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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INTRODUCTION

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 (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.

Key words: acute lymphoblastic leukemia; ABL; gene amplification; p16^INK4a^; deletion
Case Report: ALL With ABL Amplification and p16$^{INK4a}$ Deletion

**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 × 10$^9$/L, and a platelet count of 342 × 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.

**DISCUSSION**

Regulatory or structural alterations of cellular oncogenes (proto-oncogenes) have been implicated in the causation of various types of cancers [11].
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 [7]. 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 $p16^{INK4a}$ 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.

REFERENCES