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Cryptic chromosome abnormalities in a patient with mixed phenotype acute leukemia

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Acute leukemias of ambiguous lineage are rare and incompletely characterized. They include acute undifferentiated leukemia, which lacks lineage-specific antigens, and mixed phenotype acute leukemia (MPAL), in which blasts express antigens of more than one lineage, so that assignment to a specific lineage-related category is not possible. MPAL cases, containing either distinct blast populations, each of a different lineage, or one population with multiple antigens of different lineages on the same cells, or a combination, may be further classified as B/myeloid or T/myeloid depending on their immunophenotypic features [1]. Knowledge about MPAL biology and clinical presentation is limited. Moreover, there is no agreement on how the disease should be treated. Most patients receive induction chemotherapy for either acute lymphoid leukemia (ALL) or acute myeloid leukemia (AML) based on blast morphology. There is frequent resistance to induction chemotherapy, and patients who achieve complete remission have a high risk of relapse and are therefore considered for allogeneic hematopoietic stem cell transplant when possible.

The recent World Health Organization (WHO) classification of hematopoietic malignancies recognizes that chromosome aberrations are major and reliable diagnostic markers, and specific genetic aberrations are independent predictors of disease response and patient survival. Cytogenetic findings have been used as a key factor in the classification of AML and ALL. In contrast, only a few chromosome abnormalities have been found to be associated with MPAL, including a 9;22 translocation resulting in *BCR/ABL1* fusion, 11q23 translocations rearranging the *MLL* gene [1] and a recurrent t(6;14) (q25;q32), which we previously reported in patients with T/myeloid leukemia [2]. Additional cytogenetic abnormalities remain to be identified in order to improve knowledge and guide management

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of this rare clinical entity. We report here our finding of cryptic chromosome abnormalities in a patient with T/myeloid MPAL.

Our patient was a 75-year-old man who presented with a white blood cell count of $11.8 \times 10^9/L$. Morphologic analysis favored a diagnosis of T-lymphoblastic leukemia. Flow cytometric analysis detected expression of the stem cell marker CD34 (69%), the T-cell markers cytoplasmic CD3 (78%), CD4 (50%) and CD5 (59%) and the myeloid markers CD13 (73%) and CD117 (71%). Initially, myeloperoxidase appeared to be equivocal, but was positive when repeated, leading to the diagnosis of T/myeloid MPAL. Unfortunately the patient's leukemia was refractory to induction chemotherapy that included cyclophosphamide, doxorubicin, vincristine and prednisone, and did not respond to subsequent chemotherapy regimens. He succumbed to acute leukemia 5 months after diagnosis.

Cytogenetic analysis was performed on a bone marrow specimen at the time of diagnosis. Giemsa banding-based karyotype analysis detected a 7q deletion, with a karyotype designation of 46,XY,del(7)(q34q36)[20]. In order to refine the deletion boundary and to identify potential submicroscopic cytogenetic abnormalities, we took an oligonucleotide-based microarray approach to examine the genome of this patient's leukemia cells from his bone marrow. As shown in Figure 1, the microarray analysis revealed submicroscopic deletions in 4q (8.9 Mb) and 12p (1.6 Mb), in addition to the visible 14 Mb deletion in 7q.

The deleted regions of 4q included three genes, *NFKB1*, *CENPE* and *TET2* [Figure 1(A)]. *NFKB1* encodes a protein that is a DNA-binding subunit of the nuclear factor κB (NF κB) protein complex, which is an important regulator of cell survival, cell cycle, cell adhesion and migration. Constitutive NF κB activation has been demonstrated in AML cells [3]. The NF κB pathway is also highly active in T-ALL, and inhibition of this pathway has suppressed tumor growth [4].

The *TET2* gene was first identified in patients with AML with deletions of chromosome 4q24, and was determined to be a tumor suppressor gene. Subsequent sequencing studies revealed *TET2* mutations in 10 – 25% of patients with myeloid malignancies [5,6]. In a recent study, *TET2* deletions were found to be recurrent in myeloid malignancies and to be frequently accompanied by *TET2* mutations on the remaining allele [7]. *CENPE* maps between *NFKB1* and *TET2*, and encodes centrosome-associated protein E, which plays a key role in the movement of chromosomes during mitosis.

The 7q deletion detected by both karyotyping and microarray was an interstitial deletion close to the terminal region of 7q. The deleted genes are shown in Figure 1(B). It is worth noting that *EZH2* was among the deleted genes. *EZH2* encodes histone-lysine N-methyltransferase for epigenetic regulation. Recent studies using single nucleotide polymorphism (SNP)-array demonstrated deletions and copy-neutral loss of heterozygosity of this region, which further led to the identification of *EZH2* mutations and the association with aggressive myeloid malignancies [8,9].

The deletion in 12p contained only one gene, *ETV6* [Figure 1(C)]. *ETV6* encodes a transcription factor required for hematopoietic development and is frequently altered in a

broad spectrum of hematologic malignancies [10]. In a recent study of genetic bases of early T-cell precursor acute lymphoblastic leukemia, Zhang *et al.* detected deletions and mutations of *ETV6* in 33% of cases of early T-cell precursor ALL and 10% of cases of non-early T-cell precursor ALL [11].

Karyotype analysis is the conventional method for demonstration of chromosomal abnormalities, but this method is often hampered by suboptimal chromosome morphology, low mitotic activity in subtypes of malignancies and inability to detect subtle (<10 Mb) abnormalities. The incorporation of fluorescence *in situ* hybridization (FISH) technology in clinical practice has significantly improved cytogenetic diagnosis and monitoring of disease progression. However, FISH can only detect abnormalities in targeted regions. The microarray-based approach allows for the detection of DNA copy number alterations in the entire genome. The micro array platform used in this study was an oligonucleotide-based microarray (Signature Genomic Laboratories, Spokane, WA). It targets > 1800 genes and genomic intervals associated with cancer at a probe spacing ranging from ~ 1 probe per 0.2 – 7.0 kb. The genomic backbone of the array had an average spacing of ~ 1 probe per 35 kb. Various types of microarray platforms have been used in the detection of copy number aberrations in hematologic malignancies [12,13]. SNP-array offers added advantage for the detection of copy-neutral loss of heterozygosity. Microarray-based cytogenetic analysis is an important method in clinical practice to close the gap between standard karyotyping and molecular analysis at the single base-pair level.

Combining karyotyping with microarray analysis, we were able to demonstrate the presence of both microscopic and submicroscopic chromosome abnormalities in a patient with MPAL. Although the morphologic analysis and initial equivocal result of myeloperoxidase favored a diagnosis of T-lymphoblastic leukemia, the positivity of myeloperoxidase from a repeated test together with flow cytometric and molecular findings supported the diagnosis of T/myeloid leukemia. This case demonstrates that accurate characterization of cytogenetic findings at the molecular level will help to characterize cases of MPAL. Moreover, molecular analysis may ultimately help in identification of therapeutic targets in these leukemias which respond poorly to current therapies.

MPAL is a rare leukemia. In a recent retrospective analysis of 4780 patients with adult leukemia by Yan *et al.* only 117 (2.4%) patients had leukemias that met the WHO diagnostic criteria for MPAL [14]. MPAL is a complex entity with heterogeneous clinical, immunophenotypic, cytogenetic and molecular genetic features. While heterogeneity is the mainstay, detection of recurrent and cryptic chromosome abnormalities will contribute to the accurate diagnosis of MPAL, increase our understanding of the molecular basis of this rare entity and hopefully identify molecular therapeutic targets.

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Figure 1.

Chromosome abnormalities detected in mixed phenotype acute leukemia. (A) An 8.9 Mb deletion in 4q, including *NFKB1*, *CENPE* and *TET2* [arr 4q23q25(100,298,592-109,207,091)×1]. (B) A 14 Mb deletion in 7q, including *EZH2* and other genes as indicated [arr 7q34q36.3(142,128,202-156,466,525)×1]. (C) A 1.6 Mb deletion in 12p containing *ETV6* [arr 12p13.2p13.1(11,094,776-12,635,257)×1].