

인체 뇌종양조직에서 텔로머레이즈의 발현과 세포사멸*

김충현 · 정진환 · 백광흠 · 김재민 · 고 용 · 오석전

= Abstract =

Expression of Telomerase Activity and Apoptosis in Human Brain Tumors

Choong Hyun Kim, M.D., Jin Hwan Cheong, M.D., Koang Hum Bak, M.D.,
Jae Min Kim, M.D., Yong Ko, M.D., Suck Jun Oh, M.D.

Department of Neurosurgery, College of Medicine, Hanyang University, Seoul, Korea

Objective : Telomerase, a ribonucleoprotein adds telomere repeats to the ends of telomeres to compensate for the progressive loss. A favorable prognosis associated with low or no telomerase activity in some tumors, and cells transfected with antisense human telomerase lost telomeric repeats and die. We studied about the relationship between telomerase activity and apoptosis in the human brain tumors.

Material and Methods : Between July 1998 and December 1999, 62 patients with brain tumors underwent surgery and their surgical specimens were obtained. Telomerase activity was investigated by telomeric repeats amplification protocol (TRAP) assay. Apoptosis was also evaluated by DNA fragmentation analysis. Differences and correlation in data were analyzed using Mann - Whitney test and Wilcoxon - signed rank test.

Results : Expression rate of telomerase activity and apoptosis were 80% and 30% in malignant gliomas, 33% and 0% in low grade gliomas, 63% and 38% in meningiomas, 67% and 33% in pituitary adenomas, 33% and 33% in metastatic tumors, 67% and 17% in acoustic neurinomas, 100% and 100% in pineoblastomas, 100% and 0% in the hemangioblastoma, respectively. There was no significant difference of telomerase activity and apoptosis between histological types. But a significant difference was noted in the expression of telomerase activity between malignant gliomas and low grade gliomas ($p = 0.022$). Brain tumors with telomerase activity expressed the lower rate of apoptosis. A significant correlation was also found between telomerase activity and absence of apoptosis in the human brain tumors ($p = 0.005$).

Conclusions : Our data suggests that telomerase may protect from apoptosis of the human brain tumors and also may play an important role in the biological malignancy of the gliomas.

KEY WORDS : Telomerase · Apoptosis · Brain tumors · Malignancy.

서론

(telomere) 50~200 (nucleotides)가

AGGG 6 가

¹⁾ DNA ,

TT- 가

(telomerase) 6

5' - TTAGGG - 3' 가

(ribonucleoprotein)

⁹⁾

¹⁰⁾

⁵⁾

2000

70~95%
90%,
83%
20)
가
가
21)
가
가
tosis)
가
13)
가

3)17)
가
62%
4 , 15,000rpm
25
30
(homogenizer)
(BCA Protein Assay, Pierce,
UK)
가 0.5 μg/μl
CHAPS
2 μl 10 μl
5x TRAP 10 μl [Tris buffer, biotinylated
telomerase substrate oligonucleotide(b-TS primer,
5'-AATCCGTCGAGCAGAGTT-3'), reverse primer
(RP), dNTPs (dGTP, dATP, dTTP, DNP-dCTP), oligo-
mer mix(36bp internal control band)] 0.4 μl(2 units)
Taq polymerase, 37.6 μl TRAP
(PCR :
polymerase chain reaction) TRAP (30
30 ; 94 30 ; 53 30 ; 72 30 , 36)
12% TBE (gel)
400V, 25 (silver stain kit,
Bioneer) (Fig. 1). 25bp DNA
step ladder(Promega, UK)
PCR/ELISA

대상 및 방법

1. 대 상

1998 7 1999 12
가
62
70
14)
6 , 4
6 16 , 16 ,
12 , 9 , 6 ,
2 1

2. 방 법

1) 텔로머레이즈 분석

17) telomeric repeat am-
plification protocol(TRAP) assay(TRAP_{EZE} ELISA Te-
lomerase Detection Kit, Oncor, MD, USA)
- 70 phosphate -
buffered saline(PBS, pH 7.4) 3
가 2 3mm³ 1.5ml
. 200 μl RNAsin 가 CHAPS

(heat inactivation control) 85 10 가

2) DNA 절편분석(DNA Fragmentation Analysis)

Agarose DNA 29)
- 70
PBS(pH 7.4) 3 가 2 3mm³
1.5ml . 10mM Tris - HCl, 10
mM EDTA, 0.5% SDS(pH 8.0)가 200 μl
1.5ml

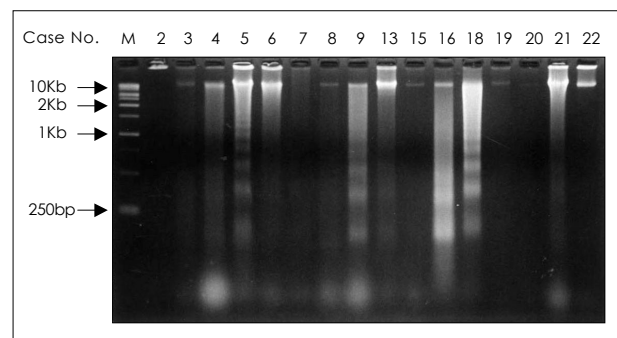


Fig. 1. DNA fragmentation in agarose gel. Fragmented DNA was isolated and electrophoresed in a 2% agarose gel containing 50ng/ml ethidium bromide. DNA fragmentation noted in lane 5, lane 9, and lane 13. Lane 1 indicates molecular weight standard.

1,500rpm 15
 10% SDS(sodium dodecyl sulfate, Bio - Rad, Richmond, CA, USA) 20 μl RNase A(10mg/ml, Sigma Chemical Co., MO, USA) 20 μl
 56 1 proteinase K (10mg/ml, Promega, UK)20 μl 37 1
 3 M sodium acetate 100 μl ethanol 950 μl -20 DNA
 12,000rpm 15 DNA 70% 12,000rpm 15 DNA
 30 μl TE(Tris - EDTA, pH 7.4) DNA 6x loading buffer 5 : 1
 2% agarose 50 100V ethidium bromide (50mg/ml) DNA
 (Fig. 2).
 DNA가 180~200bp ladder

3) 통계학적 분석

(SPSS 8.0 for Windows)
 Kruskal - Wallis test
 Mann - Whitney test
 Wilcoxon - signed rank test
 Pearson correlation test

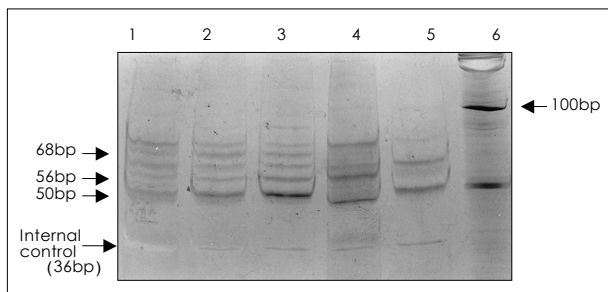


Fig. 2. Telomerase activity by silver staining. Telomerase oligonucleotides were amplified by PCR in the presence of an internal TRAP assay standard. Reaction products were resolved on a 10% polyacrylamide gel. Lane 1, case 7 ; lane 2, case 8 ; lane 3, case 9 ; lane 4, case 10 ; lane 5, DNA marker. Typical ladder pattern of telomerase activity noted in the lane 1 - 3.

결 과

1. 조직학적 분류에 따른 텔로머레이즈 발현정도

62	39 (63%)	가
63%	가	
4 (83%)	3 (75%),	6
5 (83%)	6 2 (33%)	
2		
6 (63%)	4 (67%),	16
10 (63%)	12 8 (67%)	
(56%)		9 5

가 (Table 1).

2. 조직학적 분류에 따른 세포사멸

62	16 (26%)
6 (50%)	3 (50%)
12 (33%)	4 (33%),
16 (38%)	9 3 (33%)
2	

가 (Table 1).

3. 악성 뇌교종과 저등급 뇌교종에서 텔로머레이즈 발현 및 세포사멸

Table 1. Expression of telomerase activity and apoptosis in 62 cases with brain tumors*

Histological type	No. of positive expression	
	Telomerase	Apoptosis
Astrocytoma (n = 6)	2(33%)	0(0%)
Anaplastic astrocytoma (n = 4)	3(75%)	0(0%)
Glioblastoma (n = 6)	5(83%)	3(50%)
Pituitary adenoma (n = 12)	8(67%)	4(33%)
Meningioma (n = 16)	10(63%)	3(38%)
Acoustic neurinoma (n = 6)	4(67%)	1(17%)
Pineoblastoma (n = 2)	2(100%)	2(100%)
Hemangioblastoma (n = 1)	0(0%)	0(0%)
Metastatic cancer (n = 9)	5(56%)	3(33%)

*p >0.05 in the difference of telomerase expression and apoptosis between histological types. Mean percentage of telomerase activity and apoptosis in 62 cases with brain tumors are 63% and 25% respectively.

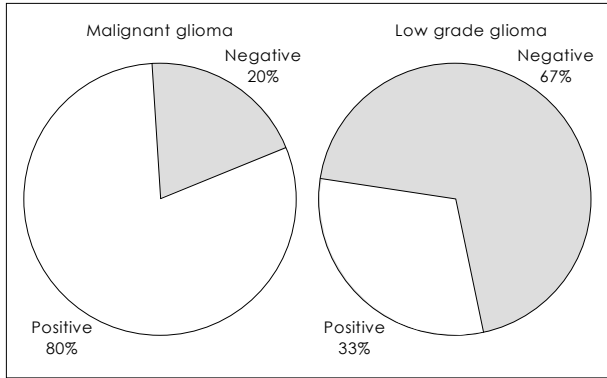


Fig. 3. Comparison of telomerase activity between malignant astrocytoma and low grade astrocytoma ($p = 0.022$, Mann-Whitney test).

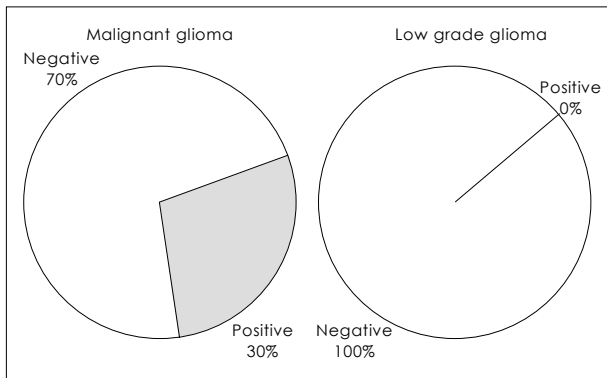


Fig. 4. Comparison of apoptosis between malignant glioma and low grade glioma ($p > 0.05$).

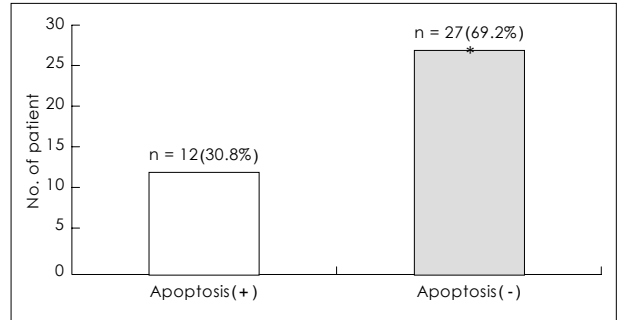


Fig. 5. Bar graph showing apoptosis in the brain tumors with telomerase activity ($*p = 0.016$, Wilcoxon-signed rank test).

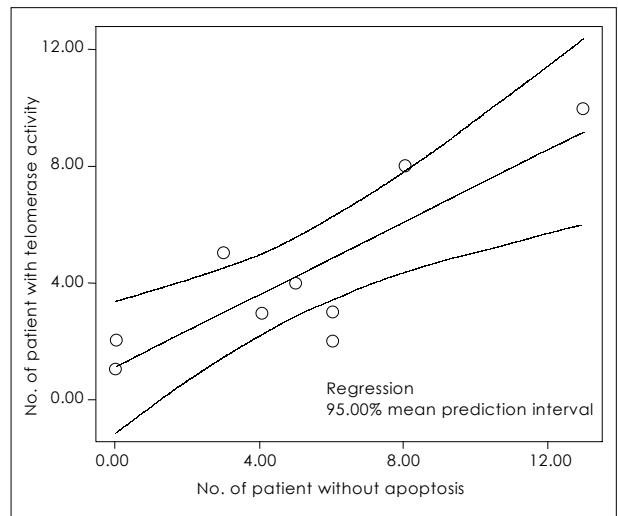


Fig. 6. Scatterplot showing the significant correlation between telomerase activity and absence of apoptosis in brain tumors ($r^2 = 0.71$, $p = 0.005$).

80% 가
33%
($p = 0.022$)(Fig. 3).
33% 0%
가 (Fig. 4).

4. 텔로머레이즈 발현과 세포사멸과의 관계

가 39 12 (30%)
27 (69%)
($p = 0.016$,
Wilcoxon - signed rank test)(Fig. 5).

($p = 0.005$, Pearson correlation test)(Fig. 6).

고 찰

가
가
(telplate) RNA RNA
telomerase reverse transc-
riptase(TERT) 4)27).
가 1997 Harrington
8)
25) 가
가 가 24).
S 가

G₂/M

bcl-2, myc, p53

가

S¹⁸⁾

G₂/M

APO - 1/Fas

70~80%

³⁾ Hiraga ¹¹⁾

(cytokine)

Bcl - 2

(cytochrome) C가

107 66

apoptosis protease activating factor - 1(Apaf - 1)

가

grade

caspase

, , TNF Fas

20%,

40%

72.3%

(death receptor)

. Kudoh ²⁰⁾ 1999

(adapter)

caspase , , DNA

90%,

62%,

14%,

p53, Ataxia - telangiectasia mutated(ATM) kinase

83%,

47%

caspase Bcl - 2

7%

⁶⁾

15.4%, grade

14.3%

가

42.9%

가

Bcl - 2

가

가

²⁾

가

²³⁾

가

antisense

가

가

²⁸⁾

Cisplatin

가

가

¹⁹⁾

가

가

가

69.2%

가

30.8%

¹²⁾

⁷⁾

가

²²⁾

1972 Kerr, Wyllie Currie ¹⁵⁾

가

가

²⁶⁾

결 론

¹⁶⁾

- Telomeres, telomerase and cancer : is the magic bullet real ?*
Anticancer Res 16 : 511-516, 1996
- 26) Thompson CB : *Apoptosis in the pathogenesis and treatment of disease. Science 267 : 1456-1462, 1995*
- 27) Weinrich SL, Pruzan R, Ma L, Ouellette M, Tesmer VM, Holt SE, et al : *Reconstitution of human telomerase with the template RNA component hTR and the catalytic protein subunit hTERT. Nat. Genet 17 : 498-502, 1997*
- 28) Yashima K, Piatyszek MA, Saboorian HM, Virmani AK, Brown D, Shay JW, et al : *Telomerase activity and in situ telomerase RNA expression in malignant and non-malignant lymph nodes. J Clin Pathol 50 : 110-117, 1997*
- 29) Yin D, Kondo S, Takeuchi J, Morimura T : *Induction of apoptosis in murine ACTH-secreting pituitary adenoma cells by bromocriptine. FEBS Lett 339 : 73-75, 1994*