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Cytogenetic and molecular characterization of T-cell acute lymphoblastic leukemia as a second tumor after anaplastic large-cell lymphoma in a boy

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Abstract

We report a case of acute T-cell lymphoblastic leukemia which developed in a boy 8.5 years after successful treatment for anaplastic large-cell lymphoma. Cytogenetic and molecular characterizations of the second tumor were performed. The cytogenetic investigation revealed a complex pattern of karyotypic alterations, including double minutes, ring chromosomes, and a duplication of the p21-32 region of chromosome 1. The microsatellite DNA analysis excluded rearrangement or deletion of the *TAL1* gene in the tumor cells; rearrangements of the *MLL* gene were excluded by Southern blot analysis. To the best of our knowledge, this is the first report of T-cell lymphoblastic leukemia arising after treatment of CD 30+ anaplastic large-cell lymphoma. The different T-cell receptor rearrangement evidenced in the two tumors indicates that this second malignancy most likely emerged *de novo*, but was plausibly related to the previous radiation and chemotherapy.

Key words: secondary T-cell lymphoblastic leukemia, childhood cancer, cytogenetics, DNA analysis

Second malignant neoplasms are one of the most common causes of morbidity and death in young adults who have survived a childhood cancer, occurring with an incidence of 2-12% at 25 years.[1,2]

A number of treatment modalities, although highly effective, may be associated with a high rate of late sequelae in survivors. Secondary leukemia represents a well-recognized late complication of chemotherapy (accounting for 20% of all secondary neoplasms), and is typically acute myeloid leukemia (AML),[3,4] while acute lymphoblastic leukemia (ALL) accounts for only 5-10% of cases.[5] Whereas in most instances secondary AMLs are induced by alkylating agents and topoisomerase II inhibitors, the pathogenesis of secondary ALL is less well defined, although a relationship with DNA topoisomerase II inhibitors has been demonstrated.[4] We describe here the cytogenetic and molecular characterizations of a secondary T-cell acute lymphoblastic leukemia (T-ALL) occurring in a young patient who had been successfully treated for CD 30+ anaplastic large-cell lymphoma 8.5 years earlier.

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Case Report

In November, 1986, a 7-year-old boy was diagnosed as having a Ki-1+ (CD30+) anaplastic large-cell lymphoma with T-cell receptor-γ (TCR-γ) rearrangement. According to Murphy's staging system, the patient was classified as being in stage III, since the tumor arose in pelvic bones and there was large soft tissue spread, and also involvement of soft tissues of the skull. Treatment was started according to the ongoing protocol for stage III non-Hodgkin's lymphoma, consisting of induction chemotherapy and irradiation to the site of bulky tumor (30 Gy to the right hemipelvis), followed by maintainance chemotherapy, as described elsewhere.[6] Overall, treatment lasted 24 months. A complete remission was obtained; this lasted until April, 1997, when the patient complained of fever, asthenia and pain in the lower extremities. On clinical examination, ubiquitous peripheral lymph-node enlargement, hepatosplenomegaly and perimalleolar skin infiltration were evident. Abdominal ultrasound and computerized tomography documented the involvement of retroperitoneal lymph nodes. Blood tests showed pancytopenia with 490/mL leukocytes (390 lymphoblasts and 100 neutrophils), platelets 51,000/mL and hemoglobin 9.5 g/DL. LDH value was 742 U/L (normal upper value 460 U/L). MRI of the CNS and cerebrospinal fluid cytology were negative. Bone marrow biopsies and smears were performed for histopathologic diagnosis, and karyotypic and molecular characterizations. The diagnosis was acute lymphoblastic leukemia; T-cell type FAB 2, CD2+, CD5+, CD7+, TdT+ (80% bone marrow replacement) and CD30- with TCR-γ rearrangement. The patient received another

course of chemotherapy following our institution's protocol for high-grade NHL relapses.[7] A second complete remission was obtained in September 1997. As consolidation treatment, the boy received a cord blood stem cell transplantation in April 1998 and is alive in complete remission at the time of this report.

Karyotypic investigation, carried out as described,[8] disclosed the presence of a diploid clone with a dup(1)(p21p32) as the sole cytogenetic abnormality in 10% of the metaphases analyzed, and a hyperdiploid clone carrying several copies of the dup(1) chromosome plus double minutes and ring chromosomes in 90% of the cells (Figure 1). The chromosomal region 1p32, where the *TAL1* gene is mapped, was investigated both by Southern blot analysis and by PCR amplification of microsatellite sequences. Southern blot analysis of *TAL1* gene rearrangements, performed by *Hind*III digestion of tumor DNA and hybridization with a SILDB probe,[9] did not show rearrangements of the gene (data not shown). Analysis of the 1p32 region was performed using four highly informative microsatellite markers closely linked to the *TAL1* gene. The relative position from telomere to centromere is D1S211, D1S197, D1S200 and D1S220, *TAL1* being localized between D1S197 and D1S200.[10] The microsatellite analysis showed an alleleic imbalance at the D1S211 locus, indicating a trisomy of the telomeric region of chromosome 1p (Figure 2), as indicated by the cytogenetic analysis and further confirmed by fluorescence *in situ* hybridization (FISH) by a painting probe for the whole chromosome 1 and a q arm-specific probe[11] (Figure 3). The *MLL* status was investigated by Southern blot analysis carried out by digestion by *Bam*HI of tumor DNA and hybridization with a B859 probe.[12] No MLL rearrangements were detected (data not shown). Moreover, the TCR-γ chain gene rearrangements of the LCL and of the ALL were investigated by PCR amplification as previously described.[13] The analysis showed the presence of a clonal T-cell population in both tumors: however, the clones observed had different electrophoretic migration patterns, indicating that they represent two distinct populations (Figure 4).

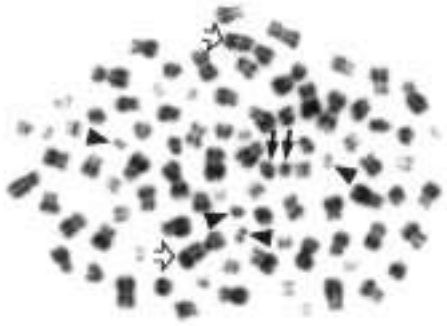

Figure 1. Representative hyperdiploid metaphase showing the dup(1)(p21p32) (open arrow), double minutes (arrowhead), dicentric chromosomles (arrow).

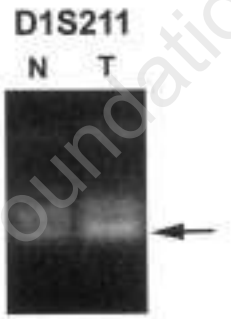

Figure 2. Allelic imbalance at the D1S211 locus; N: normal DNA, T: tumor DNA. The arrow indicates the duplicated allele in the tumor sample.

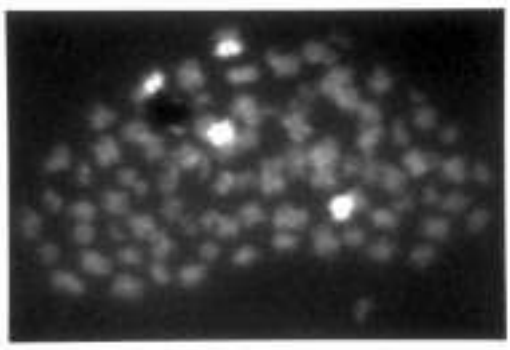

Figure 3. Representative hyperdiploid metaphase hybridized with the q arm-specific probe of chromosome 1. The probe specifically paints (white signals) only the q arm of chromosome 1, excluding that the duplicated region on the p arm of chromosome 1 derives from the q arm of the same chromosome.

Discussion

As the cure rate for childhood malignancies improves, the long-term effects of therapy become evident and are cause of increasing concern. Second neoplasms, particularly acute myelogenous leukemia, are well-known late complications in patients who have been treated for childhood cancer. Second tumors may result from an increased genetic susceptibility, from the mutagenic effects of chemotherapy and/or radiotherapy, or might develop by chance. The dose-dependent association of alkylating agents with secondary leukemia and non-Hodgkin's lymphoma has been reported.[14,15] Interestingly, the risk of leukemia appears to reach a plateau level at 10 years. ALL has been infrequently reported as a second tumor in both adults and children, and represents only 5 to 10% of secondary leukemias, as reviewed by Hunger *et al*.[5] Here we report the clinico-pathologic, cytogenetic and molecular characterizations of a T-ALL occurring in a patient successfully treated for

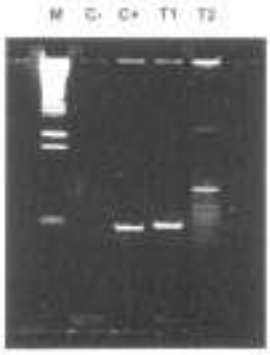

Figure 4. TCR-γ chain gene rearrangements of the LCL (T1) and of the ALL (T2). C+: positive PCR control; C–: negative PCR control; M: 1Kb molecular weight marker.

anaplastic large-cell lymphoma. The cytogenetic investigation evidenced a karyotypic pattern (double minutes, ring chromosomes) probably induced by the previous antineoplastic combination therapy. This therapy included – as the sole alkylating agent – cyclophosphamide to a cumulative dose of 6.5 g/m^2, the total dose of 300 mg/m^2 of adriamycin, and 30 Gy radiotherapy to pelvic bones. The relative roles of chemotherapy and radiotherapy are difficult to asses: it has been shown that radiation therapy for childhood cancer is not an important risk factor for the development of subsequent leukemia,[14] although secondary ALL was reported in one patient receiving high dose cyclophosphamide and total-body irradiation.[16] On cytogenetic and molecular grounds, a variety of distinct chromosomal translocations were observed in the T-ALL malignant cells in our patient as shown in Table 1.[17] The chromosomal region 1p32 is frequently involved in deletions and translocations in T-ALL,[17] and one of the most common genetic alterations is rearrangement of the *TAL1* gene.[9,18-27] As a result of the translocation, *TAL1*, originally described to be involved in t(1;14)(p32;q11), is juxtaposed with sequences from the TCR α/δ chain locus.[18-23] Although this translocation occurs in less than 3% of T-ALL cases,[23] deletions of *TAL1* are reported in 12-26% of T-ALL with apparently normal karyotype.[9,24-27] The molecular investigation of TAL1 genomic structure in our patient did not reveal abnormalities; furthermore, no microdeletions of the 1p32 chromosomal band were detected. A duplication of the p21-p32 region of chromosome 1 was identified by cytogenetic, FISH and microsatellite analyses. Moreover, no MLL gene rearrangements were detected. After a Medline® database research we believe that this is the first report of T-ALL arising as a second tumor after treatment for anaplastic large-cell lymphoma. Because the TCR-γ chain gene rearrangements differed in the two tumors, we can suppose that the T-ALL emerged *de novo*, perhaps as a consequence of the previous radiochemotherapy. The T-ALL was negative for CD30 immunoreactivity and T-cell receptor rearrangements were not identical in the former (CD30$^+$ ALCL) and latter (T-ALL) samples.[28] Moreover, CD30$^+$ ALCL is a subtype of non-Hodgkin's lymphoma that, at least in children, very rarely relapses later than one year after diagnosis.[6,29,30] The prognosis of secondary ALL appears to be dismal;[31-33] in fact, almost all of the reported cases died of their disease or because of treatment-related complications. At the time of the present report, our patient remains alive and in complete remission after allogeneic stem cells transplantation.

Table 1. Chromosomal rearrangement in T lineage of acute lymphoblastic leukemia.

Rearrangement	*Typical immunophenotype*
t(1;7)(p32;q34)	T lineage
t(1;7)(p34;q34)	T lineage
t(1;14)(p32-34;q11)	T lineage
t(7;9)(p34-36;q34)	T lineage
t(7;9)(q34;q32)	T lineage
t(7;10)(q34-36;q24)	T lineage
t(7;11)(q34-36;p13)	T lineage
t(7;19)(q34;p13)	T lineage
+8	B or T lineage
t(8;14)(q24;q11)	T lineage
t or del(9p)	T lineage
del(9p)	B or T lineage
t(10;11)(p13-14;q14-21)	T lineage
t(10;14)(q24;q11)	T lineage
t(11;14)(p15;q11)	T lineage
t(11;14)(p13;q11)	T lineage
t/del(12p)	B or T lineage
t or del(14q11)	T lineage

Contributions and Acknowledgments

DPe was the main investigator, carried out all the molecular analyses, managed the data, and with AF, MC and MM performed the literature revision, and wrote the article. GS contributed to cytogenetic descriptions. AM performed FISH analyses. FP and AB contributed to Southern blot investigations. DPa evaluated the TCR-γ rearrangements. RG was the pathologist who reviewed the case. FG and PM gave technical support to the work. AF, MC, FFB and MM managed the clinical data. The criteria for the order in which the names of the authors appear are based on the author's contribution to the design, analysis, interpretation of the data, and execution of the study.

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Disclosures

Conflict of interest: none.

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