INHIBITION OF TELOMERASE ACTIVITY DURING INDUCTION OF HL-60 CELLS BY RETINOID Ro13-7410

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Abstract

Objective: To investigate the effects of Ro13-7410 on telomerase activity and cell cycle distribution. Methods: Telomerase activity of HL-60 cells induced by retinoid Ro13-7410 was detected by telomerase PCR-ELISA-kit. The cell cycle was analyzed by flow cytometry. Results: Telomerase activity declined gradually after 10^{-6} mol/L Ro13-7410 treatment, and the inhibition of telomerase activity at day 5 of treatment with Ro13-7410 was less effective than with Retinoid Acid (RA). DNA flow cytofluorimetric analysis revealed that Ro13-7410 caused partial cells arrest in the G_2/M phase after 4-days treatment. Conclusion: Telomerase activity declined gradually and partial cells were arrested in the G_2/M phase after Ro13-7410 treatment.

Key words: Telomerase, Leukemia cell, Cell cycle, Retinoid.

Telomeres form the ends of eukaryotic chromosomes consisting of tandem arrays of highly conserved hexameric (TTAGGG) repeats. Telomeres have been implicated in protecting the ends of chromosomes against exonuclease and ligases, preventing the activation of DNA-damage checkpoints, and countering the loss of terminal DNA segments that occurs when linear DNA is replicated.[1] Telomerase, a ribonucleic acid complex, adds hexameric repeats of 5'-TTAGGG-3' to the ends of mammalian chromosomal DNA (telomerase) to compensate for the progressive lose that occurs with successive of DNA replication.[2] Telomerase activity had been detected in a few normal somatic cells and vast majority of tumor cells. Differentiation of most tumor cells is associated with a marked down regulation of telomerase activity.[3] In previous studies, telomerase activity can be detected in HL-60 cell line and its inhibitions is associated with the differentiation induced by RA and Dimethyl Sulfoxide (DMSO).

We have recently demonstrated that HL-60 cells treated with Ro13-7410 exhibited apoptosis and differentiation.[5] In this study the effects of Ro13-7410 on telomerase activity and cell cycle distribution were investigated by PCR-ELISA and flow cytometry.[6]

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MATERIALS AND METHODS

Chemicals

Ro13-7410 ($C_{24}H_{28}O_2$ supplied by Chongqing Huabang Co. Ltd) and RA (sigma) were dissolved in ethanol as a 10^{-2} mol/L stock solution, which was stored at -20°C and protected from light.

Cell Culture

HL-60 cells were grown in RPMI-1640 medium supplemented with 10% (V/V) fetal bovine serum (FBS), 100 units/ml penicillin and 100 μg/ml streptomycin and maintained at 37°C in a humidified atmosphere of 5% CO_2 in air and used for experiments during the exponential phase of growth.

Telomerase Assay by PCR ELISA

Telomerase PCR ELISA-kit from Boehringer Mannheim Cop. Pellets of 2×10^6 were collected, and

cell extracts were prepared. The PCR-based TRAP assay for assessing telomerase activity was used. Add telomerase substrate, primers, nucleotides, and Taq polymerase and sterile water to a final volume of 50 μl. Reactions were incubated for 30 min at 25°C. Then reverse-transcriptive products were placed in a thermal cycle for 30 cycles at 94°C for 30s, 50°C for 30s, 72°C for 90s, 72°C for 10 min. ELISA procedure were used. Telomerase activity were tested using a microtiter plate (ELISA) reader, measure the absorbance of the samples at 450 nm within 30 min after addition of the stop reagent.

Cell Cycle Analysis

The cell cycle was analyzed by flow cytometry after incubation of HL-60 cells with Ro13-7410 and RA respectively between 1 day and 4 days. Briefly, the cells were fixed in cold methanol and incubated for 30 min at 4 in the dark with a solution of 50 μg/ml propidium iodide, 1 μg/ml RNase (sigma). Analysis was performed immediately after stain.

RESULTS

Telomerase Activity

Telomerase PCR-ELISA assay showed that untreated control HL-60 cells had significant telomerase activity (OD 0.606). Telomerase activity inhibited by Ro13-7410 was time-dependent and changes becoming evident at 1 day. Figure 1 showed that telomerase activity was 0.504, 0.392, 0.250 (OD) respectively at 1, 3, 5 day following exposure to 10^{-6} mol/L Ro13-7410. Telomerase activity at day 5 of treatment with RA was also inhibited heavily.

Cell Cycle Distribution

Analysis of the cell cycle distribution of HL-60 after exposure to either Ro13-7410 or RA between 1-day and 4-day is shown in Table 1. Results of these studies showed complex changes. No significant change in cell cycle distribution occurred after cells treated with Ro13-7410 or RA at 1 day. By day 2, partial cells were arrested in the G_2/M phase and the percentage of cells arrested in the G_2/M phase increased after 4-day treatment. With RA-treated cells, a reduction in the percentage of cells in the G_2/M phase was observed after 2-day of treatment.

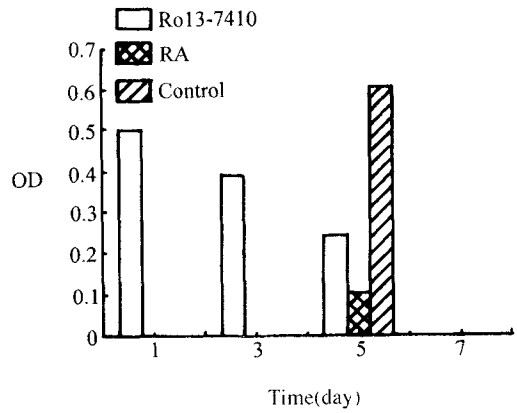

Fig. 1. Telomerase activity of HL-60 cells incubated with Ro13-7410 or RA

Table 1. Cell cycle distribution in HL-60 cells after Ro13-7410 and RA treatment

Days of treatment		Cells in cell cycle phase (%)		
		G_1	S	G_2/m
Control	1	26.9	18.2	54.9
	2	17.4	28.5	54.1
	3	18.4	19.7	61.9
Ro13-7410	1	23.8	29.1	47.1
	2	20.2	19.9	59.9
	3	16.2	5.9*	77.9
RA	1	27.5	20.5	52.0
	2	15.3	37.1	47.6
	3	44.0	4.7*	51.3

x^2 test *$P<0.05$ compared with control and RA *$P<0.005$ compared with control

DISCUSSION

Telomerase is a complex enzyme containing both protein and an RNA component that acts as a template for telomere alongation. Reduction of telomeres occurs during cell differentiation and normal aging. Telomerase activity has been strongly associated with immortalization of cells and malignant tumors. In the last few years, results from many laboratories have shown that telomerase activity is downregulated in a

large number of tumors induced differentiation and is considered as a target for cancer treatment.

Ro13-7410, the benzoic acid, derivative of retinoic acid, was found to have less mucocutaneous side effects than other retinoids in the treatment of retinoid-responsive dermatoses.[6] Our results demonstrated that apoptosis and differentiation of Ro13-7410 treated HL-60 cells is accompanied by a loss of telomerase activity and alteration of cell cycle distribution. Telomerase activity declined gradually and changes becoming evident at 1 day. These results suggest that inhibition of telomerase activity by Ro13-7410 is complex and may be indirect.

Several reports have documented a functional relationship between telomerase and the cell cycle.[7] In tumor cell lines, telomerase activity changes as cells progress through the cell cycle. As cells progress through the G_1/S phase of the cell cycle, telomerase activity gradually increases. The highest level of telomerase activity is present at the replicative S phase, with a virtual loss of activity at the G_2/M phase. Our results showed that Ro13-7410 arrested the HL-60 cells at the G_2/M phase of the cell cycle, and inhibited the telomerase activity. However, HL-60 cells arrested in G_1 phase by RA were also accompanied with the loss of telomerase. The data indicate that Ro13-7410 was different from RA in inducing HL-60 cells and further studies should be required to evaluate the effects of Ro13-7410 on leukemia cells.

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