demonstrated an abnormal clone with a chromosomal complement of a whole number multiple of 26 chromosomes (i.e., 52 chromosomes). Given the allele dosage patterns showing either diploidy or tetraploidy, this is consistent with an original clone that had 26 chromosomes including two copies of chromosomes 14 and 21 and a single copy of all other chromosomes, including the X and Y. The presence of heterozygosity in chromosomes 14 and 21 with a copy number dosage of 4 (Fig. 4a), combined with the loss of heterozygosity in the remaining diploid chromosomes (Fig. 4b), is a conclusive evidence of near-haploid origin.

Discussion

This is an interesting case of childhood B-ALL that required SNP array data for proper subclassification and designation of the patient to the appropriate prognostic subgroup. On initial review, the karyotype appears hyperdiploid with 52

Fig. 3 *ETV6/RUNX1* (*TEL/AML1*) FISH. Four *RUNX1* signals (chromosome 21, red) and two *ETV6* signals (chromosome 12, green) are identified

chromosomes and suggests that the patient should be risk-stratified into the relatively common and prognostically favorable hyperdiploid subgroup. There are several features of the patient's karyotype, however, that are not entirely consistent with the usual hyperdiploid profile. In common hyperdiploidy, gain of chromosome X but not chromosome Y is seen, whereas this patient showed diploidy for both. Furthermore, unlike this patient who had tetrasomy for chromosomes 14 and 21, standard hyperdiploid clones usually have tetrasomy 21 without other tetrasomic chromosomes [21]. Typical hyperdiploid clones also show gain of chromosomes 4, 10, and 17, and the absence of this finding, as seen in this case, would be highly unusual [10, 13]. Thus, the karyotypic findings in this case suggest evolution of a near-haploid clone in spite of the fact that such a clone was not detected by conventional cytogenetics.

Hypodiploidy, and especially near haploidy, confer an extremely poor prognosis and high risk of relapse in B-ALL [8, 15]. In children, the 3-year event-free survival (EFS) rate for the low-hypodiploid and near-haploid groups

Fig. 2 Representative karyotype showing tetraploidy of chromosomes 14 and 21 and diploidy of all remaining chromosomes including X and Y

52,XY,+X,+Y,+14,+14,+21,+21[cp20].

Fig. 4 SNP analysis of representative disomic and tetrasomic chromosomes. The *top y-axis histogram* represents copy number state and the *bottom y-axis histogram* segregates allele calls based on dosage ($A=+0.5$, $B=-0.5$). **a** SNP analysis of chromosome 14. The *top histogram* shows signal levels consistent with four copies of all loci analyzed. The *bottom histogram* demonstrates the midline (0 line) heterozygosity line throughout the analyzed region. **b** SNP analysis of chromosome 2. The *top histogram* shows signal levels consistent with two copies of all loci analyzed. The *bottom histogram* demonstrates homozygosity at the vast majority of loci analyzed, illustrated by the absence of AB alleles on the center (0) line. This is consistent with loss of heterozygosity

is 30 %, compared with 66 % in the high-hypodiploid group [9, 10, 15, 16, 23, 24]. In contrast, hyperdiploid B-ALL patients have up to an 80 % 5-year EFS [25]. Thus, the distinction between a hyperdiploid clone and a near-haploid clone with evolution is critical both for the prediction of prognosis and more importantly for the guidance of therapy in these children. Current recommendations are that children with near-haploid B-ALL proceed directly to allogeneic stem cell transplant at the first complete remission [26], whereas treatment for hyperdiploid B-ALL is a more standard multiphase chemotherapy regimen.

Although hypodiploidy can be seen in 5–8 % of B-ALL, cases that fall into the near-haploid group are extremely rare, accounting for <1 % of B-ALL [12, 13, 15, 27]. This frequency has likely been underestimated due to the propensity of these clones to undergo clonal evolution by chromosomal duplication resulting in an apparently hyperdiploid karyotype [15, 17, 28]. This genetic phenomenon highlights an important limitation of karyotyping and FISH in cases where the original clone is no longer present, as is often the case in near haploidy and severe hypodiploidy. Thus, as in this case, apparent hyperdiploidy detected via conventional cytogenetics can potentially mask an underlying hypodiploid or near-haploid state [15, 17, 28]. As demonstrated here, the use of SNP array confirmed the suspicion of a doubled, near-haploid

clone that was not directly detected in conventional karyotypic or FISH analyses.

Near-haploid B-ALL is characterized by several unique genetic attributes including the rarity of concurrent structural abnormalities, preferential retention of chromosomes 14, 18, 21, and X/Y, as well as frequent duplication of the near-haploid genome [15–17]. In a recent case series of near-haploid B-ALL, chromosome 21 was retained in 8/8 cases, followed by chromosome 14 in 6/8 and X/Y in 5/8, thus highlighting the nonrandom nature of chromosome retention [29]. Our case highlights a very typical example of the uncommon near-haploid B-ALL, made especially diagnostically challenging by the lack of the original hypodiploid clone.

Low-hypodiploid B-ALL has a tendency to exhibit retention of the same chromosomes as in near-haploid B-ALL but, in addition, often shows retention of chromosomes 1, 11, and 19. Additionally, duplication of chromosome number is also frequently observed in low hypodiploidy, yet unlike near haploidy, this entity frequently exhibits structural abnormalities, including translocations. High-hypodiploid B-ALL is quite distinct from the former two groups in that reductions in chromosomal number are typically due to loss of whole chromosome or unbalanced translocations. Most of these patients have complex karyotypes and do not show duplicated hyperdiploid states [15].

Due to the relative rarity of hypodiploid B-ALL, little is known about the specific genetic features that confer a predilection for disease relapse. Whole-genome and FISH analysis of relapsed B-ALL patients suggest that the relapsed clones represent subpopulations of the original leukemia that have undergone selection via the patient's therapeutic regimen [22, 30]. In the cases of ALL at diagnosis when near haploidy was initially observed within a mixture of other karyotypes, the near-haploid clone later predominated in the relapsed marrow [22]. Retention of particular chromosomes in near haploidy might affect the expression of genes that ultimately confer a survival or proliferative advantage to these cells or induce drug resistance [31–33]. Haploinsufficiency of certain genes on monosomic chromosomes has also been suggested to potentially contribute to the transformation and relapse in both near-haploid and low-hypodiploid clones [28]. Additional investigation into the identity of the affected genes may help elucidate the mechanism by which near haploidy and hypodiploidy lead to treatment failure and relapse.

Recent sequencing studies reveal that many patients with near-haploid B-ALL have deletions and mutations affecting the *Ras* signaling pathway as well as specific members of the *IKAROS* lymphoid transcription factor family [8, 11, 12, 34]. Of note, this case did not show a deletion in the *IKAROS* gene. In addition, these studies have demonstrated that near-haploid cases show mutations in a different subset of genes than those seen in other low-hypodiploid cases [8, 11, 12, 34]. These genetic studies clearly demonstrate the potential for identification of targeted therapies and the further subclassification of this poorly understood subtype of B-ALL.

In summary, this case represents a typical example of the genetic features seen in near-haploid B-ALL and the potential for misidentifying these cases, as many present as doubled, near-haploid clones. As such, SNP array analysis, which can easily distinguish heterozygosity from homozygosity on a genomic scale, provides critical information to aid in this distinction.

Conflict of interest The authors declare that they have no conflict of interest.

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