

The activity of human telomerase in the cells of acute leukaemias

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Telomeres are the end fragments of chromosomes formed by a number of non-coding double-stranded TTAGGG repeats in vertebrates. During cell division the number of repeats decreases, leading to cell senescence or apoptosis. In immortal cells, including cancer cells, the telomere length is stable and maintained by, among other factors, telomerase. The aim of the study is to compare telomerase activity in normal lymphocytes and in leukaemic cells. Samples of acute leukaemia cells, HL 60 cell line and the lymphocytes of healthy volunteers were examined. Telomerase analysis was performed using TeloTAGGG Telomerase PCR ELISApplus (Roche). The relative telomerase activities (RTA) in leukaemic and normal cells were analysed. A high level of RTA was observed in leukaemic cells.

Key words: telomeres, telomerase, human acute leukaemia cells

INTRODUCTION

One of the functions of telomeres, the end fragments of chromosomes formed by a number of non-coding double-stranded TTAGGG repeats in vertebrates, is the protection of chromosome ends from inappropriate DNA repair mechanisms [1]. During cell division the number of repeats decreases (at a rate of about 150 nucleotides per division), leading to cell senescence or apoptosis [3]. In immortal cells, including cancer cells, the telomere length is stable and maintained by a number of telomere protection mechanisms. Probably the most important of these mechanisms is telomerase [5]. This enzyme is able to maintain a sufficient number of telomeric repeats for cell survival [8]. Leukaemic cells, including ALL, ANLL-M1, CLL and CML, have, in a number of studies, shown higher telomerase activity than the control lymphocytes [2, 6].

The aim of the study is to compare telomerase activity in normal lymphocytes with that in leukaemic cells (HL-60 cell line, AML and APL).

MATERIAL AND METHODS

Bone marrow cells were obtained from 3 patients with suspected acute myeloid leukaemia from the Department of Haematology of the Medical University of Lublin. Cells were also obtained from human HL-60 leukaemic cell line and normal peripheral blood lymphocytes were obtained from 3 healthy volunteers.

Telomerase analysis was performed using TeloTAGGG Telomerase PCR ELISApplus (Roche). Cells were counted 2×10^5 per single reaction and were pelleted at 3000xg for 5 min at 4°C. The cells were suspended in PBS and the centrifugation step was repeated. The pelleted cells were then resuspended in Lysis reagent and were incubated on ice for 30 min. The lysate was centrifuged at 16000xg for 20 min at 4°C and 3 µl cell extract was added to the PCR tubes. For each sample tested and the control template 25 µl Reaction mixture (containing biotinylated telomerase substrate P1-TS, optimised anchor primer P2, nucleotides and Taq DNA poly-

merase) and 5 μ l of the Internal Standard were transferred into the PCR tubes. Negative controls were prepared by heat treatment of cell extracts for 10 min in 85°C. The control template was prepared by adding commercial control template and Lysis reagent into 2 separate tubes. PCR were performed in PerkinElmer 2400 Thermal Cycler: 30 cycles — 94°C for 30 s, 50°C for 30 s and 72°C for 90 s. The amplification product was added to 2 separate tubes containing 10 μ l of denaturation reagent. 100 μ l hybridisation buffer T was added to one vial and 100 μ l hybridisation buffer IS was added to the other. Each mixture was transferred into pre-coated MTP modules, incubated in 37°C for 2 h, washed and incubated with 100 μ l Anti-DIG-HRP. MTP modules were incubated at 20°C for 30 min and 100 μ l TMB was added for colour development. The absorbance of the samples was measured at 450 nm (with a reference wavelength of 620 nm) using a Microtiter plate (ELISA) reader. The relative telomerase activities (RTA) were obtained using the following formula:

$$RTA = [(A_s - A_{s0}) : A_{s,IS}] : [(A_{TS8} - A_{TS8,0}) : A_{TS8,IS}] \times 100$$

(A_s — absorbance of sample, A_{s0} — absorbance of heat- or Rnase-treated sample, $A_{s,IS}$ — absorbance of internal standard (IS) of the sample, A_{TS8} — absorbance of control template (TS8), $A_{TS8,0}$ — absorbance of Lysis buffer, $A_{TS8,IS}$ — absorbance of internal standard (IS) of the control template (TS8).

RESULTS

The relative telomerase activities (RTA) in leukaemic and normal cells were analysed (Table 1). A high level of RTA was observed in leukaemic cells.

DISCUSSION

As it was mentioned above, telomerase is present in human germ cell lines (testis and ovary), cancer cells and immortal cell lines. However, it has not been detected in normal adult somatic tissues or cultured human diploid cells, with the exception of renewal tissues that contain stem cells (e.g. blood, skin and intestine) [3, 4]. Immortal cells, including cancer cells, avoid the progressive loss of telomeric DNA by expressing telomerase. Surprisingly, while cancer cells have high levels of telomerase, their telomeres are generally short, although their length remains stable [5]. Reactivation of telomerase activity may be the critical, if not rate limiting step in the development of neoplastic cells

Table 1. The relative telomerase activities

Sample	Origin	RTA
1	Acute promyelocytic leukaemia	14,400.0
2	Acute promyelocytic leukaemia	16,440.0
3	Acute myeloid leukaemia with t(8;21)	10,600.0
4	HL-60 cell line	9,010.0
5	Healthy donor	112.5
6	Healthy donor	117.5
7	Healthy donor	168.8

[5]. The previous studies of the leukaemic cells mentioned above [2, 6, 7] have demonstrated significantly higher telomerase activity than in normal lymphocytes. The results of this study confirm these findings. Thus we may conclude that telomerase activity is worth further investigation as one of the markers of tumour development.

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