Acute Lymphoblastic Leukemia

Characterization of the t(17;19) translocation by gene-specific fluorescent *in situ* hybridization-based cytogenetics and detection of the *E2A-HLF* fusion transcript and protein in patients' cells

This is the first report to comprehensively characterize the *E2A-HLF* fusion generated from the t(17;19)(q22;p13) translocation in childhood B-lineage acute lymphoblastic leukemia. *E2A* gene rearrangement and *E2A-HLF* transcript and protein expression were determined using conventional cytogenetics, fluorescent *in situ* hybridization, reverse transcriptase polymerase chain reaction and Western blotting in leukemic cells from three patients.

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The t(17;19)(q22;p13) translocation, which is found in a subset of childhood B-lineage acute lymphoblastic leukemia (ALL), generates the *E2A-HLF* chimeric transcription factor and is associated with a poor prognosis.[1,2] All patients reported to date, with *E2A-HLF* expression in their leukemic cells, have died.[3] Two different genomic rearrangements that generate *E2A-HLF* have been described.[4] The type I rearrangement arises from a breakpoint within intron 13 of *E2A* and intron 3 of *HLF* and is associated with disseminated intravascular coagulation (DIC). Type II rearrangements involve a breakpoint within intron 12 of *E2A* and intron 3 of *HLF* and are associated with hypercalcemia. Three patients with pre-B/common ALL who had the t(17;19) translocation were identified in this study. All patients had greater than 90% blasts in the bone marrow at diagnosis (Table 1). Patients 2 and 3 had abnormal coagulation times or DIC at diagnosis. Patients 1 and 2 had DIC at relapse. Only patient 1 showed elevated serum calcium at relapse. Patients 1 and 2 relapsed and died 15 to 18 months after diagnosis

Table 1. Information on the patients and analysis of leukemic cells by conventional cytogenetics and FISH.

Pat.	Clinical information	Conventional Cytogenetics		FISH with E2A (TCF3) break-apart probe			
		% abnormal metaphases	Karyotype	% metaphase cells N	% metaphase cells R	% interphase cells N	% interphase cells R
1	**Diagnosis** 5 year 8 month old female WBC: 1.1×10^9/L Treatment: UKALL 97/99 Regimen A[8] Remission at day 15 Normal coagulation Normal serum calcium	92.8	46,XX,t(17;19)(q22;p13)[13]/46,XX[1]	9.6	90.4	17.8	82.2
	Relapse 18 months from diagnosis WBC: 11.1×10^9/L DIC Elevated serum calcium Died	0.0#	46,XX[12]	100.0	0.0§	20.0	80.0
2	**Diagnosis** 5 year 10 month old female WBC: 88.6×10^9/L Treatment: UKALL 97/01 Regimen B, but switched to Regimen C8 Remission at day 29 Abnormal coagulation Normal serum calcium	N/A	N/A	N/A	N/A	N/A	N/A
	Relapse 15 months from diagnosis WBC: 71.6×10^9/L DIC Normal serum calcium Died	33.3	47,XX,i(9)(q10),t(17;19)(q22;p13),+21[4]/46,XX[8]	20.0	80.0	16.3	83.7
3	**Diagnosis** 14 year old male WBC: 3.5×10^9/L CNS disease present Treatment: UKALL 97/99 Regimen B with cranial radiotherapy[8] Remission at day 28 DIC Normal serum calcium Patient well at week 49 of Regimen B	91.0	46,XY,t(17;19)(q21-q22;p13)[9]/46,XY[1]	–	–	9.0	91.0

Patient 1 was identified at Great Ormond Street Hospital for Children, London, UK. Patient 2 was identified at Our Lady's Hospital, Dublin, Ireland. Patient 3 was identified at the Royal Victoria Infirmary, Newcastle-upon-Tyne, UK. #There was a population of metaphase cells which had poor chromosome morphology and were unsuitable for GTG-band analysis. §Sample displayed a low mitotic index. WBC: white blood cell count; BM: bone marrow; DIC: disseminated intravascular coagulation; N/A: not available; CNS: central nervous system; N: Normal; R: rearranged.

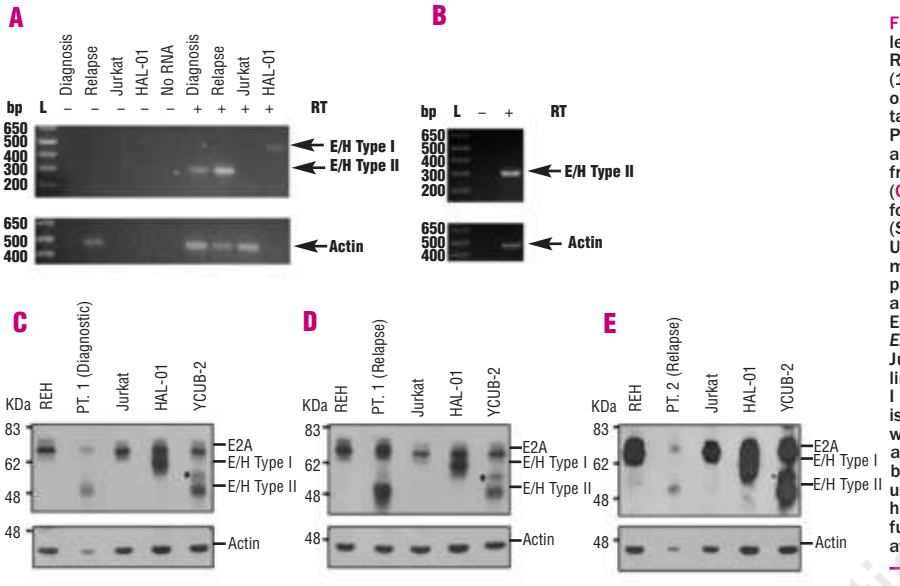

Figure 1. Expression of *E2A-HLF* in the leukemic cells from patients 1 and 2. (A) RT-PCR was performed using primers (19F and 17B) to amplify *E2A-HLF* (E/H) or actin in samples from patient (PT.) 1 taken at diagnosis and relapse. (B) RT-PCR was performed using primers to amplify *E2A-HLF* and actin in a sample from patient 2 taken at time of relapse. (C) Western blotting analysis was performed using anti-*E2A* (Yae) antibody (Santa Cruz Biotechnology, California, USA) on up to 5.5 μg of protein from bone marrow mononuclear cells taken from patient 1 at diagnosis (C) and relapse (D) and from patient 2 at relapse (E). Equivalent amount of protein from the *E2A-HLF*-negative ALL cell lines, REH and Jurkat, and *E2A-HLF*-positive ALL cell lines, HAL-01 and YCUB-2. A variant, type I *E2A-HLF* protein present in YCUB-2 cells is indicated by the asterisk.[7] Membranes were subsequently reprobed with anti-actin (Santa Cruz Biotechnology). Western blotting analysis could not be carried out using an anti-HLF antibody, which would have confirmed the presence of the fusion, as it was no longer commercially available at the time of this study.

and patient 3 is currently well.

Conventional cytogenetics and fluorescent *in situ* hybridization (FISH) analysis using an *E2A (TCF3)* dual color break-apart probe (DakoCytomation, Ely, UK) was performed on bone marrow cells from the three patients (Table 1). GTG-band investigation of metaphase cells from the bone marrow from patient 1 revealed that a t(17;19) was present at diagnosis, and FISH analysis detected an *E2A* rearrangement both at diagnosis and relapse. Patient 2 showed an abnormal karyotype by GTG-band analysis, with clonal changes in addition to the t(17;19). Rearrangement of *E2A* in the relapse sample from patient 2 was confirmed by FISH analysis. The leukemic cells from patient 3 revealed that the t(17;19) translocation was the sole karyotypic change at diagnosis and that this involved rearrangement of the *E2A* gene. The expression of types I or II *E2A-HLF* fusion was determined by reverse transcriptase polymerase chain reaction (RT-PCR) using a sense primer (19F) which corresponds to a sequence within exon 11 of *E2A* and a reverse primer (17B) which corresponds to a sequence within exon 4 of *HLF*.[5] RT-PCR was performed on RNA samples extracted from bone marrow cells from patient 1 taken at the time of diagnosis and at relapse and from patient 2 at the time of relapse. No material was available from patient 3 for RT-PCR analysis. A 293 bp PCR product was amplified in the diagnostic and relapse samples from patient 1 and in the relapse sample from patient 2 (Figure 1A,B). These products were consistent with the expected size generated from a type II rearrangement. Sequence analysis confirmed that the RT- PCR products were type II E2A-HLF transcripts (*data not shown*). The HAL-01 E2A-HLF-positive cell line, which has a type I *E2A-HLF* rearrangement, generated a larger (~450 bp) product. Western blot analysis using a monoclonal antibody that recognizes E2A and also E2A-HLF, was used to determine whether the E2A-HLF protein was expressed in the patients' cells. E2A was detected in all the patients' samples and cell lines. E2A-HLF was detected in HAL-01 and YCUB-2 cell lines, both of which have a t(17;19), but not in REH and Jurkat cell lines which do not (Figures 1D,E,F). A 52 KDa protein that had the expected size of a type II E2A-HLF protein was detected in the cells from patient 1 at diagnosis (Figure 1D) and at relapse (Figure 1E) and in patient 2 at relapse (Figure 1F). The molecular weight of the 52 KDa protein detected in the patients' samples, taken together with the FISH and RT-PCR data, strongly suggests that this protein is a type II E2A-HLF protein. In this study, RT-PCR and FISH analysis were able to detect the rearrangement and expression of the E2A-HLF fusion in the cells from patient 1 taken at relapse when abnormal metaphases could not be detected due to a very low mitotic index. RT-PCR and FISH have also been used to detect *E2A-HLF* in an ALL patient with DIC who had an apparently normal karyotype using conventional cytogenetics.[6] The sensitivity of RT-PCR for the detection of *E2A-HLF* would also allow this method to be used for detecting minimal residual disease and could be used to determine effectiveness of treatment.[5] The precise and rapid identification of patients with a t(17;19), by using a combination of RT-PCR for the *E2A-HLF* fusion and FISH for parallel confirmation of a *E2A* rearrangement in addition to conventional cytogenetics, could significantly aid selection of appropriate treatment strategies and could also anticipate clinical problems.

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