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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

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Deletions and chromosomal translocations involving the 1p32 region, are frequently observed in T cell acute lymphoblastic leukemia (T-ALL). One of the most common genetic changes is the breakage of the *TAL1* gene, which was originally described to be involved in the T-ALL carrying the t(1;14)(p32;q11) translocation. Site-specific deletions in the *TAL1* gene are reported to occur in 12–26% of T-ALL with apparently normal karyotype. In order to investigate the presence of other subkaryotypic abnormalities involving the 1p32 chromosomal region, where *TAL1* gene is mapped, we assessed losses of heterozygosity (LOH) for microsatellite markers, in a series of 22 children with T-ALL. Microsatellite polymorphic markers flanking the *TAL1* gene (D1S211, D1S197, D1S200 and D1S220) were analyzed to detect LOH. Eight patients displayed LOH for at least one of the markers, suggesting the existence of hot spot regions at the short arm of chromosome 1. Two out of 11 with no molecular evidences of *TAL1* gene involvement, compared to six out of 11 in the group of *TAL1* rearranged gene, showed LOH at 1p32. *TAL1* gene rearrangements and clonal LOH may represent two independent events, but could be related to the same causes. For the first time this study provides evidences that LOH at 1p32 region, occurs in T-ALL in the same region known to be involved in chromosomal deletions and translocations. LOH mapping may help to define the location of a new putative tumor-suppressor gene implicated in the trasformation and progression of children T-ALL.
Keywords: childhood ALL; T-ALL; *TAL1*; rearrangements; LOH

Introduction

A variety of distinct chromosomal translocations are observed in the malignant cells of patients with T cell acute lymphoblastic leukemia (T-ALL).[1] In most of the cases different proto-oncogenes are activated as a result by juxtaposition with sequences from the T cell receptor (TCR) loci on chromosome 7 or 14.[2] The chromosomal region 1p32 is frequently involved in deletions and chromosomal translocations observed in T-ALL.[1] One of the most common genetic changes is the breakage of the *TAL1* gene.[3–13] As a consequence of the translocation, the *TAL1* gene, originally described to be involved in the t(1;14)(p32;q11) translocation, is juxtaposed with sequences from the TCR α/δ chain locus.[3–8] Although this translocation occurs in less than 3% of T-ALL patients,[8] site-specific deletions in the *TAL1* gene are reported to occur in 12–26% of T-ALL with apparently normal karyotype.[9–13]

In order to investigate the presence of other subkaryotypic abnormalities involving the 1p32 chromosomal region, where *TAL1* gene is mapped, we evaluated losses of heterozygosity (LOH) in a series of pediatric T-ALL patients. Our findings indicate that LOH at 1p32 occurs in T-ALL in the same region known to be involved in chromosomal deletions and translocations. Interestingly clonal LOH appears to be more frequent in T-ALL carrying *TAL1* gene rearrangements.

Materials and methods

Patient samples

Twenty-two childhood T-ALL were selected from a non-random group of 71 T-ALL screened at diagnosis for the presence of *TAL1* gene rearrangements, based on the availability of frozen bone marrow (BM) at diagnosis and during the remission phase. Main clinical and laboratory data of the patients are reported in Table 1. Diagnosis was established according to standard morphologic and immunological criteria. In all the subjects mononuclear cells from each BM sample were isolated by centrifugation on Ficoll–Hypaque density gradient and viably frozen in liquid nitrogen until use. At this stage, all the preparations contained more than 90% of malignant cells. These samples were used for immunophenotyping and DNA analyses. The immunophenotype according to the stage of thymocyte maturation was determined using a panel of monoclonal antibodies (moAbs) including CD1, CD2, CD3, (either surface -sCD3 and cytoplasmic -cyCD3, CD3), CD5, CD7. Reactivity with moAbs was assessed by indirect immunofluorescence as previously described.[14]

Patients were enrolled in 8803/33 and 9103/33 ALL protocols of the Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP) study group, in which a Berlin–Frankfurt–Munster (BFM) modified strategy was adopted.[15]

Molecular analysis of TAL1 gene rearrangements

High molecular weight DNA was extracted according to standard procedures.[16] Ten micrograms of DNA were digested with *Hin*dIII, size-fractionated on 0.7% agarose gels and transferred to nylon membranes (Gene Screen Plus; Biotechnology System New Research Products, Boston, MA, USA) as described.[14] *TAL1* deletion was studied using a ^{32}P random-labelled SILDB probe.[13] PCR amplification of the specific *TAL1* deletion junctions was performed using 1 μg of DNA, 12 pmol of the 5′ SILDB primer (5′ GGGGAGCTCGTGGGAGAAATTAAG 3′) and 12 pmol of the 3′ *TAL1* db primers (tal 1 dbl 5′ GCCTCGAAGGGTCCACATCTAC 3′; tal 1 db2 5′ TTGTAAAATGGGGAGATAATGTCGAC 3′; tal 1 db3 5′-TGCATGCACTCTGATGAGCAGCC 3′; tal 1 db4 5′ GGATTATAGGTGCCTGTCACCAC 3′; tal 1 db5 5′ GTCCTGCTAGCCCCATCCAGG 3′),[13] 1 U Amplitaq DNA polymerase (Perkin Elmer Cetus, Norwalk, CT, USA) in 100 μl of PCR reaction mixture. The PCR amplification was performed in a

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Table 1 Clinical and laboratory features at diagnosis with respect to *Tal-1* gene rearrangements

Patient No	*Age/sex (years)*	*WBC 10^9/l*	*Immunophenotype*[a]	*PDN response*	*Clinical follow-up*
TAL-1 not rearranged					
429	13.3/M	50.4	mature	poor	CCR +59 mo (post-BMT)
500	13.3/M	325.7	inter/mature	poor	relapse +7 mo; death +16 mo
623	13.6/F	121	intermediate	good	relapse +21 mo; death +33 mo
754	3.4/F	19.8	intermediate	good	CCR +39 mo
836	8.5/M	112.2	intermediate	unknown	resistant; death +5 mo
850	6.5/F	15.3	intermediate	good	CCR +32 mo
870	2.5/M	195	intermediate	good	relapse +15 mo; death +29 mo
878	4.8/M	14.5	intermediate	good	CCR +28 mo
918	14.5/M	23.7	intermediate	good	death +16 mo (post-BMT)
936	9.2/M	34.8	intermediate	good	CCR +26 mo
954	15.2/M	290	intermediate	unknown	relapse +12 mo; death +24 mo
TAL-1 rearranged					
100	2/4/F	113	not done	good	CCR +12 mo
341	10.9/F	124	mature	good	CCR +58 mo
403	5/M	840	early	good	CCR +62 mo (post-BMT)
548	3.4/M	280	early	good	death +1 mo
604	12.1/M	300	mature	unknown	relapse +6 mo; death +14 mo
607	3.9/F	173.6	mature	poor	CCR +49 mo
637	11.9/M	210	mature	poor	death +7 mo
792	3.5/M	50.9	intermediate	poor	relapse +13 mo
813	8.3/M	384	intermediate	good	relapse +5 mo; death +23 mo
834	7.1/M	760	inter/mature	poor	relapse +7 mo; death +8 mo
917	14.1/M	144.6	mature	good	relapse +4 mo; death +13 mo

[a]Early T-ALL, Tdt-positive, CD7 and cyCD3 positive, CD1 and surface CD3 negative; intermediate T-ALL, Tdt-positive, CD1 and CD7 positive; mature T-ALL, Tdt +/–, sCD3 and CD7 positive, CD1 negative.[14]

thermal cycler (Perkin Elmer Cetus) for 40 cycles using the following parameters: denaturation at 94°C for 5 min during the first cycle, then for 30 s, annealing at 60°C for 30 s and extension at 72°C for 30 s. After the last cycle an additional extension step of 72°C for 10 min was performed. The PCR products of *TAL1* deletion types were fractionated by 1.6% agarose gel and visualized by UV lamp after ethidium bromide staining.

Analysis of LOH at 1p32

Analysis of LOH at 1p32 was performed by PCR amplification of microsatellite sequences using primer pairs encompassing the following loci: D1S211, D1S197, D1S200 and D1S220.[17] The microsatellite sequences were selected according to the high degree of constitutional heterozygosity and to the location tightly linked to the *TAL1* gene. The relative position from telomere to centromere was the following: D1S211, D1S197, *TAL1*, D1S200 and D1S220. The relative distance from D1S211 and D1S220 was calculated to be approximately 15 cm.

PCR amplification for microsatellite analysis was performed in a total volume of 50 μl and included 100 ng DNA, 25 pmol of each primer, 200 μm/l of each dNTP and 0.2 U of Amplitaq DNA polymerase. The conditions used were the following: initial denaturation at 95°C for 5 min was followed by 28 cycles of PCR: 1 min at 95°C (denaturation), 45 s at 55°C (annealing) and 45 s at 72°C (extension). Microsatellite analysis was performed on a 10% polyacrylamide gel followed by detection by silver staining detection, as previously described.[18] LOH was assessed by densitometric laser scanning on the stained gels and was defined as a reduction of more than 50% in the signal intensity of one allele in the leukemic DNA relative to its counterpart in constitutional DNA.

Results

Screening of 71 T-ALL by Southern blot analysis revealed *TAL1* gene rearrangements in 16 cases (22.5%); the exact location of the deletions was determined by PCR amplification using SILDB and TALDB specific primers (see Materials and methods) and resulting in the identification of 13 type 1 and one type 2 *TAL1* deletions.[13] In one case extensive molecular analysis revealed the presence of a new coding sequence juxtaposed 3′ to the first SIL exon (R Amaru, personal communication). Eleven patients with *TAL1* gene rearrangements and the same number without, were selected for the present study, based on the availability of frozen BM samples during the remission phase. As previously reported in other series,[12,19] *TAL1* rerrangements did not seem to confer a distinct clinical phenotype, with the exception for a higher white blood cell count in the cases with *TAL1* gene rearranged as compared with those without detectable *TAL1* alterations (Table 1).

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 BM at diagnosis were compared with corresponding matched samples obtained during remission. Figure 1 illustrates the results of allelic losses in representative cases. As summarized in Table 2, analysis of LOH allowed the detection 1p changes in eight patients with at least one examined marker. LOH was more frequently observed in cases of T-ALL

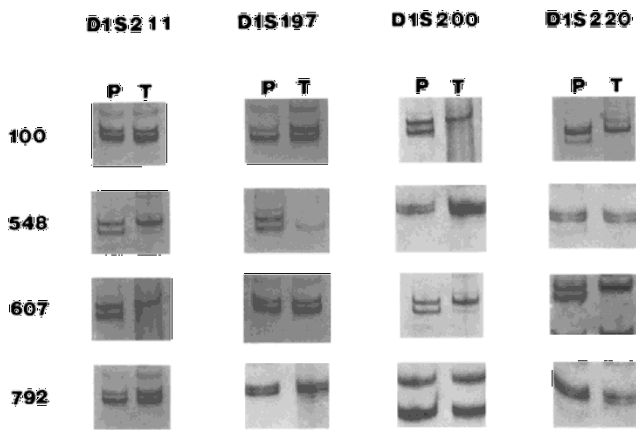

Figure 1 Loss of heterozygosity in pediatric T-ALL. Microsatellite band patterns in DNA extracted from bone marrow mononuclear cells at diagnosis (T) were compared with those seen in DNA from bone marrow of the same patient during remission phase (P).

Table 2 Allelic losses of markers located at chromosome 1p32

Patient No.	*D1S211*	*D1S197*	*D1S200*	*D1S220 cent*
429	—	—	NI	—
500	—	—	—	—
623	—	NI	—	—
754	—	—	—	LH
836	NI	NI	—	—
850	—	—	—	—
870	—	—	—	—
878	NI	—	—	NI
918	—	—	—	LH
936	—	—	—	—
954	—	—	—	—
100	—	—	LH	LH
341	NI	—	—	—
403	LH	NI	NI	—
548	LH	LH	—	—
604	—	—	—	NI
607	LH	—	LH	LH
637	LH	NI	LH	—
792	—	—	—	—
813	—	—	—	—
834	—	—	—	NI
917	LH	NI	LH	LH

—, heterozygote; NI, non-informative; LH, loss of heterozygote.

carrying *TAL1* rearrangements, with allelic losses detectable in six out of 11 cases with *TAL1* alterations as compared with two out of 11 cases without evidence of *TAL1* gene involvement. Comparison of LOH among different patients permitted the definition of two of the most commonly deleted regions of LOH as defined by D1S220 and D1S211.

Discussion

In the present study we were able to demonstrate that LOH at the 1p32 chromosomal region is a recurring event in pediatric T-ALL. LOH of a marker in tumor DNA may reflect the presence of a putative tumor suppressor gene (TSG) in the deleted fragment whose inactivation or loss accounts for tumor initiation and progression.[20] Different types of event can lead to TSG inactivation with the most frequent being loss of the entire chromosome, deletion, mutation or disruption by translocations. According to Knudson's hypothesis, at least two 'hits' are required to inactivate a TSG.[21] One of them usually involves gross chromosomal rearrangements and leads to LOH. Although the number of patients limited any definitive conclusions on the rate of frequency of LOH in childhood T-ALL, our findings indicate that LOH occurs in a region 1p32 known to be involved in translocations and site-specific deletions.[3–13] The two most useful markers in identifying LOH have been D1S200 and D1S211 suggesting the existence of hot spot regions of deletion.

The demonstration of subkaryotypic abnormalities occurring in T-ALL is not limited to the 1p32 region. Several groups, including ours, have demonstrated that genetic deletions of different extension occur on the short arms of the 9 chromosome pairs, in most of the cases of pediatric T-ALL (>90%), even in the absence of cytogenetically detectable 9p aberrations.[22–27] The p15^{INK4B} deletion involved at least two putative TSGs (p15^{INK4B} and p16^{INK4A}) codifying for highly homologous small problems able to specifically inhibit two cyclin dependent serin-threonine kinases (CDK4 and CDK6).[28–30] Of interest a new number of the p16^{INK4A} gene, namely p18 gene,[31] has been isolated and mapped on chromosome 1p32. Even the p18 gene loss may occur in T-ALL, although detectable in a minor but significant percentage of cases (14%)[27] as compared to p16 and p15 homozygous deletions.[22–27]

Inactivation of multiple cell cycle-related TSG may occur more frequently than previously considered in patients with lymphoid tumors and may possibly result in predominant growth of the clones with multiple defects of the genes.[32] Whether inactivation of a TSG affects inactivation of the others, or alternatively multiple defects may occur almost incidentally, is still unknown.

The recurrence of LOH in the T-ALL subgroup carrying the *TAL1* rearrangements, indicates that multiple genetic lesions may occur at the chromosomal breakpoint cluster region. The relationships between the two events are still intriguing, with the site-specific deletions involving the *TAL1* and SIL genes possibly mediated by the same V (D) J recombinase system involved in immunoglobulin/T cell receptor (TCR) gene rearrangements.[10,33] Of interest LOH occurring at the region 12p12–13 partially overlaps with the region containing the t(12;21) translocation breakpoints, and both events are very common in childhood ALL of B lineage.[34–36] More recently Pabst *et al*[37] showed that LOH is a very common event occurring in adult leukemias and that it involves different chromosomes.[37] It is noteworthy that 25% of the cases displayed LOH at D1S209, which were mapped in the investigated area by the present study.

Finally, it should be considered that the region 1p32 explored by the polymorphic markers used in the present study, has been indicated for the presence of TSG involved in other neoplasias.[38] The identification of the smallest deleted region and the subsequent identification of new putative genes may help to define other genetic lesions relevant for tumor transformation and progression of pediatric T-ALL.

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