GENETICS

Genetic Analysis of Changes in Size of Telomeres and Antioncogens p53 and RB in Children with Pre-B Acute Lymphoblastic Leukemia

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Changes in telomere length and mutations in p53 and RB genes were analyzed in 6 children with pre-B acute lymphoblastic leukemia. Telomere length was reduced in 4 patients. One patient had mutation in p53 and in one patient loss of heterozygosity of the BR gene was found; in both samples telomere length was reduced.

Key Words: *acute lymphoblastic leukemia; telomeres; antioncogens*

Acute lymphoblastic leukemia (ALL), a clonal malignant disease of the blood [1], is the most frequent form of leukemia in children. ALL is accompanied by molecular changes in DNA of blast cells [3,4,11], in particular, inactivation of p53 and RB antioncogens.

Telomeres forming the ends of eukaryotic chromosomes protect them from degradation, fusion, and recombination. Telomere length in normal cells decreases with each cell division. At the same time, in immortalized cells with unlimited division potential, telomere length remains unchanged. It is likely that maintenance of telomere length is essential for infinite cell proliferation.

The aim of the present study was to analyze changes in telomere length and activity of p53 and RB genes and possible associations between these parameters in children with pre-B ALL.

MATERIALS AND METHODS

Blood and bone marrow samples were obtained from 6 children aged 1.5-11 years (patients of the Hematology Department, Sochi Children's Hospital) after diagnosis of pre-B ALL. Lymphocytes and bone marrow cells were separated by density gradient centrifugation (Lymphocyte Separation Medium, Flow). All samples were analyzed by panning with monoclonal antibodies to differentiation antigens. DNA from bone marrow cells was isolated as described previously [7] and its purity was controlled spectrophotometrically by the ratio of optical densities at 260 and 280 nm.

Telomere length was measured as described elsewhere [8]. Cell DNA (5 μg) was digested by Alu I and Hinf I endonucleases (Boehringer Mannheim) and restriction fragments were separated by gel electrophoresis in 0.7% agarose. After standard depurination, denaturation, and neutralization, the DNA fragments were transferred to nylon membrane and fixed. The telomere-specific probe $[TTAGGG]_n$ was synthesized by PCR using $[GGGTTA]_n$ and $[TAACCC]_n$ primers. The probe was labeled with uridine-digoxygenin (Boehringer Mannheim) during synthesis. Hybridization was performed in 50% formamide and 50% hybridization buffer. The spots were visualized using a Dig luminescent detection kit (Boehringer Mannheim) in accordance with manufacturer's protocol.

Mutations at codons 3-9 of p53 antioncogene was analyzed by denaturing gradient gel electrophoresis (DGGE). Primers for PCR and DGGE conditions were

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TABLE 1. Molecular Biological Analysis of Cell DNA from Children with Pre-B ALL

No.	Age, years	Telomere length, kb	Mutations of p53	Loss of heterozygosity	
				p53	RB
1	1.5	9.5	n.f.	n.f.	n.f.
2	8	6.3	n.f.	n.f.	n.f.
3	5	11.7	n.f.	n.d.	n.d.
4	11	6.4	n.f.	n.f.	Loss of heterozygosity
5	4	6.5	m3-4, del.ex7/in7	n.d.	n.f.*
6	3	4.0	n.f.	n.f.	n.f.

Note. m3-4: mutation of a fragment containing exons 3-4; del. ex7/in7: deletion of a fragment containing exon 7-intron 7 junction region; n.f.: not found; *recurrence after 23 months.

described previously [5]. The loss of p53 gene heterozygosity was determined by analyzing restriction fragments in intron 7 after Apa I digestion of PCR products. The loss of RB gene heterozygosity was determined by single strand conformation polymorphism (SSCP) analysis [9] and DGGE of PCR products [2] (for introns 25 and 20, respectively). DNA restriction fragment in PAG visualized by silver staining.

RESULTS

The mean telomere length in 4 bone marrow samples from patients was reduced (to 4-6.5 kb *vs.* 10 kb in normal bone marrow from healthy children and *vs.* 8.2 kb in lymphocytes from adults). In 2 samples the telomere length was only slightly decreased (9.5 kb) or even increased (11.7 kb).

Exons 3-4, 5, 6, 7, and 8-9 and a fragment containing intron 7 were amplified by PCR. No loss of heterozygosity was found. However, one patient had mutation in the fragment containing exons 3-4. Analysis for heterozygosity showed changes in the length of Apa restriction fragments corresponding to deletion of an exon 7-intron 7 junction region of the p53 gene.

The DNA sequence containing introns 25 and 20 was amplified by PCR and analyzed by SSCP and DGGE, respectively. Both tests showed loss of RB gene heterozygosity in one patient. Experimental data for all patients are summarized in Table 1.

Leukemia cells are immature due to blocked differentiation process. In the majority of human cells, including blood cells, the length of telomeres decreases by 50-100 bp after each division. In accordance with the telomere hypothesis, malignant cells with short telomeres can be considered as cells passed a great number of divisions. Stabilization of the telomere length even at a low level results from reactivation of telomerase, which synthesizes telomeric DNA by copying a template sequence within the RNA moiety of the enzyme. All immortalized cells have active telomerase [6], while normal somatic cells lack telomerase activity.

In 4 children with pre-B ALL, the length of telomeric DNA was considerably decreased. This agrees with previous data on a reduced (to 4-6 kb) telomere length in adult patients with ALL [10]. In healthy children, the telomere length in bone marrow cells is about 10 kb. Hence 35-60% telomeric DNA was lost in children with ALL. It can be concluded that these cells passed 40-70 extra divisions before telomerase reactivation and stabilization of the telomere length. Unlike adult patients with ALL, where the telomere length was decreased in 100% cases, 2 children with ALL had unchanged (9.5 kb) or even slightly elongated (11.7 kb) telomeres. This can be attributed to early telomerase reactivation. It should be noted that hemopoietic stem cells possess active telomerase and differentiation block at the early stages (pre-B) led to transformation and the development of malignant tumors in the presence of active telomerase.

Antioncogen p53 encodes a protein involved in various cell processes including transcription, DNA repair, aging, cell cycle control, and apoptosis. Mutations in the p53 gene in cancer patients are usually associated with poor prognosis (Table 1).

Thus, genetic studies of some antioncogens in patients with pre-B ALL are important for choosing treatment strategy.

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