TEL rearrangements in acute lymphoblastic leukemia: association with p16 deletions in relapsed cases

We report the frequency of TEL rearrangements in a series of 52 children with B-cell lineage acute lymphoblastic leukemia (ALL9. Twenty-seven percent of samples at diagnosis and 24% at relapse harbored a TEL rearrangement. p53 mutations were absent in this molecular subgroup. By contrast, p16 was deleted in 3 samples with TEL rearrangements at relapse (2 homozygous and one heterozygous).

The t(12;21) (p13;q22), which fuses the TEL gene on chromosome 12p13 and the AML1 gene on chromosome 21 q22, is the most common single gene abnormality in childhood acute lymphoblastic leukemias (ALL).[1-3] This molecular lesion has been associated with a favorable outcome.[4,5] We studied the prevalence of TEL rearrangements in a consecutive group of pediatric leukemias from a single Institution. Moreover , in order to clarify the role of tumor-suppressor gene inactivation in this setting, we performed a p53 mutational analysis in B-cell lineage ALL and searched for p16 deletions in the TEL positive group.

Ninety-two consecutive children with acute leukemias, who were referred to our hospital from 1988 to 1997, were included in the study. The diagnosis of B-cell lineage ALL (52 cases:15 at diagnosis and 37 at relapse) was based on standard morphologic and immunophenotypic criteria. Most patients at relapse were enrolled in protocols including bone marrow transplantation. Leukemic infiltration was always greater than 30% and was determined by morphologic and immunophenotypic methods. Southern blot, reverse transcription (RT) polymerase chain reaction (PCR) for TEL/AML1 rearrangements and p53 mutational studies were performed following well established protocols. TEL rearrangements were investigated by means of the pSB probe.[4] The p16 probe used was a fragment of exon 2 obtained by PCR using previously described primers.[6]

TEL rearrangements, determined by Southern blot analysis, were found in 12/52 cases. In one case, the TEL rearrangement was documented exclusively by RT-PCR, and Southern blot was in the germ line configuration. In 6 additional cases with available RNA, the RT-PCR was positive. This represents 13/52 B-cell lineage ALL (25%). Of 15 cases analyzed at diagnosis we found TEL rearrangements in 4 cases (27%). TEL rearrangement frequency in samples analyzed at relapse was 24% (9/37). We did not find p53 mutations in the TEL+ group. In four cases (11%) of B-cell lineage ALL at relapse, p53 mutants were identified. In three of these cases a cytogenetic study was available. All three cases showed a very complex karyotype with multiple abnormalities involving 17 p in one of them. Two patients had the same mutation at codon 245. In TEL+ leukemias, there was a homozygous deletion of p16 in two cases and a heterozygous loss in one case (Figure 1). Results of p16 deletions were assessed taking into consideration the following criteria: the percentage of neoplastic cells determined by flow cytometric methods, the use of the same amount of DNA on enzyme digests and the comparison of the germ line band with that obtained for the pMC41 3RC probe (c-myc), which was tested in all cases.

TEL/AML1 rearrangement is the commonest molecular lesion encountered in pediatric leukemia. In most studies, its frequency was found to be around 25%. However, important geographic differences have been reported. García-Sanz *et al*. found a low frequency of TEL /AML 1 gene rearrangements in Western Spain (3%).[7] These findings are in sharp contrast to the present series obtained from Catalonia (25%), whose population is mainly urban and more industrialized. Patients with TEL/AML1+ acute leukemias seem to form a group with a good prognosis. Nevertheless, even with the use of very effective protocols some patients relapse and die. Tumor-suppressor gene inactivation could account for some cases of relapses or treatment failures in ALL. The p53 gene is inactivated in a wide range of neoplasms. In hematologic malignancies, p53 mutations are found in BL, CLL, transformed follicular lymphoma and in some cases of relapsed ALL.[8] In this series, p53 mutations do not seem to contribute to treatment failures in TEL/AML-1+ leukemias. Our findings in the group of TEL/AML-1 negative leukemias are in line with previous reports and p53 mutations are associated with very complex karyotypes and a very bad outcome.

Table 1. Clinical and biological findings in patients with TEL rearrangements.

Pt	Sex	Age	Sample	Immunophenotype	Time	RT-PCR	Outcome
1	F	10 y	BM	$CD10^+CD19^+CD20+TdT^+$ CD13-CD33-	Relapse	ND	Died,9m
2	M	5 y	BM	$CD10^+CD19^+CD20^+$ $HLA\text{-}Dr^+TdT^+$	Diagnosis	Pos	Alive, CR
3	M	11 y	Testicle	$CD10^+CD19^+CD20^+$CD13-CD33-	Relapse	ND	Alive,CR
4°	F	12 y	BM	$CD10^+CD19^+CD20^+TdT^+$ $CD13^+CD33^+$	Relapse	Pos	Died, 6m
5	M	3 y	BM	$CD10^+CD19^+CD20$- $HLA\text{-}Dr^+CD13^+CD33$-	Diagnosis	Pos	Alive,CR
6°	M	8 y	BM	$CD10^+CD19^+CD20$-$HLA\text{-}Dr^+$ TdT^+CD13-CD33-	Relapse	Pos	Died, 4m
7	F	3 y	BM	$CD10^+CD19^+CD20$- $HLA\text{-}Dr^+$ $CD34^+TdT^+CD13^+CD33^+$	Relapse	Pos	Died,11m
8	M	8 y	BM	$CD10^+CD19^+$ $HLA\text{-}Dr^+$ $TdT^+CD13^+CD33^+$	Relapse	ND	Died, 4m
9	F	13 y	BM	$CD10^+CD19^+$ $HLA\text{-}Dr^+$ $CD13^+$ $CD34^+$	Relapse	ND	Alive,CR
10	F	2 y	BM	$CD10^+CD19^+HLA\text{-}Dr^+CD22^+$ TdT^+CD20- CD33-CD13-	Diagnosis	ND	Alive, CR
11°	F	15 y	BM	$CD10^+CD19^+CD22^+$ TdT^+ CD20- $CD33^+CD13^+CD34^+$	Relapse	ND	Died, 6m
12	F	3 y	BM	CD10-$CD19^+CD20^+$Hla-Dr^+ $CD13^+CD33^+$	Relapse	Pos*	Died, 7m
13	M	5 y	BM	$CD10^+CD19^+$CD20- $HLA\text{-}Dr^+$ $CD13^+CD33^+CD79a^+TdT^+$ $CD22^+$ CD117- CD66- $CD45^+$	Diagnosis	Pos	Alive,CR

Diagnosis based exclusively on PCR findings. °Cases 4,6 and 11 showed p16 deletions. M = male; F = female; BM = Bone marrow; ND = not done; CR = complete remission.

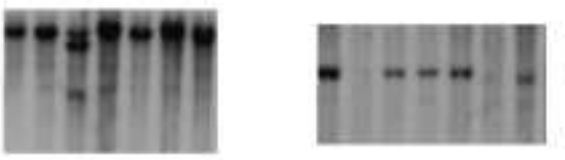

Figure 1. Left: Detection of TEL rearrangement in a testicular relapse. Lane 3 shows two rearranged bands. Right: p16 deletion in three cases with TEL rearrangements. Lanes 2 and 6 correspond to homozygous deletions and lane 7 to a heterozygous deletion .

Deletion of p16INK4a was associated with a poor outcome in series of B-cell lineage ALL.[9] In this series of ALL with TEL/AML-1 rearrangements, we detected three samples with p16 deletions: a homozygous deletion in two cases and a heterozygous deletion in the third case

Rubnitz *et al.*[5] analyzed the presence of TEL/AML1 rearrangements at diagnosis in a group of consecutively enrolled children with ALL and found 35 positive cases. In 31 of these, p16 deletion was investigated and only 3 positive cases were reported. In that paper most p16 deletions were found in T-ALL. There were 21 cases with B-cell lineage ALL with p16 deletion.[5] Anguita *et al.* reported a single relapsed case of TEL/AML1 rearrangement with p16 deletion.[10]

Further studies are needed to compare the frequency of p16 inactivation at diagnosis and at relapse, and to ascertain whether patients with this inactivation have a different disease evolution compared to patients with TEL+ leukemia with normal p16.

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Key words: acute lymphoblastic leukemia, TEL/AML1, p53, p16, molecular biology.

Funding: this study was supported by a grant from the FIS (97/1118). We are grateful to Prof. G. Grosveld for providing the pSB probe and thank Dr. E. Campo for the gift of the p16^{INK4a} probe.

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