

실험적으로 유발된 중이진주종에서 Telomerase의 발현

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Expression of Telomerase Activity in Experimental Cholesteatoma

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—ABSTRACT—

Background and Objectives : Telomerase is a marker of cell proliferation in malignant tissues and also can be expressed in benign disease such as cholesteatoma. There are two opposite opinions about the expression of telomerase in cholesteatoma. The purpose of this study is to evaluate whether telomerase is expressed in experimentally induced cholesteatoma. **Materials and Methods** : Cholesteatoma was induced by ligation of EAC of ten Mongolian gerbils. The telomerase activity was detected by the telomerase repeat amplification protocol (TRAP) assay method in both cholesteatoma and normal control retroauricular skin. **Results** : 10 of 10 (100%) cholesteatoma cases expressed telomerase activity, whereas 3 of 10 (30%) retroauricular normal skin detected telomerase activity. **Conclusions** : The high expression of telomerase in experimentally induced cholesteatoma suggests activation of telomerase may be related to the proliferative nature of cholesteatoma as in human tissues. (J Clinical Otolaryngol 2004;15:250-255)

KEY WORDS : Telomerase · Cholesteatoma · Animal experimentation.

서론

Telomeres are DNA-protein complexes that are located at the ends of eukaryotic chromosomes. They consist of a DNA sequence (TTAGGG in humans) and associated proteins. Telomeres play a role in DNA strand break capping and telomerase activity. Telomerase is a ribonucleoprotein complex that maintains telomere length. The expression of telomerase is up-regulated in many types of cancer. In this study, we investigated the expression of telomerase activity in experimentally induced cholesteatoma in Mongolian gerbils. We found that telomerase activity was expressed in cholesteatoma but not in normal retroauricular skin. These results suggest that telomerase activation may be related to the proliferative nature of cholesteatoma.

1) ...
2) ...
3) ...
4) ...

Belair telomerase가
telomerase가
가 가

: 2004 10 5
: 2004 11 23
: , 602 - 739 1가 .
: (051) 240 - 7536 · : (051) 246 - 8668
E - mail : entgate@pusan.ac.kr

Telomerase

가 가 . Ketamine(40 mg/kg) Rumpun(8 mg/kg) 4~0 silk

가 . 3 telomerase

연구방법

가 telomerase TRAP telomerase

가 가 . 22 Telomerase Kim ⁷⁾ TRAP assay

17 (77.3%) telomerase가 TRAPEZETM Telomerase Detection Kit (Oncor Co., USA) (Fig. 1).

(0%) 15 telomerase가 , primer , TRAP , poly-

⁵⁾ Rudolph ⁶⁾ 29 , acrylamide gel , phosphorimager

1 (3.4%) telomerase가

9 6 (66.7%) telomerase

telomerase 4

gerbil 50~100 mg microcentrifuge tube

telomerase 100 μl 1 × CHAPS lysis buffer(10 mM Tris - HCl, pH 7.5, 1 mM MgCl₂, 1 mM EGTA, 0.1 mM benzamidine, 5 mM -mercaptoethanol, 0.5% CHAPS, 10% Glycerol) 가 가 , pestle Pellet pestle motor (Kontes Co., USA)가

대상 및 방법

연구대상

65~70 g 10 Mongolian gerbils 10

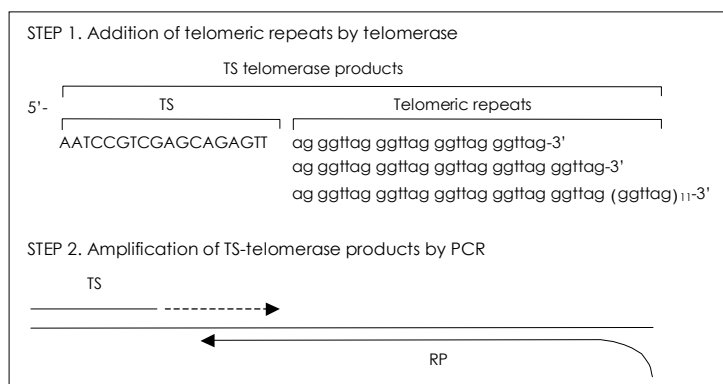


Fig. 1. TRAP assay.

30 , 5417R microcentrifuge(Eppendorf Co., Germany) 4 12,000×g 20 80 µl tube kit(Biorad Co., U.S.A.) 1× CHAPS lysis buffer 1 µl/µl가 TRAP TS primer(5' - AATCCGT-CGAGCAGAGTT - 3') 5' - labeling 20 µl ³²P - ATP (3000 Ci/mmol, 10 uCi/ml) 2.5 µl, TS primer 10.0 µl, 10× kinase buffer 2.0 µl, T4 polynucleotide kinase(10 units/µl) 0.5 µl 5.0 µl 37 20 85 5 가 4 TRAP 2 µg 25 µl 가 10× TRAP buffer(200 mM Tris - HCl, pH 8.3, 15 mM MgCl₂, 630 mM KCl, 0.5% Tween 20, 10 mM EGTA, 0.1% BSA) 2.5 µl, 50× dNTPs Mix(25 mM each dATP, dTTP, dGTP and dCTP) 0.5 µl, ³²P - TS primer 1 µl, TRAP primer mix(RP primer, K1 primer, TSK1 template) 0.5 µl, Taq polymerase(5

units/µl, Takara Co., Japan) 0.2 µl 18.3 µl (1 µg/µg) 2 µl 25 µl가 mimeral oil 20 µl PCR heating block(Mastercycler 5330, Eppendorf Co., Germany) 30 30 telomerase가 94 30 가 94 30 , 60 30 30 4 10 1 loading dye(0.25% bromophenol blue, 0.25% xylene cyanol, 50% glycerol, 50 mM EDTA, pH 8.0) 12.5% polyacrylamide gel(acrylamide : bis - acrylamide=19 : 1) 0.25× TBE buffer 20 V 3 gel autoradiography phosphorimager(Molecular Dynamics Co., USA) TRAP PCR Taq polymerase , 10× CHAPS lysis buffer 36 bp band , 36 bp 50, 56, 62, 68 6 bp

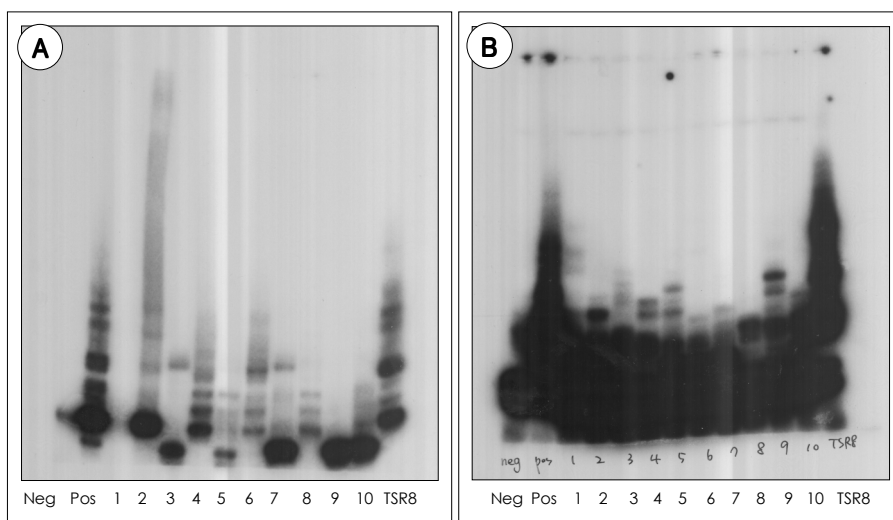


Fig. 2. Telomerase expression in experimentally induced cholesteatoma. A : control group, Three of 10 (No. 4, 6, and 8) showed telomerase activity. B : cholesteatoma group. Ten of 10 expressed telomerase activity.

Telomerase

TRAP . Mutirangura ¹¹⁾ 16
telomerase가 14 telomerase가 (87.5%) ,
85 5 가 RNase (erythroplakia)
TRAP 38.5% .
telomerase (hyperplasia) (dysplasia)
3 가 . Ka-
nnan ¹²⁾ 75% telo-
merase가 telo-
merase가 ¹³⁾ 8
telomerase
10 10 (100.0%) (Fig. 2), 7
10 3 (30%) telomerase가 .
Kyo ¹⁴⁾
telomerase (prolife-
rative phase) telomerase 95% 가
(secretory phase) 42%
Belair ⁴⁾ Kyo ¹⁴⁾
Telomere telomerase가
가 가 .
telomere DNA
strand가 DNA
mere telo-
¹⁾ .
Simian Virus 40
tumor antigen PCNA, EGF, EGFR 가
¹⁵⁾¹⁶⁾ Shinoda ¹⁷⁾
telomerase telomere
가 telomerase
, telomere 가 wide type
⁸⁾ Telomerase telomere p53 가 wide
가
⁹⁾ type p53 apoptosis
¹⁸⁾ Fas apop-
18 16 (89%) telomerase가 tosis 가
. Cheng ¹⁰⁾ 85% 가 .
telomerase가 가
apoptosis가
(60%) telomerase .

Holly ¹⁹⁾ 가 c- myc 가 telomerase 가

myc 가 c - myc telomerase 가

가 telomerase가 telomerase

Belair ⁴⁾ telomerase 가 telomerase가 가

가 가 telomerase

Goh ⁵⁾ telomerase가

Rudolph ⁶⁾ telomerase apoptosis가 telomerase

가 Kojima

²⁰⁾ telomerase telomere 가 telomerase

가 telomerase가 22 telomerase가 telomerase

결 론 telomerase

중심 단어 : Telomerase

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