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# *ABL* Oncogene Amplification with *p16*$^{INK4a}$ Gene Deletion in Precursor T-Cell Acute Lymphoblastic Leukemia/Lymphoma: Report of the First Case

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**Gene amplification is a relatively rare event in hematologic malignancies. The *ABL* gene on chromosome band 9q34 is a proto-oncogene and is the well-known translocation partner of the *BCR* gene on 22q11 giving rise to t(9;22)(q34;q11), which is the hallmark of chronic myeloid leukemia and is the most common chromosomal abnormality in adult acute lymphoblastic leukemia (ALL). Amplification of *ABL* is an exceedingly rare event, with only less than 5 cases reported in the literature. The *p16*$^{INK4a}$ (or *CDKN2A*) gene on 9p21 is a tumor suppressor gene, and deletion thereof is recently recognized as one of the most common genetic abnormalities in ALL. The authors herein describe an 8-year-old male patient with precursor T-cell ALL harboring both *ABL* gene amplification and *p16*$^{INK4a}$ gene deletion. Fluorescence in situ hybridization (FISH) analysis using *BCR/ABL* probes revealed five or more *ABL* signals, indicating amplification in 51.5% of interphase nuclei. FISH using *p16*$^{INK4a}$ gene probes showed heterozygous *p16*$^{INK4a}$ deletion in 71.0%. On conventional cytogenetic analysis, however, only 10 metaphases were available, which showed the normal karyotype, 46,XY[10], serving no evidence for the findings on FISH. This is the first report of an ALL case with *ABL* amplification, and the authors speculate that both *ABL* proto-oncogene amplification and the *p16*$^{INK4a}$ tumor suppressor gene deletion have been implicated in leukemogenesis in the present case, although whether the *ABL* amplification truly contributes to the leukemogenesis or merely an epiphenomenon representing underlying genomic instability remains to be determined. Am. J. Hematol. 76:360–363, 2004.** **© 2004 Wiley-Liss, Inc.**

**Key words: acute lymphoblastic leukemia; *ABL*; gene amplification; *p16*$^{INK4a}$; deletion**

## INTRODUCTION

Gene amplification is a term used to indicate the production of multiple copies of a specific gene [1] and represents a common genomic event in human cancer cells. It is a well-characterized genetic event in solid tumors, and detection thereof has recently been incorporated as a routine workup in, for example, breast cancer, due to its prognostic and therapeutic relevance [2,3]. In hematologic malignancies, on the other hand, it is relatively a rare event and its pathogenic implication is still elusive. This is partly because in hematologic malignancies, other chromosomal aberrations such as reciprocal translocations or deletion of genetic segments are far more common and their roles in leukemogenesis have been extensively studied. The *ABL* gene on 9q34 is a well-known proto-oncogene with tyrosine kinase activity, and its constitutive activation via fusion with the *BCR* gene on 22q11, generating the *BCR/ABL* chimeric transcript, is the molecular hallmark of chronic myeloid leukemia (CML) and is one of the most common genetic events identified in adult acute lymphoblastic leukemia (ALL). Detection of the *BCR/ABL* rearrangement is the prototype of

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leukemia-specific therapeutic approach by the institution of an *ABL*-specific tyrosine kinase inhibitor, STI571, and thus has become an essential part of routine leukemia workup not only at initial diagnosis but also during follow-up to monitor minimal residual disease and to detect molecular evidence for resistance of leukemic cells to STI571 therapy, such as *BCR/ABL* amplification [4,5].

Deletion of tumor suppressor genes constitutes another principal genetic pathway toward tumorigenesis and the loss of heterozygosity for the $p16^{INK4a}$ gene or *CDKN2A* (cyclin-dependent kinase inhibitor 2A) gene by the HUGO Gene Nomenclature Committee (http://www.gene.ucl.ac.uk/nomenclature/) on 9p21 (MIM 600160) via homozygous or heterozygous deletion is a well-known genetic aberration in various solid tumors including melanoma. Recently, the $p16^{INK4a}$ deletion has been shown to be one of the most common genetic events in ALL [6]. A growing line of evidence shows that the $p16^{INK4a}$ deletion heralds poor prognosis of the disease [7]. Detection of the $p16^{INK4a}$ deletion needs incorporation of a molecular cytogenetic approach such as fluorescence in situ hybridization (FISH), as the deletion may be submicroscopic [8].

Recently, amplification of the *ABL* gene has been described in two reports: one in a series of treatment-related acute myeloid leukemias and the other in a case of CML in blast phase [9,10]. Here we describe the first case of precursor T-ALL harboring both *ABL* amplification and $p16^{INK4a}$ deletion as determined by FISH with a review of the previously reported cases.

## PATIENTS AND METHODS

### Case Report

An 8-year-old boy was admitted because of dyspnea and palpable neck masses that had developed 2 months prior to admission. On admission, cervical, axillary, and inguinal lymphadenopathy were noted and chest X ray revealed mediastinal widening, bilateral pleural effusion, and pericardial effusion. The laboratory findings included a hemoglobin level of 12.5 g/dL, a leukocyte count of $6.4 \times 10^9$/L, and a platelet count of $342 \times 10^9$/L. Peripheral blood film revealed left-shifted neutrophils with blasts counted at 2.5%. A bone marrow aspirate smear showed medium-sized leukemic cells with a high N/C ratio and convoluted nuclei up to 58% of all nucleated cells. Flow-cytometric analysis of the blast cells demonstrated expression of CD3, CD7, and TdT and negativity for CD2, CD5, B-lymphoid markers, and myeloid markers. Cytospin preparations of the pleural effusion demonstrated malignant cells at 89%. These findings established the diagnosis of precursor T-cell acute lymphoblastic leukemia/lymphoma. Induction therapy for ALL with vincristine, prednisolone, daunorubicin, L-asparaginase, and cyclophosphamide was initiated, and the patient was documented to have attained complete remission of disease at day 28. He remains in continuous remission on routine follow-up 17 months after the initial diagnosis.

### Conventional Cytogenetic Analysis and FISH studies

Unstimulated isolated bone marrow cells were cultured for 24 hr and G-banded according to standard procedure. Metaphases were analyzed and karyotyped according to the nomenclature system proposed by the International System for Human Cytogenetic Nomenclature, 1995.

FISH study was performed with commercial *BCR/ABL* probes (LSI® BCR/ABL ES Dual Color Translocation Probe, Vysis, Downers Grove, IL) and $p16^{INK4a}$ gene probes (LSI® p16/CEP®9 Dual Color Probe, Vysis) according to the manufacturer's instructions. The *BCR/ABL* probes generate a fusion signal with an extra orange signal from the derivative chromosome 9 when the *BCR/ABL* fusion (major form) occurs. Homozygous or heterozygous deletion of the $p16^{INK4a}$ gene is represented by loss of one or two orange *p16* signal(s), respectively, while retaining two green centromeric signals from chromosome 9. Gene amplification was determined by five or more signals per nucleus as previously described [2]. Two hundred nuclei were counted in each test.

## RESULTS

On conventional cytogenetic analysis, only 10 metaphases were available, each of which revealed the normal karyotype, 46,XY[10]. *BCR/ABL* FISH did not show fusion signals for *BCR/ABL* translocation, but revealed five or more *ABL* signals indicating *ABL* amplification in 51.5% of 200 interphase nuclei analyzed: 2 in 18%, 4 in 30.5%, 5 in 8.5%, 6 in 25%, and 7 or more in 18% (Fig. 1A). For cytogenetic characterization of the *ABL* gene amplification, we further analyzed *ABL* FISH signal patterns on metaphases, however, only to find rare metaphases with the normal signal pattern. *p16* FISH revealed heterozygous $p16^{INK4a}$ gene deletion in 71.0% (142/200) interphase nuclei (Fig. 1B). All the analyzed nuclei, including those with $p16^{INK4a}$ deletion, exhibited two green signals from the centromeric probes for chromosome 9.

## DISCUSSION

Regulatory or structural alterations of cellular oncogenes (proto-oncogenes) have been implicated in the causation of various types of cancers [11].

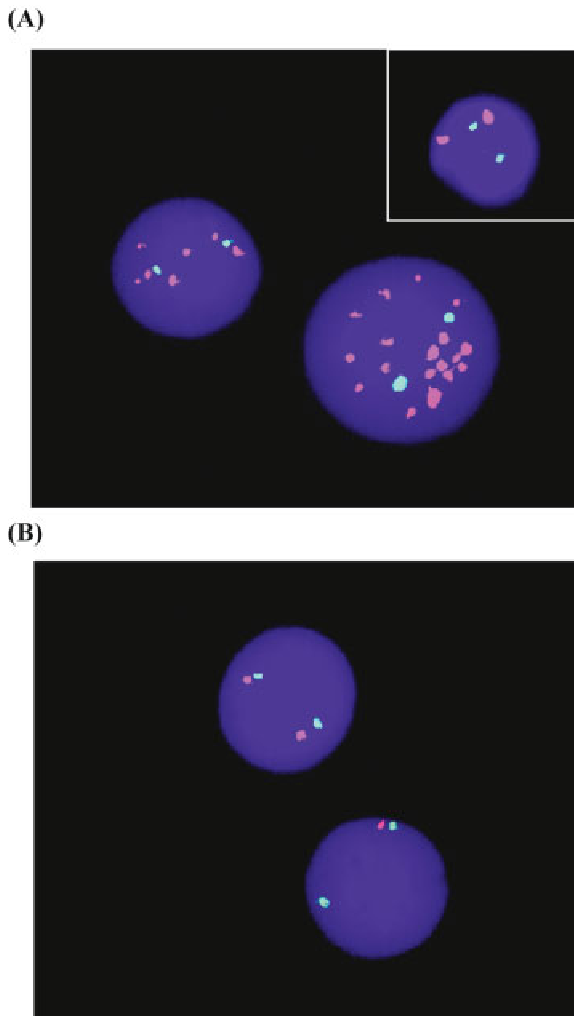


**Fig. 1.** (A) *BCR/ABL* FISH on bone marrow cells. The number of orange signals from *ABL* exceeded four in 51.5% of interphase nuclei. Part of the excessive orange signals could not be captured because they were out of focus at a given plane. The normal *BCR/ABL* signal pattern is shown in the inset (two orange and two green signals from *ABL* and *BCR*, respectively). (B) *p16* FISH on bone marrow cells. Deletion of one of the two orange signals from $p16^{INK4a}$ is noted, while retaining two green centromeric signals from chromosome 9 (bottom right). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Gene amplification represents one of the major molecular pathways by which gene expression is constitutively enhanced above the level of physiologically normal variation. Determination and identification of the underlying genetic events including the amplified genes have recently gained unprecedented clinical relevance due to the advent of molecularly targeted therapy. Cytogenetic manifestations of gene amplification in tumor cells include the homogenously staining regions (HSR) and double-minute chromosomes (dmin). Another robust technique to determine gene amplification status is FISH, which enumerates specific gene copies simply by counting fluorescent signals from interphase or metaphase cells. According to the criteria by Pauletti et al., a ratio of the number of specific gene signals to the number of corresponding centromere signals per cell greater than 2 or a ratio of specific gene signals per cell greater than 4 represents gene amplification [2]. The centromeric signals serve as internal control to rule out increased number of gene signals due to polysomy. The expanding application of molecular cytogenetic technique has rendered amplification of such genes as *MYC*, *MLL*, and *AML1* more readily detectable [12–15]. The cases hitherto reported on gene amplification in hematologic malignancies mostly involved acute myeloid leukemia (AML), and *CMYC* appears to be the most frequently amplified gene in AML. Tanaka et al. first reported *ABL* amplification in three of 7 secondary leukemia cases determined by FISH [9]. They considered three or more (not 5 or more) *ABL* signals to represent amplification, and indeed, the proportions of nuclei with five or more *ABL* signals in two of the three cases were as low as 0.8% (1/122; one with 6 *ABL* signals) and 3.8% (7/183; six with 5 *ABL* signals and one with 6 *ABL* signals), respectively. On metaphase FISH, the extra signals in these cases were isolated onto each marker chromosome or derivative chromosome. Jumping translocation was considered to be the underlying genetic mechanism for the extra *ABL* genes. Thus, the two cases may represent a different category for increased gene copy number (depicted as partial amplification by Busson-Le Coniat et al.) via jumping translocation from that for a high-level amplification encountered in HSR or dmin [16]. More recently, Kenner et al. added another case of *ABL* amplification in a case of CML in B-lymphoid blast phase determined by Southern blot analysis. The patient presented with blast-phase CML with variant Philadelphia chromosome as t(19;22)(p13;q11.2) without precedent chronic phase of the disease. In this report, the *ABL* gene of the leukemic cells was documented to be rearranged with *BCR* as determined by the presence of *BCR/ABL* chimeric transcript by polymerase chain reaction, and to be amplified as well by Southern blot hybridization. FISH study was not done in the case. Ours is the third report on *ABL* amplification, in which *BCR/ABL* FISH revealed five or more *ABL* signals in 51.5% of interphase nuclei. It was difficult to determine the exact number of numerous *ABL* signals in some of the interphase nuclei because part of the signals were out of focus at a given plane and also because of coalescing adjacent fluorescent signals. Apparently, high-level amplification with 10 or more signals was evident in a subpopulation of cells. Of note, up to 30.5% nuclei exhibited four *ABL* signals,

while *p16* FISH incorporating centromere probes for chromosome 9 demonstrated that all the leukemic cells were disomic for chromosome 9. Indeed, the cutoff level of 5 in determining gene amplification may seem somewhat arbitrary, albeit it was from the consideration that the maximum number of FISH signals from a gene in normal somatic cells being 4 [2]. Dichotomization based upon the criteria, accordingly, may seem rather factitious in some cases, considering that the gene amplification event per se would involve a continuous spectrum in a quantitative sense. In any case, the identification and consideration of underlying (cyto)genetic events giving rise to the increased gene copy number would be most relevant. We searched for the cytogenetic evidence of the gene amplification from both G-banded metaphases and metaphase FISH, however, the number of available metaphases was quite limited and both approaches failed to serve any clue.

Heterozygous *p16*$^{INK4a}$ deletion was observed in 71.0% of bone marrow cells. Both homozygous and, less commonly, heterozygous deletions of the *p16*$^{INK4a}$ gene, with or without cytogenetically discernible deletion of the short arm of chromosome 9 (9p−), are observed in ALL, especially of T-lineage [6]. In the case of heterozygous *p16*$^{INK4a}$ deletion, as in this patient, loss of heterozygosity may occur via DNA mutation in or epigenetic modification of gene expression such as promoter methylation of the non-deleted copy [17]. Because the studies on prognostic relevance of *p16*$^{INK4a}$ deletion have mostly involved homozygous *p16*$^{INK4a}$ deletion, both leukemogenic and clinical implications of heterozygous *p16*$^{INK4a}$ deletion are less clear than in the homozygous counterpart [7].

The implication of the *ABL* amplification in leukemogenesis in this case might have been further supported by revealing its co-occurrence with *p16*$^{INK4}$ deletion by FISH in leukemic cells through more sophisticated studies or by showing increased expression of the gene through quantitative analysis of the mRNA copies, for example, at the downstream level.

In conclusion, we described the first case of precursor T-ALL harboring *ABL* gene amplification as determined by *BCR*/*ABL* FISH. FISH study using *p16*$^{INK4a}$ probes revealed that the leukemic cells also had heterozygous *p16*$^{INK4a}$ gene deletion. Both the *ABL* oncogene amplification and the *p16*$^{INK4a}$ tumor suppressor gene deletion might have been implicated in leukemogenesis in this case, although whether the *ABL* amplification truly contributes to the leukemogenesis or occurs merely as an epiphenomenon representing underlying genomic instability remains to be determined. Recognition of the rare occurrence of gene amplification in hematologic malignancies is important for its detection in routine practice with the rapid expansion of application of molecular cytogenetics and ultimately can accumulate data to delineate its pathogenic and clinical significance.

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