Prognostic Implication of Telomerase Activity in Patients with Brain Tumors

Telomerase adds telomeric repeats to the ends of telomeres to compensate for their progressive loss. A favorable prognosis is associated with low or no telomerase in some tumors. The authors investigated whether telomerase activity is associated with survival of patients with brain tumors. Sixty-two consecutive patients with brain tumors underwent surgery, and their surgical specimens were investigated. The patients were pathologically categorized as group I (aggressive group) and group II (non-aggressive group). Telomerase activity was examined by the telomeric repeat amplification protocol (TRAP) assay. The median time was calculated in association with overall survival and progression-free survival in each group. The significant difference was noted in telomerase activity between high-grade gliomas and low-grade gliomas (p=0.022). Telomerase activity was significantly associated with the median overall survival and progression-free survival in all tumors of the aggressive group. On the other hand, the median overall survival in the non-aggressive group was not dependent on telomerase activity, while the median progression-free survival was. Our data suggests that telomerase is an important prognostic indicator of survival in patients with brain tumors.

Key Words : *Brain Neoplasms; Glioma; Survival; Telomerase*

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INTRODUCTION

The telomere, located at the end of the eukaryotic chromosome, is a specialized structure that consists of six TTAGGG bases in a repetitive pattern (1). Its role is to protect the chromosomes from DNA degradation, end-to-end fusions, rearrangement, and loss (2). For every cell division, 50-200 nucleotides are decreased during the aging of normal cells. As the telomere becomes shorter, it finally reaches a stage when apoptotic cell death is inevitable. This shortening process of the telomere may control the proliferative capacity of normal cells, and may be associated with aging process (3-6).

Telomerase is a type of ribonucleoprotein which adds hexameric repeats of 5´-TTAGGG-3´ to the end of telomeres to preserve the length of the chromosome. It does not express on normal somatic cells, but is instead expressed on immortalized cells, such as tumor cells (7, 8). Telomerase activity can be an index of malignant potential or malignancy itself in brain tumors. The presence of telomerase in a certain tumor results in a worse prognosis than a tumor without telomerase. A tumor with less or no telomerase activity has been found to have a better prognosis (9-11). On the other hand, cells that are telomerase positive have stronger survival and higher resistance against apoptosis (5). The fact that these cells are more susceptible to death when the action of telomerase is blocked proves that telomerase has a definite role in cell death and prognosis.

In order to investigate whether the expression of telomerase can be used as a prognostic factor, we studied the relationship between telomerase activity and survival in 62 patients with brain tumors.

MATERIALS AND METHODS

Patients and pathological diagnosis

Between March 1998 and December 1999, 93 consecutive patients with brain tumors were treated with surgical resection. A total of 62 of the cases with brain tumors had available surgical specimens. All tumor tissue was obtained at the time of surgery, frozen as rapidly as possible, and stored at -80℃ until subjected to assay.

The pathological types of brain tumors were classified according to World Health Organization (WHO) classification (13). They consisted of 16 astrocytic tumors, including 6 astrocytomas, 4 anaplastic astrocytomas, and 6 glioblastomas, 16 meningiomas, 12 pituitary adenomas, 9 metastatic brain tumors, 6 acoustic neurinomas, 2 pineoblastomas, and a hemangioblastoma. We also categorized the patients patho-

logically as aggressive group (group I) and non-aggressive group (group II). In the aggressive group, astrocytic tumors, pineoblastoma, and metastatic tumors were included. The remaining tumors, including pituitary adenoma, meningioma, acoustic neurinoma, and hemangioblatoma, were categorized as the non-aggressive group. Gliomas were also classified into high-grade gliomas, including anaplastic astrocytoma and glioblastoma, and low-grade glioma including astrocytoma.

After surgery, clinical follow-up data were obtained from patients. Follow-up imaging studies were requested at 1, 3, 6, and 12 months for the first year and at 6-month intervals thereafter. The follow-up period was censored on December 2003. Survival time was measured as the time from the date of the initial surgery to the date of death. Progression-free survival time was calculated from the date of the initial surgery to the onset of clinical deterioration or radiologically confirmed tumor recurrence.

Telomerase assay

As described previously, the telomerase assay was performed by a method of the telomeric repeat amplification protocol (TRAP) (8). The tumor tissues, which had been preserved at -80°C, were thawed, washed three times with phosphate-buffered saline (PBS; pH 7.4), dissected into 2-3 μL fractions with sterile scissors, and inserted into the 1.5 mL tubes. Tumor tissues were homogenized in 200 μL ice-cold CHAPS lysis buffer [10 mM Tris/HCl pH 7.5, 1 mM $MgCl_2$, 1 mM EDTA, 0.1 mM PMSF, 5 mM β-mercaptoethanol, 0.5% CHAPS, 10% glycerol], supplemented with 150 units RNasin (Promega, Madison, WI, U.S.A.), and incubated for 30 min on ice. The lysates were centrifuged at 12,000 g for 20 min at 4°C. The supernatants were rapidly frozen and stored at -80 °C. The protein concentration of the supernatant was determined with the BCA protein assay kit (Pierce, Rockford, IL, U.S.A.). Supernatant samples equivalent to 200 ng/μL of protein were assayed in 50 μL of reaction mixture comprised of 20 mM Tris/HCl pH 8.3, 1.5 mM $MgCl_2$, 63 mM KCl, 0.05% Tween 20, 1 mM EGTA, 50 μM deoxynucleoside triphosphates, 0.1 μg TS primer (5´-AATCCGTCGAGCAG-AGTT-3´), 0.1 μg TRAP primer mix, and 2 units Taq DNA polymerase (Gibco-BRL, Gaithersburg, MD, U.S.A.). After the reaction mixture was incubated for 30 min at room temperature for telomerase-mediated extension of the TS primer, it was then subjected to 36 polymerase chain reaction (PCR) cycles at 94°C for 30 sec, at 53°C for 30 sec, and at 72°C for 30 sec. The PCR products were then electrophoresed on 12.5% polyacrylamide gels at 400 V for 25 min and silver staining (Silver stain kit, Bioneer, Cheongwon, Korea) was performed. 25 bp DNA stepladders (Promega, Southampton, U.K.) were used as markers. For every reaction, telomerase-positive extracts and PCR/ELISA-positive controls were used. For each specimen, heat inactivation controls were made at 85°C for 10 min.

Statistical analysis

For statistical analysis, we used a commercially available program, SPSS version 9.0 (SPSS Inc., Chicago, IL, U.S.A.) for Windows. The Kruscal-Wallis test was used to correlate the expression of telomerase according to the pathological types of the tumor. The difference among the positive rates of telomerase activity in the different grades of glial cell tumors was analyzed by the Mann-Whitney test. Survival of the patients was plotted by the Kaplan-Meier method, and their difference was compared by the Log-rank test. A probability value of less than 0.05 was considered statistically significant.

RESULTS

Patient characteristics

We included 24 (39%) men and 38 (61%) women in the study. Their mean age was 41.9 ± 18.5 yr (range, 2-71 yr). At the time of last follow-up, 11 patients were dead and 22 patients were progressed clinically or radiologically. The median follow-up period was 26 months (range, 3-44 months).
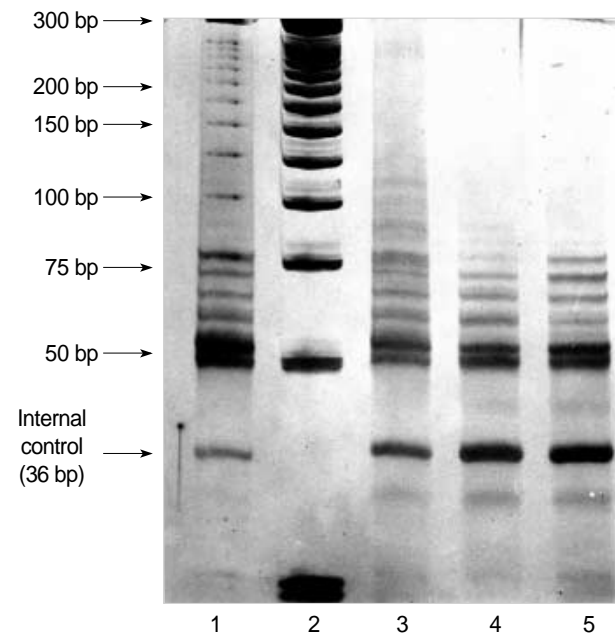

Fig. 1. TRAP assay for telomerase activity in surgical specimens of brain tumors. TS oligonucleotides were amplified by PCR, with the downstream primer CX [5´-(CCCTTA)₃ CCCTAA-3´] and the upstream primer TS, in the presence of internal TRAP assay standard. Reaction products were resolved on a 12.5% polyacrylamide gel. The positive control is shown in lane 1 and the molecular weight marker in lane 2. Telomerase activity is detected as a typical laddering pattern in glioblastomas (lane 3 and 4) and meningioma (lane 5).

Telomerase activity and pathological types

Telomerase activity was detected in 39 (63%) out of 62 brain tumors (Fig. 1). Of the 16 cases of astrocytic tumors, 10 cases (63%) showed expression of telomerase activity. Five (83%) of 6 glioblastomas, 3 (75%) of 4 anaplastic astrocytomas, and 2 (33%) of 6 astrocytomas were positive in telomerase activity. Two pineoblastomas showed telomerase activity; eight (67%) of 12 pituitary adenomas, four (67%) of six acoustic neurinomas, and 10 (63%) of 16 meningiomas showed telomerase activity, suggesting a relatively high expression rate. Nine metastatic brain tumors were found to express telomerase activity in five (56%) cases. Overall, the expression rate of telomerase activity showed no significant difference among pathological types ($p>0.05$; Table 1). However, telomerase activity of the glioma showed a definite association with the pathological grade (Fig. 2).

Correlation between telomerase activity and survival time

The median survival time of patients with and without telomerase activity was 21 and 34 months, respectively. The
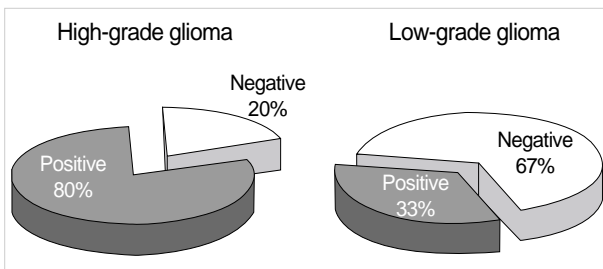

Fig. 2. Comparison of telomerase activity between high-grade and low-grade gliomas (p=0.022). Gliomas were divided into two groups, high-grade gliomas including anaplastic astrocytoma and glioblastoma, and low-grade glioma including astrocytoma.
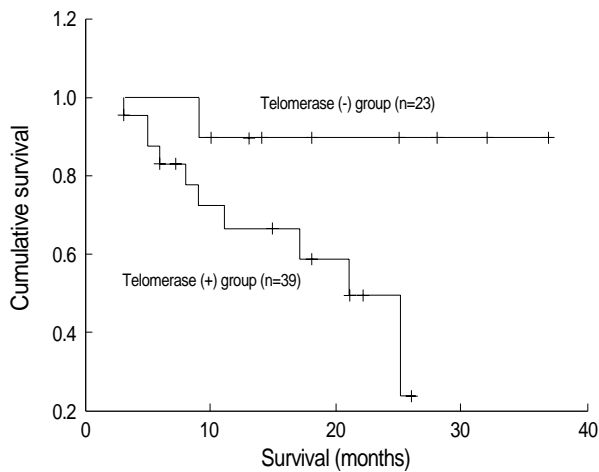
difference was statistically significant (Fig. 3). There was also a difference in the median progression-free survival time of patients with and without telomerase activity (Fig. 4). In group I, the median survival of patients with and without telomerase activity was 9 and 18 months, respectively. There was a significant difference between telomerase-positive and negative groups (Table 2).

Among group I tumors, the difference in median progression-free survival time was significant, according to the presence or absence of telomerase activity. On the other hand, telomerase activity did not affect the overall survival duration for patients with group II tumors. In the median progression-free survival of group II, there was a significant difference between telomerase-positive and negative tumors (Table 2).

Our data suggest that telomerase activity has a significant prognostic impact on both overall survival and progression-free survival for all tumors in the aggressive group. Telomerase activity does not affect overall survival in non-aggressive tumors, although it is associated with this group's progression-free survival.

Table 1. Pathological types, expression of telomerase activity, and status of 62 patients with brain tumors

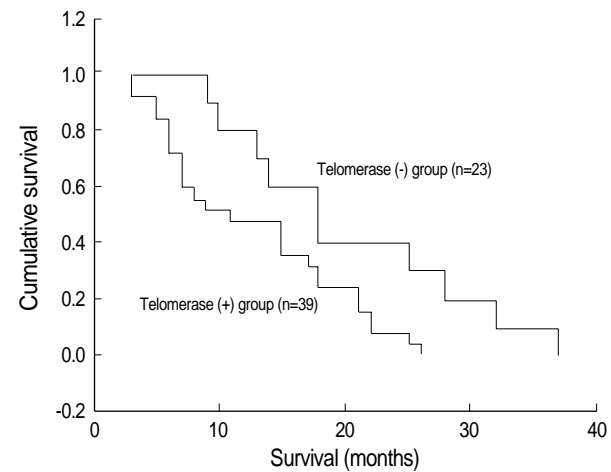| Pathological types	No. of positive expressions (%)	No. of deaths
Astrocytic tumors (n=16)	10 (63)	7
Astrocytoma (n=6)	2 (33)	2
Anaplastic astrocytoma (n=4)	3 (75)	2
Glioblastoma (n=6)	5 (83)	3
Pituitary adenoma (n=12)	8 (67)	0
Meningioma (n=16)	10 (63)	1
Acoustic neurinoma (n=6)	4 (67)	0
Pineoblastoma (n=2)	2 (100)	0
Hemangioblastoma (n=1)	0 (0)	0
Metastatic tumor (n=9)	5 (56)	3

Fig. 3. Kaplan-Meier curves demonstrating correlation of telomerase activity to the overall survival time in patients with brain tumors (p=0.0362).

Fig. 4. Kaplan-Meier curves showing the correlation of telomerase activity to the progression-free survival time in patients with brain tumors (p=0.0202).

Table 2. Overall and progression-free survival time of the patients with brain tumors

Brain tumors	No. of patients	Overall survival (months)			Progression-free survival (months)		
		Median	95% CI*	p-value	Median	95% CI*	p-value
All	62			0.0362			0.0202
Telomerase (+)	39	21	14.99±27.01		11	4.15±17.85	
Telomerase (-)	23	34	28.99±39.41		18	11.93±24.07	
Group I	25			0.0053			0.0071
Telomerase (+)	15	9	5.15±12.85		7	5.73±8.27	
Telomerase (-)	10	18	11.53±25.61		14	9.38±18.62	
Group II	37			0.2207			0.0058
Telomerase (+)	24	25	24.81±26.19		21	12.23±29.77	
Telomerase (-)	13	37	20.65±48.01		34	22.60±51.40	

*CI, confidence interval.

DISCUSSION

Telomerase consists of RNA which acts as a template for the simple base pair sequence tracts. A catalytic subunit acts as a reverse transcriptase on the RNA template. The catalytic subunit of telomerase was first identified in humans by Harrington et al. (14). Telomerase exists in its inactive state in normal somatic cells, except for adult stem cells and early embryonic cells. The complete mechanism of its activation is currently not understood. However, its activity has been found to increase in neoplastic cells, especially in tumors on the stomach, lung, brain, breast, hematopoietic system, and thyroid gland (10, 15-19). Telomerase activity was detected in 74.1% cytologic specimens and in 85.2% tissue specimens of breast cancers (16). Moreover, Poremba et al. (20) found that in neuroblastomas, telomerase activity is a powerful, independent prognostic marker. It is capable of differentiating between good and poor outcomes in putative 'favorable' clinical or biological subgroups. In neuroepithelial tumors, telomerase activity was detected in 66 (61.7%) of 107 cases. It was expressed in 20% for grade II astrocytoma, 40% for anaplastic astrocytoma, and 72.3% for glioblastomas, respectively (10). In addition, Kudoh et al. (21) found telomerase activity in 98% of glioblastomas, 86% of anaplastic astrocytomas, 54% of low-grade astrocytomas, 83% of anaplastic meningiomas, 47% of atypical meningiomas, and 7% of benign meningiomas. Telomerase activity in brain tumors including glioma, meningioma, and pituitary adenoma has been related to their prognosis (9, 11). Moreover, from the fact that telomerase activity increases in tumors with high metastatic potential (22), it is suggested that it could be used as an index in the prediction of metastasis. In our study, telomerase activity was higher in high grade gliomas than in low-grade tumors. This suggested that telomerase can not only act as a biological marker for malignant potential of the glial tumors but can also act as a significant prognostic factor. This is supported by evidence that tumors with absent or low telomerase activity hold a better prognosis. However, as we found in the present study, the differences in the activity of telomerase, according to the degree of histological malignancy, was not statistically significant. This shows that telomerase activity cannot be the sole factor in predicting the prognosis of patients with brain tumors. Therefore, preexisting prognostic factors such as age, performance status of the patient, and tumor recurrence should still be taken into account.

Telomerase-positive cells with elongated telomeres increased survival ability and resistance to apoptosis over cells with shorter telomeres. Telomerase activity and maintenance of telomere stability are associated with high resistance to apoptosis (12). It is known that the response to the treatment of epithelial cell tumors found in the head and neck decreases when the activity of telomerase is high. Also, the survival period of lung cancer patients was decreased as the activity of telomerase was increased (18, 23, 24). In the present study, the telomerase-positive tumor group attained significantly shorter overall survival and progression-free survival durations compared to the telomerase-negative tumor group. On the other hand, the overall survival duration in the non-aggressive tumor group, containing pituitary adenoma, meningioma, hemangiblastoma, and acoustic neurinoma, was not influenced by telomerase activity. However, the progression-free survival was significantly different between the telomerase-positive and negative groups. This may also suggest that telomerase activity is an important prognostic factor in the progression of brain tumors.

Since this study shows such dysmorphic results in dealing with all types of brain tumors, the prognostic significance of telomerase activity will be made clear if we provide more confident results using a pathologically homogenous type of brain tumor.

In conclusion, telomerase activity can be an important prognostic factor in predicting the progression and survival of patients with brain tumors. However, the median overall survival is not affected by telomerase activity in non-aggressive tumors. Telomerase may also play a role in the malignant progression of gliomas.

REFERENCES

1. Blackburn EH. *Structure and function of telomeres. Nature 1991; 350: 569-73.*

2. de Lange T. *Activation of telomerase in a human tumor. Proc Natl Acad Sci USA 1994; 91: 2882-5.*

3. Greider CW. *Telomerase activity, cell proliferation, and cancer. Proc Natl Acad Sci USA 1998; 95: 90-2.*

4. Harley CB, Futcher AB, Geider CW. *Telomeres shorten during ageing of human fibroblasts. Nature 1990; 345: 458-60.*

5. Harrington L, McPhail T, Mar V, Zhou W, Oulton R, Bass MB, Arruda I, Robinson MO. *A mammalian telomerase-associated protein. Science 1997; 275: 973-7.*

6. Hanh WC. *New functions for telomerase. Cancer Res Treat 2003; 35: 467-71.*

7. Greider CW, Blackburn EH. *Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell 1985; 43: 405-13.*

8. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrieh SL, Shay JW. *Specific association of human telomerase activity with immortal cells and cancer. Science 1994; 266: 2011-5.*

9. Falchetti ML, Larocca LM, Pallini R. *Telomerase in brain tumors. Childs Nerv Syst 2002; 18: 112-7.*

10. Hiraga S, Ohnishi T, Izumoto S, Miyahara E, Kanemura Y, Matsumura H, Arita N. *Telomerase activity and alterations in telomere length in human brain tumors. Cancer Res 1998; 58: 2117-25.*

11. Yoshino A, Katayama Y, Fukushima T, Watanabe T, Komine C, Yokoyama T, Kusama K, Moro I. *Telomerase activity in pituitary adenomas: significance of telomerase expression in predicting pituitary adenoma recurrence. J Neurooncol 2003; 63: 155-62.*

12. Holt SE, Glinsky VV, Ivanova AB, Glinsky GV. *Resistance to apoptosis in human cells conferred by telomerase function and telomere stability. Mol Carcinog 1999; 25: 241-8.*

13. Kepes JJ. *Review of the WHO's proposed new classification of brain tumors. Proceedings of the XIth International Congress of Neuropathology, Kyoto, September 2-8, 1990. Japanese Society of Neuropathology, Kyoto, Japan.*

14. Harrington L, Zhou W, McPhail T, Oulton R, Yeung DS, Mar V, Bass MB, Robinson MO. *Human telomerase contains evolutionarily conserved catalytic and structural subunits. Genes Dev 1997; 11: 3109-15.*

15. Harada K, Kurisu K, Arita K, Sadatomo T, Tahara H, Tahara E, Ide T, Uozumi T. *Telomerase activity in central nervous system malignant lymphoma. Cancer 1999; 86: 1050-5.*

16. Park WC, Jung SS, Kim IC. *Diagnostic significance of telomerase activity in breast cancer. J Korean Surg Soc 2000; 58: 767-74.*

17. Lee HJ, Myung SJ, Park YH, Cho YK, Jung HY, Lee GH, Hong WS, Yang SK, Kim JH, Min YI. *Measurement of telomerase activity and telomerase reverse transcriptase expression in gastric fluid and tissue for early diagnosis of stomach cancer. Korean J Gatroenterol 2003; 42: 183-9.*

18. Wu TC, Lin P, Hsu CP, Huang YJ, Chen CY, Chung WC, Lee H, Ko JL. *Loss of telomerase activity may be a potential favorable prognostic marker in lung carcinoma. Lung Cancer 2003; 41: 163-9.*

19. Okayasu I, Mitomi H, Yamashita K, Mikami T, Fujiwara M, Kato M, Oshimura M. *Telomerase activity significantly correlates with cell differentiation, proliferation and lymph node metastasis in colorectal carcinoma. J Cancer Res Clin Oncol 1998; 124: 444-9.*

20. Poremba C, Hero B, Heine B, Scheel C, Schaefer KL, Christiansen H, Berthold F, Kneif S, Stein H, Juergens H, Boecker W, Dockhorn-Dworniczak B. *Telomerase is a strong indicator for assessing the proneness to progression in neuroblastomas. Med Pediatr Oncol 2000; 35: 651-5.*

21. Kudoh C, Sugiura K, Hisatomi H, Detta A, Yeh J. D*etection of telomerase RNA component and telomerase activity in human brain tumors. 49th Annual Meeting of Congress of Neurological Surgeons, Boston, USA, 1999, Abstract p645.*

22. Kleinschmidt-DeMasters BK, Shroyer AL, Hashizumi TL, Evans LC, Markham N, Kindt G, Shroyer KR. *Part I. Telomerase levels in human metastatic brain tumors four-fold logarithmic variability but no correlation with tumor type or interval to patient demise. J Neurol Sci 1998; 161: 116-23.*

23. Liao CT, Tung-Chieh Chang J, Wang HM, Chen IH, Lin CY, Chen TM, Hsieh LL, Cheng AJ. *Telomerase as an independent prognostic factor in head and neck squamous cell carcinoma. Head Neck 2004; 26: 504-12.*

24. Koscielny S, Dahse R, Sonntag J, Riese U, Theuer C, Hoffman ME, von Eggeling F, Claussen U, Beleites E, Ernst G, Fiedler W. *Clinical implications of telomerase activity and inactivation of the tumor suppressor gene p16 (CDKN2A) in head and neck cancer. Otolaryngol Pol 2000; 54: 291-5.*