Telomerase as a therapeutic target for malignant gliomas

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Telomerase, a ribonucleoprotein enzyme, is considered as a potential target of cancer therapy because of its preferential expression in tumors. In particular, malignant gliomas are one of the best candidates for telomerase-targeted therapy. It is because malignant gliomas are predominantly telomerase-positive, while normal brain tissues do not express telomerase. In theory, there are two telomerase-associated therapeutic approaches for telomerase-positive tumors. One approach is the anti-telomerase cancer therapy to directly inhibit telomerase activity, resulting in apoptotic cell death or growth arrest. Two major components of the telomerase holoenzyme complex, the RNA template (hTER) and catalytic subunit (reverse transcriptase, hTERT) are well considered as therapeutic targets. The other approach is the telomerase-specific cancer therapy by targeting telomerase-expressing tumor cells as a means to directly kill the cells. Strategies using the transfer of therapeutic gene under the hTERT promoter system as well as immunotherapy directed against telomerase-positive cells are generally included. These telomerase-associated therapies are very promising for the treatment of malignant gliomas.

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Introduction

Gliomas are histologically classified into four grades (Grades I, II, III, and IV of World Health Organization). Grades I and II indicate low-grade glioma, and Grades III (anaplastic astrocytoma, AA) and IV (glioblastoma multiforme, GBM) are malignant glioma (Burger *et al.*, 1985). Surgery, radiotherapy, chemotherapy, and immunotherapy are currently representative treatment modalities for patients with gliomas (Davis *et al.*, 1999). Advances in these therapies extended the prognosis for patients with low-grade glioma from 5 years to 10 years over the time period of 1984–1995 (Piepmeyer and Christopher, 1997). The most malignant type, GBM represents 29% of all

primary brain tumors or 5000 new cases per year in the United States (Mahaley *et al.*, 1989). The prognosis for patients with GBM is very poor, with a median survival rate of less than a year and a 5-year survival rate of 5% or less (Schoenberg, 1983; Mahaley *et al.*, 1989; Deen *et al.*, 1993). No therapeutic modality has substantially changed the outcome of patients with GBM (Mahaley *et al.*, 1989; Deen *et al.*, 1993). Therefore, in order to improve the prognosis in this invariably fatal disease, it is imperative to explore novel therapeutic modalities.

Telomerase in malignant gliomas

Telomerase, a ribonucleic acid-protein complex, adds hexameric repeats of 5'-TTAGGG-3' to the ends of telomeres to compensate for the progressive loss (Blackburn, 1991; Counter *et al.*, 1992; de Lange, 1994). Telomerase activity, which is generally undetectable in normal somatic cells, is expressed in approximately 90% of tumors (Kim *et al.*, 1994; Broccoli *et al.*, 1995; Hiyama *et al.*, 1995). In malignant gliomas, telomerase activity is very often detected (Table 1) (Langford *et al.*, 1995; DeMasters *et al.*, 1997; Sallinen *et al.*, 1997; Sano *et al.*, 1998; Kleinschmidt-DeMasters *et al.*, 1998; Chong *et al.*, 1998; Hiraga *et al.*, 1998; Le *et al.*, 1998; Weil *et al.*, 1999; Carroll *et al.*, 1999; Falchetti *et al.*, 1999; Huang *et al.*, 1999; Kleinschmidt-DeMasters *et al.*, 2000; Harada *et al.*, 2000). In Grade I or II of gliomas, telomerase activity is detected in 0% or 0 to 33%, respectively. In malignant gliomas, telomerase is positive in 10 to 100% of anaplastic astrocytomas and in 26 to 100% of GBM. Although the ratio of telomerase-positive cells in gliomas varies, most of the reports show that the incidence of telomerase expression is closely correlated with the malignancy, and telomerase is detected in the vast majority of malignant gliomas. By contrast, normal brain tissues do not express telomerase activity (DeMasters *et al.*, 1997; Le *et al.*, 1998; Huang *et al.*, 1999; Harada *et al.*, 2000), while telomerase is detectable only in human embryonic neural precursor cells at low levels (Ostenfeld *et al.*, 2000). Therefore, telomerase is expected to represent a very good candidate not only for the useful prognostic and diagnostic marker of malignant gliomas, but also for the targeted therapy of malignant gliomas. There are theoretically two approaches as telomerase-asso-

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Table 1 Ratio of telomerase-positive tumors in malignant gliomas histologically classified

Normal brain tissues (%)	Grade I (%)	Grade II (%)	Grade III (AA) (%)	Grade IV (GBM) (%)	References
0/8 (0)		2/20 (10) 0/2 (0) 1/1 (100) 0/8 (0)	38/51 (75) 2/8 (25) 0/3 (0) 2/3 (67)	10/19 (53) 4/6 (67) 3/6 (50) 11/11 (100)	Langford <i>et al.</i> , 1995 DeMasters <i>et al.</i> , 1997 Sallinen <i>et al.</i> , 1997 Sano <i>et al.</i> , 1998 Kleinschmidt-Demasters <i>et al.</i> , 1998
	0/16 (100) 0/3 (0)	3/13 (23) 3/15 (20) 3/9 (33)	3/13 (23) 6/15 (40) 5/11 (45) 3/3 (100)	10/38 (26) 34/47 (72) 36/41 (88) 3/3 (100)	Chong <i>et al.</i> , 1998 Hiraga <i>et al.</i> , 1998 Le <i>et al.</i> , 1998 Weil <i>et al.</i> , 1999
0/4 (0)		0/4 (0) 0/4 (0)	3/11 (27)	7/25 (28) 25/30 (83)	Carroll <i>et al.</i> , 1999 Falchetti <i>et al.</i> , 1999
0/2 (0)		10/12 (83)	10/10 (100) 1/1 (100)	11/11 (100) 16/18 (89)	Huang <i>et al.</i> , 1999 Kleinschmidt-Demasters <i>et al.</i> , 2000
0/1 (0)				26/42 (62)	Harada <i>et al.</i> , 2000

World Health Organization Grade. Low grade astrocytoma (Grade I or II). AA, anaplastic astrocytoma (Grade III). GBM, glioblastoma multiforme (Grade IV)

ciated therapy for malignant gliomas. One is the anti-telomerase cancer therapy to directly inhibit telomerase activity, resulting in induction of cell death or growth arrest. The other is the telomerase-specific cancer therapy by targeting telomerase-positive cells as a means to directly kill the cells.

Anti-telomerase cancer therapy

Telomerase has two major components and several associated proteins that are essential for proper function (Feng *et al.*, 1995; Harrington *et al.*, 1997; Meyerson *et al.*, 1997; Nakayama *et al.*, 1997; Nakamura *et al.*, 1997). The first major component is the human telomerase RNA (hTER) to serve as the template on which telomeric repeats are added to the ends of the chromosomes (Feng *et al.*, 1995). The second major component is the human telomerase reverse transcriptase (hTERT) catalytic subunit (Meyerson *et al.*, 1997; Nakamura *et al.*, 1997). The ability of cells to express telomerase activity is predominantly regulated at the transcriptional level of hTERT in cells (Takakura *et al.*, 1999; Cong *et al.*, 1999). The hTER and hTERT have been mainly used as therapeutic targets so far (Table 2).

Construct expressing an antisense transcript

The hTER is divided into several domains that are essential for normal enzymatic function and assembly of the telomerase enzyme (Betts *et al.*, 1995; Gilley and Blackburn, 1996; Tzfati *et al.*, 2000). Feng *et al.* (1995) demonstrated that introduction of a construct expressing an antisense transcript to the first 185 nucleotides of the RNA induced cell death in HeLa cells after 23–26 doublings. The clones that underwent crisis had reduced telomerase activity and shortened telomeric DNA. Using the same construct, malignant glioma U251-MG cells underwent apoptosis or differentiation after 30 doublings following telomerase inhibition (Kondo *et al.*, 1998a). These findings indicate that inhibition of telomerase function induced antitumor effect.

2-5A antisense system

A 5'-phosphorylated, 2', 5'-linked oligoadenylate (2–5A) is required to activate the catalytic activity of RNaseL (Silverman, 1997). 2–5A-antisense chimeras contain 2–5A covalently attached through butanediol phosphate linkers to antisense oligonucleotide. In nature, RNaseL functions as a component of the 2–5A system, implicated in the antiviral activity of interferon (IFN) against picornavirus, vaccinia virus, and reovirus (Silverman, 1997). In the 2–5A-antisense approach, RNaseL is converted to a highly specific endoribonuclease capable of selectively cleaving individual RNA targets. When applied to HeLa cells, 2–5A-antisense resulted in the ablation of PKR mRNA and enzymatic activity effectively blocking dsRNA activation of transcription factor NF- κ B (Maran *et al.*, 1994). Because RNaseL is present in most mammalian cells, it is clear that the application of this technology is a powerful method for controlling gene expression. Recently, 2–5A antisense strategy has been applied to degrade hTER specifically and effectively (Kondo *et al.*, 1998b). An antisense oligonucleotide against hTER linked to 2–5A (2–5A-anti-hTER) in the presence of cationic liposome reduced the viability of human malignant glioma cells to 20–43% within 4 days through induction of caspase-dependent apoptosis (Mukai *et al.*, 2000; Komata *et al.*, 2000). Similar antitumor effects of 2–5A-anti-hTER were demonstrated in prostate cancer, ovarian cancer, and bladder cancer cells (Kondo *et al.*, 2000; Kushner *et al.*, 2000; Koga *et al.*, 2001a). In contrast, cultured astrocytes, endothelial cells, or fibroblasts lacking telomerase were resistant to the 2–5A-anti-hTER treatment. Furthermore, treatment of subcutaneous or intracranial injections in nude mice with the frequent intratumoral injections of 2–5A-anti-hTER was therapeutically effective (Kondo *et al.*, 1998b; Mukai *et al.*, 2000; Koga *et al.*, 2001a). 2–5A-anti-hTER also enhanced the *in vitro* and *in vivo* antitumor effect of cisplatin or the recombinant adenovirus bearing the tumor suppressor p53 gene (Ad5CMV-p53) on malignant glioma

Table 2 Components of telomerase as a target of anti-telomerase cancer therapy

Components	References
RNA component of telomerase (hTER)	
Construct expressing an antisense transcript	Feng <i>et al.</i> , 1995; Kondo <i>et al.</i> , 1998a;
2-5A antisense system	Kondo <i>et al.</i> , 1998b, 2000, 2001; Komata <i>et al.</i> , 2000;
	Kushner <i>et al.</i> , 2000; Mukai <i>et al.</i> , 2000; Koga <i>et al.</i> , 2001a
Hammerhead ribozymes	Mukai <i>et al.</i> , 2000; Yokoyama <i>et al.</i> , 1998
Peptide nucleic acids	Norton <i>et al.</i> , 1996; Shammas <i>et al.</i> , 1999
2'-O-MeRNA oligomers	Agarwal <i>et al.</i> , 1997; Pitts <i>et al.</i> , 1998; Herbert <i>et al.</i> , 1999
Telomerase catalytic subunit (reverse transcriptase, hTERT)	
Dominant negative hTERT	Hahn <i>et al.</i> , 1999; Zhang <i>et al.</i> , 1999
Reverse transcriptase inhibitors	Strahl and Blackburn, 1994, 1996; Melana <i>et al.</i> , 1998;
	Multani <i>et al.</i> , 1998; Tejera <i>et al.</i> , 2001
Hammerhead ribozymes	Yokoyama <i>et al.</i> , 2000; Ludwig <i>et al.</i> , 2001

cells (Komata *et al.*, 2000; Kondo *et al.*, 2001). Therefore, targeting telomerase RNA with 2-5A-anti-hTER is expected to represent an effective and novel approach for the treatment of telomerase-positive tumors including malignant gliomas as a single or combined regimen. However, the effect of 2-5A-anti-hTER is telomere length-independent (unpublished data) and is contrary to the widely perceived need for an extensive lag phase (dependent on telomere length) prior to the induction of cellular senescence or apoptosis. Therefore, the precise molecular mechanisms underlying the antitumor effect of 2-5A-anti-hTER need to be determined.

Hammerhead ribozymes

Ribozymes are catalytic antisense RNAs that cleave RNA substrates in a sequence-specific manner (Cech *et al.*, 1981). The hammerhead-type ribozyme was originally discovered in satellite RNA of tobacco ringspot virus (Buzayan *et al.*, 1986). Recent investigations reported that a hammerhead ribozyme targeting hTER reduced telomerase activity against tumor cells (Kana-zawa *et al.*, 1996; Yokoyama *et al.*, 1998), although no change in proliferation rate was observed. Furthermore, a hammerhead ribozyme cleaves hTERT mRNA and inhibits telomerase activity (Yokoyama *et al.*, 2000; Ludwig *et al.*, 2001). Ribozyme cleavage of hTERT mRNA not only led to shortened telomere length and induction of apoptosis, but also sensitized tumor cells to inhibitors of topoisomerase (Ludwig *et al.*, 2001). These results demonstrate a hammerhead ribozyme approach for anti-telomerase cancer therapy and suggest the possibility that it might be combined advantageously with topoisomerase-directed chemotherapy.

Peptide nucleic acids

The major problems with the oligonucleotide approach are that of stability and uptake common to oligonucleotide therapeutics. Peptide nucleic acids (PNAs) are modified oligonucleotides that contain a non-ionic backbone in which the deoxyribose linkages have been replaced by N-(2-amino-ethyl) glycine units, making them resistant to degradation by endo- and exonucleases. Telomerase activity was inhibited in cells in

culture by PNAs complementary to the hTER (Norton *et al.*, 1996). When PNAs against hTER were co-electroporated into immortal human cells, the cells showed reduced telomerase activity, shortened telomeres, reduced colony size, and arrested cell proliferation after a lag period of 5-30 cell generations, consistent with suppression of their immortality (Shammas *et al.*, 1999). Because the membrane permeability of PNAs is not high, strategies to facilitate *in vivo* uptake are expected.

2'-O-meRNA oligomers

2'-O-methyl-RNA (2'-O-meRNA) oligomers bind more tightly to complementary RNA sequences than do analogous DNA oligomers and have improved resistance to degradation by nucleases, reducing the need for phosphorothioate linkages and improving the selectivity of antisense effects (Agarwal *et al.*, 1997). 2'-O-meRNA oligomers inhibited telomerase in prostate cancer cells with potencies superior to those possessed by analogous peptide nucleic acids (PNAs) (Pitts and Corey, 1998). Additionally, 2'-O-meRNA oligomers inhibited telomerase, leading to progressive telomere shortening and causing immortal human breast epithelial cells to undergo apoptosis with increasing frequency until no cells remain (Herbert *et al.*, 1999). These results indicate the feasibility of using 2'-O-meRNA oligomers for the treatment of telomerase-positive tumors.

Dominant-negative hTERT

Recently, two groups have reported that direct inhibition of hTERT was achieved by introduction of dominant-negative versions of hTERT that are mutated at critical amino acid residues within the catalytic domain (Hahn *et al.*, 1999; Zhang *et al.*, 1999). When introduced into tumorigenic human cell lines, these mutants virtually abolished telomerase activity, presumably by competing for binding to other components of the telomerase holoenzyme complex that are essential for catalysis. Loss of telomerase activity led to a reduction in telomere length and an induction of apoptosis that occurred only when telomeres became critically short. Hahn *et al.* (1999) have also noted that

Table 3 Telomerase-specific cancer therapy

System	References
hTERT promoter	
caspase-8	Koga <i>et al.</i> , 2000
bax	Gu <i>et al.</i> , 2000
diphtheria toxin A	Abdul-Ghani <i>et al.</i> , 2000
TK	Majumdar <i>et al.</i> , 2001
rev-caspase-6	Komata <i>et al.</i> , 2001
FADD	Koga <i>et al.</i> , 2001b
Immunotherapy	
hTERT-derived peptides	Vonderheide <i>et al.</i> , 1999; Minev <i>et al.</i> , 2000; Arai <i>et al.</i> , 2001
TERT RNA-transfected dendritic cells	Nair <i>et al.</i> , 2000

telomerase-inhibited human cell lines are unable to form tumors when injected into immunodeficient nude mice. The application of dominant-negative hTERT is promising to inhibit telomerase activity.

Reverse transcriptase inhibitors

Because the hTERT protein is a reverse transcriptase, several reverse transcriptase inhibitors have been tested as telomerase inhibitors (Strahl and Blackburn, 1996). Nucleotide analogs such as 3'-azido-3'-deoxythymidine (AZT) can be preferentially incorporated into DNA and result in chain termination. AZT has been the most extensively studied among reverse transcriptase inhibitors and has been shown to cause telomere shortening in *Tetrahymena* (Strahl and Blackburn, 1994). AZT inhibited telomerase activity and suppressed the growth of breast cancer cells (Melana *et al.*, 1998). Additionally, AZT induced a rapid telomere shortening in melanoma or breast cancer cells (Multani *et al.*, 1998). Interestingly, AZT-treated tumor cells have a reduced tumorigenicity in syngeneic BALB/c mice (Tejera *et al.*, 2001). Tumor incidence was reduced and survival was prolonged in animals inoculated with AZT-treated cells when comparing with control counterparts. The number and size of spontaneous metastases were also decreased in animals inoculated with AZT-treated cells. Further investigation is needed to produce more potent telomere-targeting reverse transcriptase inhibitors with tolerable toxicity profiles.

Potential problems with anti-telomerase cancer therapy

There are several considerations about anti-telomerase cancer therapy. First, induction of apoptosis or growth arrest is considered to take place when telomeres shorten to a certain critical length. Therefore, there will be an expected lag phase between when telomerase is inhibited and the time when the telomeric DNA of tumor cells shorten sufficiently to produce detrimental effects on growth. This lag phase is about 30 doublings or one month in malignant glioma U251-MG cells (Kondo *et al.*, 1998a), indicating that the antitumor effects may not occur until one month after telomerase activity is inhibited. Therefore, it is clear that simply inhibiting telomerase may not result in the anticancer

effects that were originally hypothesized when tumors with long telomeres are treated. Because 2–5A-anti-hTER functions in a telomere length-independent manner, its application is expected to abolish the time lag. Recently, it has been shown that some tumor cells undergo p53- and the ataxia telangiectasia mutated (ATM) kinase-dependent apoptosis 48 h after inhibition of the telomeric-repeat binding factor 2 (TRF2) (Karslender *et al.*, 1999). It indicates that telomeres lacking TRF2 directly signal apoptosis, possibly because they resemble damaged DNA. These telomere length-independent approaches are promising to treat tumors with long telomeres. Second, tumor cells may become resistant to telomerase inhibitors or acquire alternative mechanisms of telomere maintenance independent of telomerase during the treatment. Lastly, it is likely that inhibitors of telomerase may damage normal cells expressing telomerase such as hematopoietic stem cells, germ cells or activated T cells (Chiu *et al.*, 1996; Weng *et al.*, 1996; Wright *et al.*, 1996; Ramakrishnan *et al.*, 1998). However, these side effects might be minor because stem cells of renewal tissues typically have longer telomeres than tumor cells have and the stem cells only proliferate intermittently and their telomerase activity is negligible during quiescence (White *et al.*, 2001).

Telomerase-specific cancer therapy

Recently, there have been reported two types of exciting investigations by targeting telomerase-positive cells as a means to directly kill the cells (Table 3). First, the promoter of hTERT gene is used to restrict the expression of suicide gene or toxin to telomerase-positive tumor cells (Koga *et al.*, 2000, 2001b; Gu *et al.*, 2000; Abdul-Ghani *et al.*, 2000; Majumdar *et al.*, 2001; Komata *et al.*, 2001). Second, immunotherapy directed against telomerase-positive cells is developed (Vonderheide *et al.*, 1999; Minev *et al.*, 2000; Nair *et al.*, 2000; Arai *et al.*, 2001). Using hTERT-derived peptides or hTERT RNA, cytotoxic T cell responses and tumor immunity are elicited. Because normal cells lacking telomerase activity are kept intact, these approaches are expected as a promising targeting approach for the treatment of tumors with telomerase activity.

Figure 1 Schematic model of telomerase-associated therapies for malignant gliomas

hTERT promoter system

Utilization of a promoter that is predominantly active in tumor cells would be an ideal system to restrict the therapeutic gene expression. Telomerase is a particularly attractive target for specifying tumors, because approximately 90% of tumors have telomerase activity, whereas most normal cells do not express telomerase (Kim *et al.*, 1994; Broccoli *et al.*, 1995; Hiyama *et al.*, 1995). In human cells, telomerase activity is regulated by transcriptional control of the hTERT gene whose product is the catalytic subunit of the enzyme (Meyerson *et al.*, 1997; Nakamura *et al.*, 1997). Certainly, the transcriptional activity of the hTERT gene promoter is significantly higher in telomerase-positive cells than in telomerase-negative cells (Takakura *et al.*, 1999; Cong *et al.*, 1999). Therefore, the feasibility of using the hTERT promoter to induce tumor-specific transgene expression in cancer gene therapy has been expected. Recent investigations have demonstrated that, by using the hTERT promoter-driven expression vector of therapeutic gene such as caspase-8, bax, diphtheria toxin A, thymidine kinase, rev-caspase-6, or FADD, the cytotoxic effect is restricted to telomerase-positive tumor cells of wide range (Koga *et al.*, 2000, 2001b; Gu *et al.*, 2000; Abdu-Ghani *et al.*, 2000; Majumdar *et al.*, 2001; Komata *et al.*, 2001). These results suggest that the hTERT

promoter is a novel and promising tumor-selective promoter with potential application in targeted cancer gene therapy, although telomerase-positive normal cells may be damaged by the treatment.

Immunotherapy

If tumor-associated antigens that are universal to tumors of wide range are discovered, these antigens will be used to elicit effective immuno-response against the antigens-positive tumors. Telomerase or hTERT is considered a potential target for cancer immunotherapy because it is preferentially expressed in tumor cells, indicating it as the truly universal tumor antigen. In human systems, CD8⁺ cytotoxic T lymphocyte (CTL) specific for hTERT peptides and restricted to MHC HLA-A2 lysed hTERT⁺ tumors from multiple histologies (Vonderheide *et al.*, 1999; Minev *et al.*, 2000). This CTL response was not shown in telomerase-negative cells, cells negative for the HLA-A2 (Vonderheide *et al.*, 1999), or telomerase-positive CD34⁺ hematopoietic cells (Minev *et al.*, 2000). Additionally, the CD8⁺ CTL clones specific for hTERT-derived peptides carrying motifs for HLA-A24 exerted cytotoxicity against leukemia cells in an HLA-A24-restricted manner (Arai *et al.*, 2001). Furthermore, it has been demonstrated that induction of CTL responses and tumor immunity against unrelated tumors using TERT

RNA-transfected dendritic cells (Nair *et al.*, 2000). These findings suggest hTERT as a potentially important and widely applicable target for immunotherapy, although we need to investigate whether telomerase-positive normal cells will be affected by the CTL response.

Potential problems with telomerase-specific cancer therapy

Telomerase-specific cancer therapy is to kill telomerase-expressing cells. This approach has the advantage of abolishing the time lag that is required with the vast majority of anti-telomerase cancer therapy. If the hTERT promoter system is systemically given, telomerase-positive normal cells such as stem cells or germline cells will undergo cell death. To increase the safety of this modality, local delivery may be a reasonable approach. It is also possible that similar side effects will occur in immunotherapy. Recently, it has been shown that immunization with tumor RNA was generally more effective than immunization with TERT RNA (Nair *et al.*, 2000; Heiser *et al.*, 2001). Therefore, an optimal immunization protocol may have to include TERT as well as additional tumor antigens.

Future prospects for telomerase-associated treatment of malignant gliomas

Because malignant gliomas are predominantly telomerase-positive in the central nervous system (CNS), they might be one of the best candidates for anti-telomerase or telomerase-specific cancer therapy. As shown in Figure 1, these approaches will display low toxicity and few side effects for patients with malignant gliomas if locally administered. It is because normal brain tissues are generally telomerase-negative and local administration of reagents will permit us to save extracranial tissues expressing telomerase from toxicity or side effects. With regard to the anti-telomerase cancer therapy, it is essential to keep the concentration of

reagents of this therapy above a certain level in malignant gliomas. The application of pump systems such as osmotic minipumps combined with stereotaxic techniques (Zhu *et al.*, 1996) will allow continuous delivery of therapeutic materials into the tumors. Additionally, the use of injectable and biodegradable sustained-release formulations such as polymer or microspheres (Johnson *et al.*, 1997; Putney and Burke, 1998) may be promising. As for the telomerase-specific cancer therapy, local injection of hTERT promoter system carrying therapeutic genes using pumps or biodegradable materials is a reasonable approach as anti-telomerase cancer therapy. To treat malignant gliomas with immunotherapy against telomerase-positive cells, we need to determine whether telomerase-positive stem cells or germline cells are affected by the CTL response. Recent insights into the immunobiology of the CNS and malignant gliomas suggest that difficulties in immunotherapy may be mainly due to the following factors (Roth and Weller, 1999). (1) Presence of the blood-brain barrier (BBB) that functions to exclude elements of the immune system from the tumor or brain parenchyma, although it is partially disrupted by the tumor. (2) Lack of organized secondary lymphatic tissues supporting efficient immune response locally in the CNS. (3) Low level in MHC expression in the CNS. (4) An apparent paucity of the most efficient antigen-presenting cells (APCs). And (5) malignant glioma-derived immunosuppressive factors, such as transforming growth factor (TGF)- β , that interfere with the induction of local as well as systemic immune responses to the tumor. Recognition of these factors will permit us to establish novel immunotherapeutic approaches including hTERT-based immunotherapy.

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