TELOMERASE ACTIVITY DURING 7, 12-DIMETHYLBENZ [a] ANTHRACENE-INDUCED HAMSTER BUCCAL POUCH CARCINOGENESIS

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ABSTRACT

Objective: To investigate the roles of telomerase activity (TA) in relation to hamster buccal pouch tumor progression. Methods: male hamster were treated three times weekly with 0.5% of 7, 12-dimethyl-benzanthracene (DMBA) over a 15 weeks experimental period. Hamsters were sacrificed at 3, 6, 9, 12 and 15 weeks after treatment. Telomerase activity of hamster buccal pouch tissue were measured along with the analyses of the formation of DMBA-induced hamster buccal pouch tumors. Results: DMBA-induced squamous cell carcinomas were found at the 6th week after dosing. Telomerase activity elevation began at the 3rd week and was increasing to a plateau at the 12th week. Conclusion: Our results show that telomerase activity in the target tissue may be detected at the early stage of the DMBA-induced hamster buccal pouch tumor formation and suggests that telomerase activity may be used as a biomarker for an early clinical detection of buccal pouch cancer.

Key words: Telomerase, Hamster, DMBA-carcinogenesis

Telomerase is a ribonucleoprotein complex intimately involved in cell immortalization and carcinogenesis. Increasing evidence shows that telomerase activity has been detected in human tumor cells but not in normal somatic tissues.[1] Thus, an hypothesis has been proposed that the activation of telomerase activity is essential for cells to overcome senescence and also may result in the immortality and malignant progression of a cancer.[2] Recently, comparatively low telomerase activity has been found in non-malignant tissues from rats and mice.[3] It is suggested that there may be species differences between human and rodent telomerase. Nonetheless, rodent models are useful to study mechanisms of induction and inhibition of chemically induced tumor formation. The hamster buccal pouch mucosa provides an one of the most widely accepted experimental models of oral carcinogenesis.[4] In this experiment, we treated hamster with potent carcinogen, 7, 12-dimethyl-benzanthracene (DMBA) to induce hamster buccal pouch tumors. The DMBA-induced squamous cell carcinogenesis would be analyzed. Hamster buccal pouch telomerase was measured during the development of hamster buccal pouch tumorogenesis. The results of DMBA-induced telomerase activity was correlated with the hamster buccal pouch tumor formation.

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MATERIALS AND METHODS

Animals and Treatment

Sixty Hamsters (6—8 weeks old) were divided into 8 groups. Each DMBA-treated group contains ten hamsters, while each parallel control group contains five hamsters. Both pouches of each animals in DMBA-treated group were painted three times weekly with a mineral oil containing 0.5% DMBA (Sigma). The control groups were painted with mineral oil. Fifteen animals (10 treated and 5 control) were sacrificed at the 3rd weeks after dosing. The

remaining groups of animals were killed at the 6th, 9th, 12th and 15th week after dosing. The hamster buccal pouch tissues were applied to histological examination, while the left buccal pouch tissues were subjected to telomerase activity analysis.

Telomerase Activity Assay

The extraction of cellular protein from buccal pouch tissues and the telomeric repeat amplification protocol (TRAP) assay were performed as described previously[5] with a slight modification. Briefly, 2 μl of tissue extract (protein concentration 0.5 μg/μl) was incubated in 50 μl of reaction solution containing TRAP buffer, 50 mmol/L of dATP, dTTP, and dGTP, 1 μg TS primer, 1μg ACX primer, 2Unit Taq polymerase, 50 mmol/L of 4 μCi ^{3}H-dCTP (2.0 TBq/mmol, NEN™ Life Science Product) and non-labeled dCTP, The PCR products were then separated by 12.5% polyacrylamide gel electrophoresis (125v for 4 h). The gel was stained by silver nitrate technique and the telomerase activity was estimated by measuring the incorporation of ^{3}H-dCTP into the PCR products.

Statistic Analysis

The SSPS (version 8.0) for windows software was used to analyze the data. The comparison of means between the different groups was evaluated by ANOVA with Fisher's test. To evaluate the significance of the correlation, the Spearmen nonparametric coefficient was used.

RESULTS

Animal Model

Histologically, the tumors yielded in the present study were either exophytic squamous cell papillomas or carcinomas. The control groups were free of tumors as demonstrated under the light microscope. The pathological finding from this experiment was summarized in Table 1.

Table 1. DMBA-induced hamster buccal pouch tomorogenesis

Treatment (weeks)	No. of hamster	No. of hamster with dysplasia	No. of hamster with tumor	Incidence (%)
3	10	1	0	0
6	10	3	2	20
9	10	4	5	50
12	10	1	9	90
15	10	0	10	100
Total	50	9	26	52

Tumors were counted and the tumor incidence was calculated by No. of tumors/No. of hamster.

Telomerase Activity

To characterize the telomerase activity, a simple enzyme kinetic study of activity versus concentration was conducted. Figure 1 shows the plots of telomerase activity against different concentration of A549 cell extract. A linear relationship was obtained when cell extract was increase from 0.25 μg to 4 μg while the total ^{3}H-dCTP (sum of the radioactivity incorporated into the bans of the ladder) was proportionally increase (r=0.964, P<0.001). Using this modified TRAP assay buccal pouch telomerase activities from hamster treated with DMBA were measured. Figure 2 showed the results of telomerase activities at different time points after the hamsters received DMBA. All the data were expressed as $\bar{x} \pm s$ (n=10). At all time point, the telomerase activity in DMBA-treated group was higher than that in control group (P<0.001). Telomerase activity induced by DMBA started at the 3rd week, and increased to a plateau at the 12th week. The telomerase activity induced by DMBA is 4～5 fold greater than that in normal buccal pouch tissue.

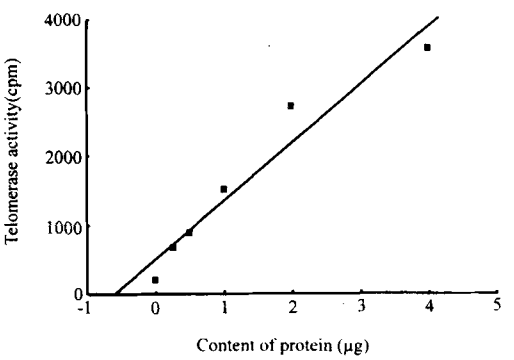

Fig. 1. Dependency of telomerase activity on the protein amount of a cell extract.

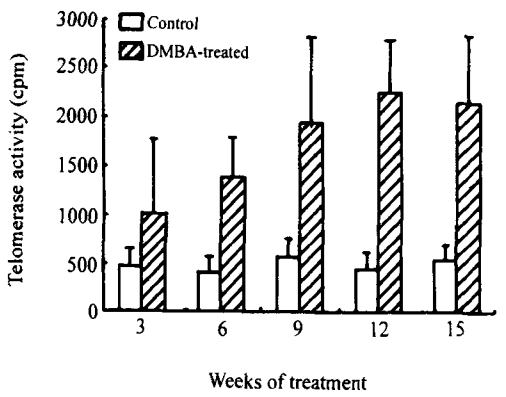

Fig. 2. Telomerase activity in control and DMBA-treated hamster huccal pouch tissues detected by the TRAP assay.

DISCUSSION

Published results show that the telomerase activities of human tissues exist in rapid proliferative cells, such as embryo, bone marrow and germ cells. However, in most differentiated normal somatic cells, the telomerase activity is hardly detected.[6] Since a high level of telomerase activity in abnormal proliferating tumor cells has been found, the hypothesis that telomerase activity may be an important factor for cancer development has been proposed. Hiyama[7] reported in a study on human gastric cancer that telomerase was activated as a late event in gastric cancer progression. It seems to be true that human telomerase activity is greater in the late stage of tumor development than the early stage since the percentage of detectable telomerse is much greater in the tissues of patients at the later stage of cancer than at the early stage.[8] In contrast to the human tissue, telomerase activity in laboratory animals, especially rodents, has been detected not only in tumor cells but also in many normal somatic tissues. Our results also show that the expression of telomerase can be detected in both normal and malignant hamster buccal pouch mucosa cells. The detection of telomerase activity in normal cells in this different stages during the tumor development. Telomerase activity has been shown to increase during tumor progression, such as mouse skin premalignant progression.[9] Our results indicates that telomerase activity may be an important biomarker during tumor promotion and progression stages. To study the induction and inhibition of telomerase activity can potentially be a novel area for cancer detection and therapy.

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