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Genomic alterations in myeloid neoplasms with novel, apparently balanced translocations

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Characterization of gross chromosomal rearrangements, particularly translocations in neoplasms, has proven to be valuable in patient management by aiding in diagnosis, defining prognosis, and leading to new therapeutic interventions. In this report, we investigate two apparently balanced translocations, t(6;17)(q23.3;p13.3) and t(2;13)(p21;q14.11), in patients with myeloid neoplasms and uncover concomitant microdeletions associated with the breakpoints. Breakpoint mapping by fluorescence in situ hybridization (FISH) detected deletions at or adjacent to all breakpoints. Subsequently, array comparative genomic hybridization on the 244 K Agilent platform refined the deletion boundaries, revealing a 1.7 Mb deletion directly adjacent to the 6q23.3 breakpoint, and a 562 kb deletion at 17p13.3 in the first case. The second case was found to harbor a 195 kb deletion at 2p21 and a 1.4 Mb deletion distal to the 13q breakpoint at 13q14.3. Additionally, a 133 kb deletion within the breakpoint region at 13q14.11 and a 265 kb deletion proximal to the breakpoint were discovered, neither of which was detected by FISH. Although a gene fusion resulting from either novel rearrangement cannot be determined from these data, formation of a fusion transcript cannot be excluded because the resolution of the techniques used does not allow definite delineation of the breakpoint locations. Although the incidence and clinical relevance of these focal imbalances remains to be evaluated, the cases presented here support high resolution evaluation of presumably balanced rearrangements in neoplasms. Such imbalances may portend important hitherto unrecognized prognostic and diagnostic categories.

Keywords Cytogenetics, array CGH, chromosomal rearrangement, hematologic malignancy

Chromosomal translocations have been reported in most hematological malignancies and represent diagnostic and prognostic markers in these diseases (1). Such genomic perturbations often result in chimeric proteins or altered protein expression, factors believed to be causative and contributory to the disease. The etiology of translocations remains elusive; however, current hypotheses focus on the formation of double-strand breaks and subsequent ligation via homologous recombination or nonhomologous end joining (2–4). Certain sequence motifs over represented at and surrounding translocation and deletion breakpoints (such as Alu sequences, polypurine runs, DNA polymerase pause sites, and immunoglobulin and T-cell receptor loci) are also thought to facilitate deletions through homologous unequal recombination and translocations via nonhomologous recombination (5–8).

Although these rearrangements seem balanced at the conventional cytogenetic level, investigation with higher-resolution technologies refutes this observation. By exploiting fluorescence in situ hybridization (FISH) and array-based comparative genomic hybridization (aCGH), several groups have uncovered deletions frequently occurring at the breakpoints of recurrent rearrangements involving *ETV6/RUNX1, BCR/ABL1, MLL, RUNX1/RUNX1T1, CBFB/MYH11,* and *PML/RARA* (9–18). Studies of constitutional chromosomal rearrangements (8,19,20) and novel rearrangements in hematological malignancies (21,22), although far less numerous, have also revealed cryptic lesions at the breakpoints. Characterization of the breakpoints in these

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rearrangements can lead to clinically useful discoveries, as demonstrated in the investigation of a t(1;4)(q44;q12) in a patient with hypereosinophilic syndrome resulting in detection of an interstitial deletion in 4q and subsequent detection of the resultant *FIP1L1-PDGFRA* fusion transcript. The chimeric transcript results in a constitutively active tyrosine kinase, now recognized as an efficacious target of imatinib and found to be present in a significant portion of patients with hypereosinophilic syndrome (23).

The clinical relevance of other translocation-associated deletions remains a controversial topic because the only patient cohorts that have been comprehensively studied are those harboring the t(9;22)(q34.1;q11.2) with a deletion on the derivative chromosome 9. Previously, several groups reported that patients with this chromosomal imbalance responded poorly to interferon (11,15). However, with the introduction of imatinib in the treatment of chronic myeloid leukemia, there are divergent opinions on the prognostic relevance of the deletion in the era of tyrosine kinase inhibitors (9,10,17,18,24).

Herein, we report two patients with myeloid malignancies harboring novel chromosomal rearrangements with genomic imbalances at or surrounding the breakpoints. Currently, the oncogenic effect of these complex rearrangements is unclear. Nevertheless, this study adds to a growing catalog of translocation-associated deletions and highlights the use of two different techniques (FISH and aCGH) in characterizing apparently balanced rearrangements and identifying candidate genes. Although some data suggest that newly identified, cryptic lesions may negatively impact clinical course (25), future studies and further clinical follow-up (26) will improve our understanding of the prognostic significance of these findings.

Materials and methods

Patient histories

Patient 1

A 68-year-old man with a history of prostatism, hypercholesterolemia, degenerative joint disease, and colon polyps sought care for total hip replacement in January 2003 and was found to be anemic. Bone marrow biopsy performed at that time revealed mild hypocellularity, dysplastic changes, and excess blasts (10−12%), consistent with myelodysplastic syndrome, subtype RAEB-2. An erythroid stimulating agent was administered without effect. Evaluation in April 2003 was remarkable for a mildly ill appearance and hematocrit of 30%, a platelet count of 148×10^9/L, and white blood cell count of 3×10^9/L, including 6% blasts. Examination of the bone marrow revealed trilineage dysplasia with about 10% blasts, which on flow cytometric analysis showed a population of immature cells positive for CD34, CD45 (dim), HLA-DR, and myeloid markers CD13, CD117, and CD33, but negative for B and T lymphoid and monocytic markers, consistent with myeloblasts. Cytogenetic analysis revealed 46,XY,t(6;17)(q2?3;p13) in all 20 metaphases analyzed (Figure 1A). The patient was placed on a phase 1 trial (LAQ824) with a histone deacetylase inhibitor (HDACi) with no major effect after four cycles, and then remained stable while receiving azacytidine between fall 2003 and the middle of 2006, when he developed pancytopenia. In January 2007, another phase I HDACi protocol (LBH589) was administered, but the marrow progressed to acute myeloid leukemia (AML) after two cycles. The t(6;17)(q23;p13) was present in all subsequent chromosome studies. However, additional related abnormal clones were observed with either del(20)(q11.2q13) or del(13)(q14q32). Furthermore, a normal cell line (46,XY) was detected, excluding the possibility of a constitutional rearrangement. In April 2007, a phase 1 trial (SAHA) of an HDACi plus a cdk2 inhibitor (flavopirodol) was initiated, but the regimen was not well tolerated; supportive care was provided until the patient's death in July 2007.

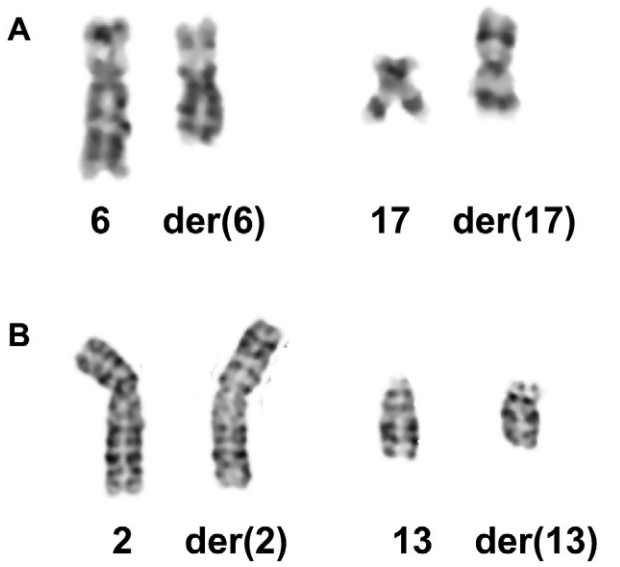

Figure 1 Partial karyotypes with GTG-banded chromosomes 6, der(6), 17, and der(17) resulting from a t(6;17)(q23.3;p13.3) for patient 1 (A), and chromosomes 2, der(2), 13, and der(13) resulting from a t(2;13)(p21;q14.11) for patient 2 (B).

Patient 2

An 87-year-old man with a history of atrial fibrillation, congestive heart failure, and stage III colonic adenocarcinoma in 1995 treated with surgery, radiation, and chemotherapy, sought care in January 2007 with weakness and pancytopenia. Complete blood count revealed a white blood cell count of 0.9×10^9/L, a hematocrit of 24%, mean corpuscular volume of 107 fl, and a platelet count of 82×10^9/L. A bone marrow biopsy revealed 60% erythroid precursors, 16% myeloid precursors, and 11% blasts, which were positive for CD33, CD13, CD117, and HLA-DR by flow cytometry. These findings led to a diagnosis of FAB M6a AML (erythroleukemia), and cytogenetic analysis demonstrated 46,XY. The patient was initially treated with daily oral 6-mercaptopurine and subsequently received induction chemotherapy (idarubicin and cytarabine), which was complicated by neutropenia with polymicrobial bacteremia. He experienced complete remission but did not receive consolidation therapy as a result of age and multiple comorbidities.

Eleven months after induction chemotherapy, the patient developed recurrent pancytopenia. Bone marrow biopsy revealed recurrent erythroleukemia. Cytogenetic analysis now demonstrated a balanced t(2;13)(p1?6.2;q1?3) in 19 of