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Unreported combination of rearrangements in a childhood B-cell acute lymphoblastic leukemia case: Coexistence of translocation t(8;14) and monoallelic loss of tumor suppressor gene *TP53*

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ABSTRACT

Introduction: B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is a clinically and biologically heterogeneous disease resulting from the accumulation of genetic alterations in B lymphoid precursor cells, and represents the most common malignant hematopoietic disease in childhood. Approximately 75% of BCP-ALL cases harbor a recurrent chromosomal alteration detectable by banding cytogenetic approaches. Some of these recurrent abnormalities define ALL subgroups and are used for risk stratification. However, coexistence of two normally independent, primary genetic aberrations within the same clone is rare in ALL.

Case presentation: Here we report an 8-year-old Syrian boy with B-ALL. He presented at diagnosis with two cytogenetic events, yet unreported to appear in common, i.e. a reciprocal translocation t(8;14) leading to *cMYC/IGH* gene fusion, and monoallelic loss of tumor suppressor gene *TP53*. This patient received treatment with prednisolone according to ALL Intercontinental Berlin-Frankfurt-Münster (IC-BFM) 2002 chemotherapy protocol but he relapsed early. Bone marrow and central nervous system were finally involved and he died within 6 months after initial diagnosis due to intracranial hemorrhage.

Conclusion: The reported combination of aberrations in a childhood case of BCP-ALL here seems to indicate an adverse prognosis, and shows that otherwise independent predictive chromosomal aberrations may arise also together.

1. Introduction

Accumulation of genetic alterations in B lymphoid precursor cells is a hallmark of B-cell precursor acute lymphoblastic leukemia (BCP-ALL) which presents otherwise as a clinically and biologically heterogeneous disease. BCP-ALL is the most common malignant hematopoietic disease in childhood (Mullighan et al., 2007; Pui et al., 2004). BCP-ALL patients have a favorable prognosis with an overall complete remission rate of 90% for children and adolescent between 1 and 15 years; this is valid especially for cytogenetic subgroups with good prognosis (Perez-Andreu et al., 2015). Approximately 75% of BCP-ALL cases harbor recurrent chromosomal alterations detectable by banding cytogenetics and can be used to define ALL subgroups and for risk stratification (Mullighan, 2012; Moorman et al., 2007).

Recurrent chromosomal abnormalities (CAs) are typical for all malignancies; one can distinguish primary from secondary CAs. Primary CAs are such which normally appear early during the course of a disease and are used to define subgroups of a malignancy. Secondary CAs are such which can be observed additionally besides primary ones and are not or less important for subgroup definition and risk stratification.

The most relevant examples for primary CAs in BCP-ALLs with prognostic impact are the translocation t(12;21)(p13;q22)/*ETV6–RUNX1*, and hyperdiploidy; both aberrations are reported in about 50% of the pediatric BCP-ALL patients and are associated with a good outcome in younger patients (Moorman et al., 2010). Philadelphia (Ph) chromosome or *BCR-ABL1* gene fusion and *MLL* rearrangements are recurrent primary CAs more frequently reported in older children and adult patients, and both are associated with poor prognosis. Another primary CA is the translocation t(8;14)(q24;q11) (*cMYC/IGH*);

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the latter is predominately observed in ~1% of Ph negative adult B-ALL patients, where it is a marker for an extremely aggressive syndrome characterized by hyperleukocytosis, lymphoma-like presentation, rapid neurological progression and a poor clinical outcome (Moorman et al., 2007; Moorman et al., 2010) with also poor response to chemotherapy (Lange et al., 1992). Overall, the coexistence of two primary genetic aberrations within the same clone is rare (Chilton et al., 2014).

Here, we are presenting for the first time a primary childhood BCP-ALL case with a two primary events yet unreported together; a reciprocal translocation t(8;14), and monoallelic loss of tumor suppressor gene *TP53*. The reported combination of aberrations in a childhood case of B-ALL here seems to indicate for an adverse prognosis.

2. Case presentation

An 8-year-old Syrian boy without significant personal or familial medical history presented with a 1.5 month history of fever, bone pain in the foot, lymphadenopathy and enlarged left testicle size. An initial evaluation revealed elevated white blood cell (WBC) count 61.3 × 10^9/l, red blood cells count was 2.48 × 10^6/mm^3, hemoglobin (Hgb) level 7.6 g/dl, and platelet count (Plt) was 388 × 10^9/l. Lymphoblasts in bone marrow (BM) aspiration were > 90%. Biochemistry analyses revealed serum lactate dehydrogenase (LDH) value was 3594 U/l (normal value up to 480 U/l), serum aspartate aminotransferase value 66 U/l (normal value up to 40 U/l). Echography of the abdomen revealed presence of a small volume of free liquid. A testicular biopsy showed infiltration of lymphoblasts. Cytogenetic and immunophenotyping analyses were also carried out (see below). Our patient was diagnosed to suffer from ALL according to World Health Organization (WHO) classification and was considered to be at high risk based on his age and WBC.

After doing the cytogenetics analysis, the patient was treated further according to ALL Intercontinental Berlin-Frankfurt-Münster (IC-BFM) 2002 chemotherapy protocol. At day seven of induction therapy the new hematological parameters were WBC: 15 × 10^9/l, Hgb: 8.3 g/dl, and Plt 166 × 10^9/l. However, the patient finished the indication phase with elevated lymphoblasts (4.5%) as detected by flow-cytometric (FCM); a few day later the patient had neutropenia and septicemia and he was treated with a wide spectrum antibiotics for 10 days; however, twenty days after the beginning of the consolidation (overall two months after start of chemotherapy), the patient relapsed. Bone marrow and cerebrospinal fluid (CSF) were involved and both revealed abnormal cells; FCM evaluation at this time point showed 90% lymphoblasts. One month later under consolidation therapy, FCM revealed of 65% lymphoblasts. One month later (overall 6 months after initial diagnosis), the patient succumbed due to intracranial hemorrhage under treatment. His mother gave consent for a scientific evaluation of her case and the study was approved by the ethical committee of the Atomic Energy Commission, Damascus, Syria.

3. Material and methods

3.1. Chromosome analysis

Chromosome analysis using GTG-banding according to standard procedures (AL-achkar et al., 2007) was performed before chemotherapy was initiated. A minimum of 10 metaphase cells derived from unstimulated bone marrow culture were analyzed. Karyotype was described according to the International System for Human Cytogenetic Nomenclature (ISCN 2013) (Shaffer et al., 2013).

3.2. Molecular cytogenetics

Fluorescence in situ hybridization (FISH) was performed before and after chemotherapy. After chemotherapy only interphase-FISH was done with locus-specific probes. Whole chromosome painting (WCP) probes for chromosomes 1, 7, 11, 13 and 17 (MetaSystems, Altlussheim, Germany), a specific probe for 17p13 (*TP53*) (Q-Biogene, USA), *MLL* break-apart rearrangement probe (Abbott Molecular/Vysis, Des Plaines, IL, USA) and an *IGH/cMYC* translocation dual fusion probe (Cytocell, UK) were applied according to manufacturer's instructions (AL-achkar et al., 2007). A minimum of 10 metaphase spreads were analyzed, using a fluorescence microscope (AxioImager.Z1 mot, Carl Zeiss Ltd., Hertfordshire, UK) equipped with appropriate filter sets to discriminate between a maximum of three fluorochromes plus the counterstain DAPI (4′,6- diamino-2-phenylindole). Image capture and processing were performed using an ISIS imaging system (MetaSystems, Altlussheim, Germany).

3.3. Flow cytometric immunophenotype

Immunophenotyping was performed using a general panel of fluorescent antibodies against the following antigens typical for different cell lineages and cell types: CD1a, CD2, CD3, CD4, CD5, CD8, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD22, CD23, CD32, CD33, CD34, CD38, CD41a, CD45, CD56, CD57, CD64, CD103, CD117, CD123, CD138, CD209, CD235a and CD243; in addition antibodies to Kappa and Lambda light Chains, IgD, sIgM, and HLADr were tested. All antibodies were purchased from BD Biosciences. Samples were analyzed on a BD FACSCalibur™ flow cytometer. Autofluorescence, viability, and isotype controls were included. Flow cytometric data acquisition and analysis were conducted by BD Cellquest™ Pro software.

4. Results

Prior to the chemotherapy treatment GTG-banding revealed a karyotype of 48,XY,+5,+21[1]/45,XY,-20[1]/46,XY,t(1;11;13),t(7;17),t(8;14)[5]/46,XY,t(1;4)[1]/46,XY,t(7;17)[1]/46,XY (Chilton et al., 2014) (Fig. 1A and B). Further studies were performed based on molecular cytogenetics (Fig. 1C–H). Results of FISH using different combinations of WCPs for chromosomes 1, 7, 11, 13 and 17 are shown in Fig. 1C to G. The probe for *TP53* confirmed the absence of the 17p on the der(17) (Fig. 1F). Results of FISH using a specific probe for *IGH/cMYC* are shown in Fig. 1H. FISH using a specific probe for *MLL* revealed no signal splitting. (Data not showed.) The following final karyotype was determined prior to and after chemotherapy treatment was determined:

Pre chemotherapy treatment:
48,XY,+5,+21[1]/45,XY,-20[1]/46,XY,der(11)t(1;11)(q25;q23),der(13)t(11;13)(q23;q33), der(17)t(7;17)(q11.2;p11.2),t(8;14)(q24.21;q32.33)[7]/45,XY,t(1;4)(q25;q25),-4[1]/ 46,XY,t(7;17)(q11.2;p11.2)[2]/46,XY[9]
and after chemotherapy treatment (after relapse):
nuc ish 8q24(cMYCx3),14q32(IGHx3)(cMYC con IGH x2)[120]

Flow cytometric analysis of BM specimen from our patient characterized this case as a B-ALL according to WHO classifications. The abnormal cell population (90% of tested cells) was positive for $CD45^{dim}$, HLADr, CD19, CD10, CD20, and CD22. Also the population was negative for CD34, CD7, CD3, CD33, CD13, CD14, Lambda, Kappa, sIgD and sIgM.

5. Discussion

We described for the first time a primary childhood BCP-ALL case with a two primary events yet unreported for appearing together; a translocation t(8;14) (*cMYC/IGH*) and monoallelic loss of tumor suppressor gene *TP53*. The patient experienced an early relapse to ALL after chemotherapy treatment; thus the combination of these two aberrations in childhood BCP-ALL seems to indicate for an adverse prognosis.