Haematologica 1999; 84:110-113 original paper

Molecular analysis of 1p32 genetic involvement in pediatric T-cell non-Hodgkin's lymphoma

DANIELA PEROTTI,* FRANCESCA PETTENELLA,° ROBERTO LUKSCH,* ROBERTO GIARDINI,#
FELICITA GAMBIRASIO,* DANIELA FERRARI,* FRANCA FOSSATI-BELLANI,* ANDREA BIONDI°
**Division of Pediatric Oncology, #Division of Anatomy Pathology and Cytology, Istituto Nazionale Tumori, Milan, Italy; and °Division of Pediatrics, Ospedale S. Gerardo, Monza, Italy*

ABSTRACT

Background and Objective. T-cell acute lymphoblastic leukemia (T-ALL) and lymphoblastic T-cell non-Hodgkin's lymphoma (T-NHL) are closely related disorders, and distinguishing between the two may be difficult. Cytogenetic investigations of large NHL series reported different recurring chromosomal alterations. Among these, aberrations of chromosome 1p seem to be associated with T-cell differentiation, the region most frequently involved in breakpoints being band 1p32-36. Deletions and translocations involving the same chromosomal region are frequently observed in T-ALL, in which one of the most common genetic changes is the breakage of the TAL1 gene, mapped to the 1p32 chromosomal region. The objective of this study was to assess the possibility of TAL1 involvement also in T-NHL.

Design and Methods. A series of 17 pediatric T-NHL patients was molecularly characterized by microsatellite markers analysis and by TAL1 gene microdeletions.

Results. TAL1 gene rearrangement was found in one case, while loss of heterozygosity (LOH) and microsatellite instability (MI) was identified in another case.

Interpretation and Conclusions. Overall our findings indicate that, differently from T-ALL, neither TAL1 gene involvement nor LOH or MI at 1p32 appear particularly relevant in the oncogenic process of T-NHL transformation.

Key words: non-Hodgkin's lymphoma, TAL1, childhood tumors, genetics, loss of heterozygosity

T-cell acute lymphoblastic leukemia (T-ALL) and lymphoblastic T-cell non-Hodgkin's lymphoma (T-NHL) are closely related disorders and although T-ALL cells are more often similar to the early stages of thymocytes than T-NHL cells, there is a considerable overlap between these two disorders.[1,2] Morphology, ultrastructure and immunocytochemistry do not allow us to distinguish them and even clinically, a clearcut discrimination between T-ALL and T-NHL is not evident.[3] In fact, T-NHL is usually differentiated from T-ALL by minimal or absent bone marrow and peripheral blood involvement, normal white blood cell count, normal hemoglobin levels and lack of organomegaly. Because this distinction does not apply in every case, a presence of less than 25% of blasts in the bone marrow is currently used to define T-NHL from T-ALL.[4]

Cytogenetic investigations of large NHL series reported abnormal karyotypes, often with complex abnormalities, in about 85% of tumors. Chromosome 1p aberrations both as structural and numerical abnormalities were found to be one of the most frequently occurring aberrations among T-cell neoplasias. The most frequent 1p breakpoints involve band 1p32-36.[5] Interestingly, both deletions and translocations frequently observed in T-ALL involve this same chromosomal region,[6] the most common genetic change observed being a 90 Kb deletion resulting in fusion of the SIL promoter to the TAL1 gene.[7-17] We performed molecular characterization of the 1p32 region by microsatellite markers analysis and of TAL1 gene microdeletions in a series of 17 children with T-NHL at diagnosis in order to evaluate whether similar genetic changes might be responsible for the oncogenic transformation in T-ALL and T-NHL. Our findings indicate that, differently from T-ALL, neither TAL1 gene deletions nor LOH or MI at 1p32 appear to be particularly relevant in the oncogenic process of T-NHL transformation.

Design and Methods

Patient population

Seventeen children with histologically confirmed T-NHL, admitted to the Division of Pediatric Oncology of the National Cancer Institute of Milan and enrolled in the Institutional treatment protocol for Childhood T-NHL, were included in the present study. The main clinico-pathologic features are summarized in Table 1. In all cases histologic diagnoses according to updated Kiel[18,19] and REAL[20] classifications were carried out on buffered formalin-fixed, paraffin-embedded tissue sections of 4 µm thickness

Correspondence: Daniela Perotti, M.D., Division of Pediatric Oncology, Istituto Nazionale Tumori, via G. Venezian 1, 20133 Milan, Italy. Fax: international +39-02-2665642 – Phone: international +39-02-2390588 – E-mail: perotti@istitutotumori.mi.it

stained with hematoxylin and eosin, Giemsa, PAS-hematoxylin and Gomori's silver impregnation. Immunophenotype on conventional paraffin or on cryopreserved tissue block sections was performed using a modified-avidin biotin-peroxidase complex (ABC) method (Vectastain ABC kit, Vector, Burlingame, CA, USA). A panel of monoclonal antibodies was used to detect the T or B lineage and the stage of differentiation of the neoplastic cells (CD2: Leu5b, CD4: Leu3a, CD7: Leu9, CD8: Leu2a, CD10: Calla, Becton Dickinson, Mountain View, CA, USA; CD3: Leu4 and antiTdT, Dako, Glostrup, Denmark). The staging procedures included careful clinical examination, chest X-ray and chest computed tomography (CT) scan, abdominal ultrasonography and CT scan, gallium and ^{99}Tc scan, bilateral bone marrow biopsy and aspirate and cerebrospinal fluid examination. The extent of disease was indicated according to the St. Jude's Staging System.[21]

Molecular characterization of TAL1 gene rearrangements

High molecular weight DNA from frozen tumor tissues obtained at diagnosis before any treatment and peripheral blood leukocytes (PBL) obtained during remission as the normal counterpart, was extracted according to standard techniques.[22] Fifteen micrograms of tumor DNA were *Hind*III digested, size-fractionated on 0.8% agarose gels, and transferred to Gene Screen Plus hybridization transfer membranes (Biotechnology System-Du Pont, Boston, Massachusetts, USA) according to the manufacturer's method. *TAL1* gene rearrangements were investigated using a ^{32}P random labeled SILDB probe.[17] Determination of the exact *TAL1* deletion junction was performed by PCR using SILDB and TALDB specific primers as described elsewhere.[23]

LOH analysis of the 1p32 chromosomal region

Analysis of the1p32 region was performed using four highly informative microsatellite markers tightly 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.[24] DNA samples from tumors and PBLs were amplified by PCR, separated and visualized on ethidium bromide-stained non-denaturing 6% polyacrylamide gels according to the method described by Watkins *et al*.[25]

Results

The clinicopathologic features of the 17 T-NHL patients included in this study are summarized in Table 1. Three cases presented with early phenotype (Stage I TdT+, CD2, CD3 and CD7+; CD4, CD8–), 11 cases showed intermediate differentiation (Stage II TdT+, CD2, CD3, CD7, CD4 and CD8+) and 3 had mature lymphoblastic T-NHL (Stage III TdT-, CD2, CD3, CD7 and CD4+; CD8–). No cases showed immunoreactivity to CD10 or CD20 antibodies. Using

Table 1. Clinico-pathological features of the 17 T-NHL cases studied.

Case no.	Sex	Age	Stage	M-mass/ BM involv.	LDH U/L	Immuno-phenotype	Outcome
1	F	9.0	2	–/–	688	A	29 mos: nodal REL
2	M	15.6	2	–/–	139	B	11 mos: nodal REL 71 mos: EX
3	M	16.0	2	+/–	336	A	4 mos: testic. PRO 22 mos: leuk. PRO 23 mos: EX
4	F	4.0	3	+/–	260	C	32 mos: med. REL 44 mos: EX
5	M	4.0	3	+/–	714	B	17 mos: nodal REL 28 mos: AWD
6	M	5.8	3	+/–	284	B	94 mos: CCR
7	M	6.5	3	+/–	811	B	4 mos: leuk. PRO 8 mos: EX
8	M	7.5	3	+/–	590	B	61 mos: CCR
9	M	7.10	3	+/–	2057	A	5 mos: nodal PRO 8 mos: leuk. PRO 12 mos: EX
10	M	9.0	3	+/–	1307	B	7 mos: CCR
11	M	9.1	3	+/–	811	B	19 mos: CCR
12	M	11.6	3	+/–	352	C	75 mos: CCR
13	M	4.2	4	+/–	638	B	42 mos: leuk. PRO 62 mos: III CR
14	M	4.10	4	–/+	379	B	8 mos: nodal PRO 11 mos: EX
15	M	5.1	4	+/+	420	B	8 mos: CCR
16	M	11.1	4	+/+	377	C	19 mos: CCR
17	F	11.9	4	+/+	1181	B	72 mos: CCR

REL: relapse; M-mass: mediastinic mass; EX: dead; MB: bone marrow; PRO: progression; AWD: alive with disease; +: involved; –not involved; CCR: continuous complete remission; A: early; B: intermediate; C: mature (29).

Southern blot analysis of *Hind*III-digested DNAs we studied DNA samples derived from 17 T-cell NHL. Rearranged bands were found only in the DNA from case #17. PCR amplification by use of sense (SILDB) and antisense (TALDB) primers showed perfect agreement with the Southern blot analysis for all samples. PCR product of case #17 was of 356 bp size, consistent with a type 1 deletion (data not shown).[17]

To determine the presence of subkaryotypic abnormalities involving the same cytogenetic region (1p32) where the *TAL1* gene is mapped, polymorphic markers spanning this band were analyzed by PCR. Results obtained with samples prepared from lymph node (LN) samples at diagnosis were compared with corresponding untreated samples of peripheral blood (PBL) obtained during remission. As summarized in Table 2 this analysis detected 1p changes in 1 of the 17 cases analyzed (5.8 %). As shown in Figure 1, the allelic pattern between the tumor and its normal counterpart was consistent with LOH and microsatellite instability in case #9.

Table 2. Microsatellite marker analysis of the 1p32 region.

Case	microsatellite			
	D1S211	D1S197	D1S200	D1S220
1	–	ni	ni	–
2	–	–	ni	–
3	ni	–	–	–
4	–	–	–	–
5	ni	–	–	ni
6	–	–	ni	–
7	–	–	–	–
8	–	–	–	–
9	mi	–	+	mi
10	ni	ni	–	–
11	–	–	ni	–
12	ni	–	ni	–
13	–	ni	ni	–
14	–	–	–	ni
15	–	–	ni	ni
16	ni	–	ni	–
17	–	–	ni	–

– constitutionally heterozygous; + loss of heterozygosity; ni: constitutionally homozygous; mi: microsatellite instability.

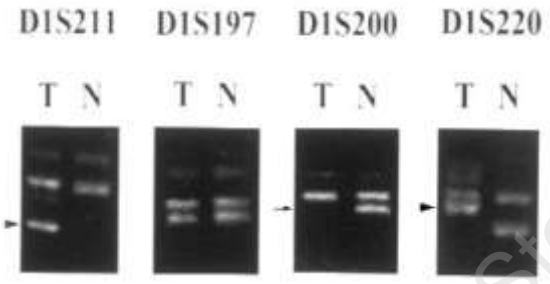

Figure 1. Microsatellite band patterns in DNA extracted from lymph node at diagnosis (T) of patient #9 compared with those seen in DNA from peripheral blood leukocytes (PBL) of the same patient during remission phase (N). The allelic patterns were consistent with microsatellite instability for D1S211 and D1S220 (arrowhead) and with LOH for D1S200 (arrow).

Discussion

The identification of *TAL1* rearrangements as a specific marker of T-ALL was first suggested in 1989 by Kurtzberg *et al.*, who described *TAL1* alterations in 8 cases of T-ALL.[27] Further studies confirmed that *TAL1* rearrangements could be identified in a significant proportion of cases of T-ALL both in adults and children. The proportion ranged from 12 to 26% in the different case series reported.[13-17] The phenotype of leukemic cells carrying *TAL1* alterations corresponds to the more mature ($CD3^+$, $CD4^+$, $CD8^+$ separately or in combination) stage of lymphocyte differentiation.[12,23] Furthermore, Breit *et al.*[17] showed in their T-ALL series that the *TAL1* rearrangements were restricted to cells of the α/β lineage.

Limited information has been reported so far on the occurrence of *TAL1* gene rearrangements in T-cell malignancies other than T-ALL.[28,29] Although most of the cases of T-NHL display an immunophenotype corresponding to intermediate and mature thymic maturation stages,[26] Kikuchi *et al.* did not find *TAL1* gene rearrangements in 18 T-NHL cases analyzed.[28] More recently *TAL1* gene rearrangement was described in 3 out of 32 cases of cutaneous T-cell lymphoma.[29] Our findings confirm and further extend the observations that despite the overlapping features between T-ALL and T-NHL, *TAL1* gene rearrangements are barely detectable in T-NHL. The only case out of 17 patients analyzed bearing a *TAL1* deletion was stage IV T-NHL with 8% bone marrow infiltration. In addition to *TAL1* submicroscopic deletions, microsatellite markers analysis at 1p32 was performed in T-ALL cases.[23] Eight of 22 T-ALL cases analyzed displayed LOH for at least one of the following markers (D1S211, D1S197, D1S200, D1S220) suggesting the existence of hot spot rearrangement on the short arm of chromosome 1.[23] When T-NHL cases were analyzed using the same markers, allelic abnormalities were detected in only one case, indicating that, although cytogenetically similar, the involvement of 1p32 region involves different genomic sequences. In conclusion, genetic lesions other than *TAL1* and LOH or MI at 1p32 might be responsible for T-NHL oncogenic transformation and for the different spectrum of T-cell neoplasms despite their similar presentation.

Contributions and Acknowledgments

DP was the main investigator, carried out all the analyses, managed the data, and with FP performed the literature revision, and wrote the article. FP contributed to Southern blot and PCR analyses. RL and FFB were involved in the clinical assessment of the patients. RG was the pathologist who reviewed all the cases. FG and DF gave technical support to the work. AB took part in the conception of the study, interpretation and writing of the paper and gave final approval of the version to be published. The criteria for the order in which the names of the authors appear are based on their contribution to the design, analysis, interpretation of data and execution of the study. The authors would like to thank Prof. A. Iolascon for providing primers for microsatellite analysis of the 1p32 region.

Funding

This work is supported by Associazione Bianca Garavaglia, Busto Arsizio (VA), Associazione Italiana per la Ricerca sul Cancro (AIRC), MURST 40%, Consiglio Nazionale delle Ricerche (PF ACRO), grant no. 9202140, PF39) and by the Fondazione Tettamanti, Monza, Italy.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

Manuscript received July 27, 1998; accepted October 27, 1998.

References

1. Reinherz EL, Nadler M, Sallin SE, Schlossman SF. Subset derivation of T-cell acute lymphoblastic leukemia in man. J Clin Invest 1979; 64:392-7.
2. Roper M, Crist WM, Metzgar R, et al. Monoclonal antibody characterization of surface antigens in childhood T-cell lymphoid malignancies. Blood 1983; 61:830-7.
3. Crist WM, Shuster JJ, Falleta J, et al. Clinical features and outcome in childhood T-cell leukemia-lymphoma according to stage of thymocyte differentiation: a Pediatric Oncology Group study. Blood 1988; 72: 1891-7.
4. Bernard A, Boumsell L, Patte C, Lemerle J. Leukemia versus lymphoma in children. A worthless question? Med Pediatr Oncol 1986; 14:148-57.
5. Offit K, Jhanwar SC, Ladanyi M, Filippa DA, Chaganti RSK. Cytogenetic analysis of 434 consecutively ascertained specimens of non-Hodgkin's lymphoma: correlations between recurrent aberrations, histology, and exposure to cytotoxic treatment. Genes Chrom Cancer 1991; 3:189-201.
6. Raimondi SC. Current status of cytogenetic research in childhood acute lymphoblastic leukemia. Blood 1993; 81:2237-51.
7. Chen Q, Cheng J-T, Tsai L-H, et al. The tal gene undergoes chromosome translocation in T cell leukemia and potentially encodes a helix-loop-helix protein. EMBO J 1990; 9:415-24.
8. Chen Q, Yang CY-C, Tsan JT, et al. Coding sequences of the TAL1 gene are disrupted by chromosome translocation in human T-cell leukemia. J Exp Med 1990; 172:1403-8.
9. Bernard O, Guglielmi P, Jonveaux P, et al. Two distinct mechanisms for the SCL gene activation in the t(1;14) translocation of T-cell leukemias. Genes Chromos Cancer 1990; 1:194-208.
10. Finger LR, Kagan J, Christopher G, et al. Involvement of the TCL5 gene on human chromosome 1 in T-cell leukemia and melanoma. Proc Natl Acad Sci USA 1989; 86:5039-43.
11. Begley CG, Aplan PD, Denning SM, Haynes BF, Waldmann TA, Kirsch IR. The gene SCL is expressed during early hematopoiesis and encodes a differentiation-related DNA-binding motif. Proc Natl Acad Sci USA 1989; 86:10128-32.
12. Carroll AJ, Crist WM, Link MP, et al. The t(1;14) (p34;q11) is non-random and restricted to T-cell acute lymphoblastic leukemia. A Pediatric Oncology Group Study. Blood 1990; 76:1220-4.
13. Brown L, Cheng J-T, Chen Q, et al. Site-specific recombination of the TAL1 gene is a common occurrence in human T cell leukemia. EMBO J 1990; 9:3343-51.
14. Bernard O, Lecointe N, Jonveaux P, et al. Two site-specific deletions and t(1;14) translocation restricted to human T-cell acute leukemias disrupt the 5' part of the TAL1 gene. Oncogene 1991; 6:1477-88.
15. Aplan PD, Lombardi DP, Reaman GH, Sather HN, Hammond GD, Kirsch IR. Involvement of the putative hematopoietic transcription factor SCL in T-cell acute lymphoblastic leukemia. Blood 1992; 79:1327-33.
16. Bash RO, Crist WM, Shuster JJ, et al. Clinical features and outcome of T-cell acute lymphoblastic leukemia in childhood with respect to alterations at the TAL1 locus: a Pediatric Oncology Group study. Blood 1993; 81:2110-7.
17. Breit TM, Mol EJ, Wolvers-Tettero IL, Ludwig W-D, van Wering ER, van Dongen JJM. Site-specific deletions involving the TAL1 and sil genes are restricted to cells of the T cell receptor α/β lineage: T cell receptor δ gene deletion mechanism affects multiple genes. J Exp Med 1993; 177:965-77.
18. Stansfeld AG, Diebold J, Kapanci Y, et al. Updated Kiel classification for lymphomas. Lancet 1988; i:292-3.
19. Stansfeld AG, Diebold J, Kapanci Y, et al. Updated Kiel classification. Lancet 1988; i:603.
20. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 1994; 84:1361-92.
21. Murphy SB. Classification, staging and end results of treatment of childhood non-Hodgkin's lymphoma: dissimilarities from lymphomas in adults. Semin Oncol 1980; 7:332-9.
22. Maniatis T, Fritsch EF, Sambrook J. Molecular cloning: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1982.
23. Iolascon A, Faienza MF, Coppola B, et al. Frequent clonal loss of heterozygosity (LOH) in the chromosomal region 1p32 occurs in childhood T cell acute lymphoblastic leukemia (T-ALL) carrying rearrangements of the TAL1 gene. Leukemia 1997; 11:359-63.
24. Weissenbach J, Gyapay G, Dib C, et al. A second generation linkage map of the human genome. Nature 1992; 359:794-801.
25. Watkins C, Warne D, Kelsell D, Beckmann J, Nyberg K, Spurr N. The use of PCR and ethidium bromide staining to detect length variation in short repeat units. Technique 1991; 3:175-8.
26. Biondi A, Champagne E, Rossi V, et al. T-cell receptor delta gene rearrangement in childhood T-cell acute lymphoblastic leukemia. Blood 1989; 73:2133-8.
27. Kurtzberg J, Waldmann TA, Davey MP, et al. CD7+, CD4–, CD8– acute leukemia: a syndrome of malignant pluripotent lymphohematopoietic cells. Blood 1989; 73:381-90.
28. Kikuchi A, Hayashi Y, Kobayashi S, et al. Clinical significance of TAL1 gene alteration in childhood T-cell acute lymphoblastic leukemia and lymphoma. Leukemia 1993; 7:933-8.
29. Neri A, Fracchiolla NS, Roscetti E, et al. Molecular analysis of cutaneous B- and T-cell lymphomas. Blood 1995; 86:3160-72.