Abstract. Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy. Approximately 84% of cases of ALL are classified as B-precursor ALL, 14% of cases are T-cell and 2% of cases are B-cell (B-)ALL. About one third of B-ALL cases show an abnormal karyotype. Combining data obtained by immunophenotyping, karyotyping and molecular cytogenetic analyses allows for a better understanding of this heterogeneous disease. This study reports an exceptional B-ALL case with a poor prognosis and unique complex chromosomal aberrations not previously observed, i.e., a translocation involving the six chromosomal regions 1q42, 4q21, 4q24, 4q35 (twice), 8q22 and 10p15.3 besides 9q34 and 22q11.2.

Introduction

Childhood acute lymphoblastic leukemia (ALL) is the most common malignancy in humans aged 18 years or younger. In a number of these children the outcome of treatment is difficult to predict and is considered to be an individual response of the patient to chemotherapy. It is likely that the observed clinical heterogeneity reflects a diverse pathogenesis of leukemia. The molecular basis of childhood ALL is to a large extent unknown, and it is likely that significant advances in the treatment of this malignancy are dependent on a better understanding of the molecular events that cause the disease (1,2). A number of investigators have attempted to identify groups of genes, termed ‘transcriptional signatures’ whose expression can be directly associated with drug resistance (3).

The immunophenotypic characteristics of precursor B-cell acute lymphoblastic leukemic cells (clonal expansion of progenitors of B-cell lymphocytes) are believed to reflect normal hematopoietic B-cell precursors. However, previous studies showed that through the simultaneous acquisition of various antigens, almost all B-precursor-ALL cases display phenotypic aberrations (4-8). The latter may be associated with specific genetic abnormalities, and it has been suggested that they are useful for a better understanding of protein expression dysregulation (9). Approximately 84% of ALL cases are B-precursor ALL, 14% are T-ALL and 2% are B-cell (B-)ALL (10). At least 32% of the ALL cases show clonal chromosomal abnormalities (11). The so-called Philadelphia (Ph) chromosome t(9;22) is present in 4% of pediatric ALL patients and confers an unfavorable prognosis, particularly when associated with either a high whole blood cell count (WBC) or slow early response to initial therapy (12-14).

This study reported a childhood B-ALL case with unique complex aberrations and six chromosomal breakpoints. In this case, the array-proven high-resolution multicolor banding (aMCB) technique was of enormous significance for detecting the genetic changes.

Materials and methods

Case report. In May 2009, a 14-year-old male patient presented with a WBC of 123.6x10^9/l, i.e., 6.1% neutrophils, 65% lymphocytes, 0.1% eosinophiles, 1.2% monocytes, 5.6% basophiles and 27.6% largely unidentified cells. The platelet count was 205x10^9/l and hemoglobin 8.7 g/dl. Serum lactate dehydrogenase (LDH) was 556 U/l (normal: up to 480 U/l), and the level of serum alkaline phosphates was 191 U/l (normal: up to 141 U/l). A physical examination showed no splenomegaly, but loss of weight was noted. No response was observed after the application of two standard protocols for ALL and acute myeloid leukemia (AML). For 2 months the patient was treated with imatinib (400 mg per day). However, one month later the patient succumbed to the disease while undergoing treatment. Cytogenetics and molecular cytogenetics. Banding cytogenetics using GTG-banding was performed according to
standard procedures (15), and 20 metaphases derived from the unstimulated bone marrow of the patient were analyzed. Patient consent for the study was obtained.

Fluorescence in situ hybridization (FISH) using commercially available probes for BCR/ABL and subtelomeric for 10p/10q (Abbott/Vysis) were applied according to the manufacturer's instructions. High-resolution aMCB, based on microdissection-derived region-specific libraries for chromosomes 1, 4, 8 and 10, was performed as previously described; method and MCB probe sets are specified (15,16). A total of 30 metaphase spreads were analyzed, each using a fluorescence microscope (AxioImager.Z1 mot, Zeiss) equipped with appropriate filter sets to discriminate between a maximum of five fluorochromes and the counterstain 4',6-diamino-2-phenylindole (DAPI). Image capturing and processing were carried out using an Isis mFISH imaging system (Meta systems, Altlussheim, Germany) for the evaluation of MCB.

Immunophenotyping. Immunophenotyping of leukemic blasts was carried out using fluorescein isothiocyanate (FITC)- and phycoerythrin (PE)-conjugated monoclonal antibodies (Becton-Dickinson, Franklin Lakes, NJ, USA). Antibodies against the following antigens were used: CD34, CD45, HLA-Dr, CD117, CD10, CD4, CD7, CD8, CD19, CD38, as well as cCD13, cMPO, cTdt, cCD2, cCD3, cCD22 and cCD79a (17). Positivity (<20%) was considered to be a negative result.

Results

Karyotyping was carried out after the initiation of chemotherapy treatment, showing certain karyotypic changes. A complex karyotype 46,XY/46,XY,der(1),del(4),der(4),t(9;22),del(10) was determined in the GTG-banding (Fig. 1) and was further studied by molecular cytogenetics (Figs. 2-4). Dual-color-FISH using a commercially available probe specific for BCR and ABL showed that the typical Ph chromosome with BCR/ABL translocation was present. The application of subtelomeric probes for 10p and 10q showed normal signals of subtelomeric 10p/10q on chromosome 10. However, only subtelomeric region 10q was present on the derivative chromosome 10, while subtelomeric region 10p was detected on the derivative chromosome 1 (Fig. 2). Thus, aMCB, using probes for the corresponding chromosomes involved according to GTG-banding, was performed (16). The result obtained was: 46,XY[4]/46,XY,der(1)[t(1;4;10)(1pter->1q42::4q21->4q35::10p15.3-10pter),del(4)(q24),t(9;22)(q34;q11.2),der(10)[t(8;4;10)(8qter->8q22::4q21->4q35::10p15.3->10qter)] (16).

Immunophenotyping of leukemic blasts was performed using FITC- and PE-conjugated monoclonal antibodies. The blasts stained positively with CD10 (88%), CD19 (86%), CD79a (80%), CD34 (86%), CD45 (95%) and HLA-DR (49%). Based on these findings and using the criteria of the European Group for the Immunological Characterization of Acute Leukemia (EGIC), the patient was diagnosed as having common precursor B-cell acute lymphoblastic leukemia.

Discussion

According to the literature, 32% of ALL cases demonstrate an abnormal karyotype, either in chromosome number (ploidy) or in structural changes such as translocations, inversions, or deletions (11-12). Not infrequently, chromosomes studied in ALL exhibit a poor morphology, tend not to spread well, and appear fuzzy with indistinct margins, making banding studies challenging or even impossible (12). Nonetheless, it is known that in ALL the most common chromosomal changes are t(12;21), t(9;22), t(4;11) and del(6q) followed by t(8;14), t(1;19) and del(9p) (18). Among the specific chromosome translocations identified and causally linked to leukemogenesis, BCR/ABL gene rearrangements are one of the best characterized rearrangements (19,20). It was shown that among B-precursor-ALL patients, this translocation is present in approximately 20-30% of adult cases while it is rarely detected in children (21-27), as found in the present patient. In this case, we described unique complex translocations associated with B-ALL, including the involvement of two...
chromosomes 4 in complex translocations, in addition to the t(9;22)(q34;q11.2). The lymphatic marker CD10 was also expressed. The patient did not respond to the standard chemotherapy protocols suggested by large prospective studies on childhood ALL (30,31), and the cytogenetic investigation after six months of chemotherapy treatment showed the aforementioned chromosomal abnormalities.

B-lineage, defined by the expression of CD19, HLA-DR, CD10 (cALLa) and other B-cell-associated antigens are observed in 80-85% of childhood ALL. Approximately 80% of B-precursor ALL cases express the cALLa, CD10 antigen (18). Thus, the observed immunophenotype of the reported case was appropriate to this group and aided in the identification of the type of malignancy present.

One study reported that the pattern of expression of a set of genes can determine resistance to common chemotherapeutic agents (3). This study identified 45 genes differentially expressed between resistant and sensitive ALL samples whose expression pattern was significantly related to treatment response (28). The 45 genes were involved in the regulation of transcription, cellular transport and cell cycle maintenance. Using patterns of gene expression, the authors were able to distinguish a subgroup of ALL tumors with cross-chemoresistance and unfavorable outcome from those which exhibited only single-drug resistance. The same study also showed that transcriptional regulation of key apoptosis genes can be linked to cellular drug resistance and prognosis in pediatric B-lineage ALL (29). The present case may reflect the same manner of drug-resistance due to the large number of chromosome breaks and aberrations involved.
Therefore, the observed complex karyotype, along with the Ph chromosome is a poor prognostic factor in B-ALL patients, since no response was observed after the application of two standard treatment protocols for ALL and AML.

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References
