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13

Development of Oncolytic Replication-Competent Herpes Simplex Virus Vectors

The G207 Paradigm

Tomoki Todo and Samuel D. Rabkin

1. INTRODUCTION

Oncolytic virus therapy is a promising new strategy for treating cancer that involves replication-competent virus vectors that can replicate *in situ* in tumor cells, exhibit oncolytic activity by direct cytocidal effects, and then spread throughout the tumor. In addition, replication-competent virus vectors are capable of transferring and expressing foreign genes in host cells. These virus vectors are either genetically engineered (e.g., herpes simplex virus type 1 [HSV-1], adenovirus, vaccinia virus), naturally attenuated (e.g., Newcastle disease virus), or nonpathogenic in humans (e.g., reovirus), so they replicate selectively in tumor cells, but do not harm normal tissues *(1)*.

HSV-1 in particular has many features that make it attractive for cancer therapy *(2)*: (1) HSV-1 infects most tumor cell types; (2) its life cycle is well studied *(3)*; (3) the HSV-1 genome has been sequenced; (4) the functions of the majority of genes have been identified *(4)*; (5) genes can be manipulated; and (6) the large size of the genome (153 kb) provides space for insertion of large amounts of deoxyribonucleic acid (DNA) *(4)*. Furthermore, HSV-1 has the following features that are well suited for clinical application: (1) total tumor cell killing in vitro can be achieved at a relatively low multiplicity of infection (MOI); (2) antiviral drugs are available that enable optional termination of the therapy *(5)*; (3) animal models are available for preclinical evaluation of safety and efficacy; (4) the viral genome does not integrate into the host cell genome; and (5) it can exist in a latent state without causing detectable damage to the infected cell *(6)*. HSV-1 is a neurotropic virus, and many of the genes necessary for neuropathogenicity are nonessential and can be mutated *(7)*. Therefore, the use of HSV-1 is especially advantageous for brain tumor therapy.

Research on oncolytic HSV-1 therapy has advanced rapidly from a basic concept to clinical studies. In the early days, replication-competent HSV-1 vectors were genetically engineered to have mutations in one nonessential gene associated with either virulence or viral DNA synthesis to restrict viral replication to transformed cells *(2,8)*. These so-called first-generation vectors demonstrated that HSV-1 vectors could in fact efficiently inhibit the growth of tumors without lethally harming the host animal. They also showed that oncolytic HSV-1 therapy could be applied not only to brain tumors, but also to a broad range of solid tumors *(9)*. There were concerns, however, regarding the use of these first-generation vectors in humans because their pathogenicity may not have been sufficiently attenuated, and a single mutation could potentially revert to wild type. To address these concerns, so-called second-generation vectors were developed that had genetically engineered mutations in two different genes.

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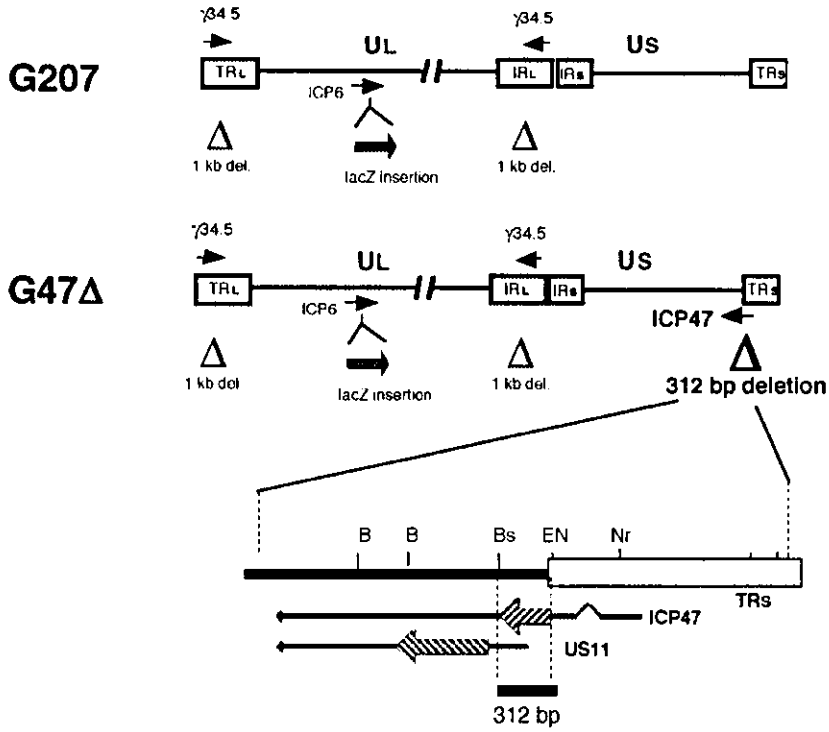

Fig. 1. Structures of G207 and G47Δ. The HSV-1 genome consists of long and short unique regions U_L and U_S, respectively, each bounded by terminal (T) and internal (I) repeat regions R_L and R_S, respectively. G207 was engineered from wild-type HSV-1 strain F by deleting 1 kb within both copies of the *γ34.5* gene and inserting the *E. coli lacZ* gene into the ICP6 coding region. G47Δ was derived from G207 by deleting 312 bp from the ICP47 locus. Because of the overlapping 3' coterminal transcripts of US11 and ICP47, the deletion also places the late gene US11 under control of the ICP47 immediate-early promoter. The ICP47 transcript contains an intron (indicated by Λ). Restriction site abbreviations: B, BamHI; Bs, BstEII; E, EcoRI, EN, EcoNI, Nr, NruI. (Modified from ref. *80*.)

2. G207

G207 was the first of the second-generation HSV-1 vectors *(10)*. It was originally designed for clinical application in patients with brain tumors, with an emphasis on employing ample safeguards. G207 has deletions in both copies of the γ34.5 gene (Fig. 1), the major determinant of HSV-1 neurovirulence *(11)*. The *γ34.5*-deficient HSV-1 vectors are considerably attenuated in normal cells, but retain their ability to replicate in neoplastic cells *(9)*.

In normal cells, HSV-1 infection induces activation of double-stranded RNA-dependent protein kinase R (PKR), which in turn leads to phosphorylation of the α-subunit of eukaryotic initiation factor 2α (eIF-2α) and a subsequent shutdown of host and viral protein synthesis *(12)*. The product of the *γ34.5* gene antagonizes this PKR activity. However, in tumor cells with an activated Ras signaling pathway, it has been suggested that PKR activity is already inhibited, thereby allowing *γ34.5*-deficient HSV-1 vectors to replicate *(13,14)*. Many of the oncolytic HSV-1 vectors currently used have deletions in the *γ34.5* gene *(8)*, including R3616 *(11)*, the parent of G207, and 1716 *(15)*.

G207 also has an insertion of the *Escherichia coli lacZ* gene in the infected-cell protein 6 (ICP6) coding region (UL39), inactivating ribonucleotide reductase, a key enzyme for viral DNA synthesis in

nondividing cells, but not in dividing cells *(16)*. This double mutation greatly minimizes the chances of G207 reverting to wild type or a pathogenic phenotype. It also confers favorable properties on the virus for treating human cancers: G207 replicates preferentially in tumor cells and is harmless in normal tissue because of attenuated virulence, G207 is about 10-fold more sensitive to ganciclovir/acyclovir than its parent virus R3616, and the reporter gene *lacZ* allows easy histochemical detection of G207-infected cells *(10)*. 3616UB is a similar, second-generation vector except uracil DNA glycosylase was inactivated instead of ICP6 *(17)*.

2.1. *Antitumor Efficacy*

G207 has been tested in more than 60 different cell lines, which revealed that the vast majority, although not all, of human tumor cell lines are susceptible to G207 infection and replication *(18)*. In human glioma and malignant meningioma cell lines, for example, G207 can achieve destruction of the entire cell population in culture within 2 to 6 days at an MOI of 0.1 *(10,19)*. In contrast, at the same MOI, G207 manifests no effect on primary cultures of rat cortical astrocytes or cerebellar neurons *(10)*.

This difference in G207 cytopathic effect observed in vitro between tumor cells and normal cells is directly reflected in the results of in vivo studies. In athymic mice harboring U87MG glioma or F5 malignant meningioma tumors intracranially or subcutaneously, a single intraneoplastic inoculation of G207 significantly inhibited tumor growth and prolonged animal survival *(10,19)*. Prominent *lacZ* expression from G207 replication within tumors could still be observed 24 days postinoculation *(19)*.

Besides brain tumors, G207 has proven efficacious in a variety of other animal tumor models in which human, mouse, rat, or hamster tumors have been generated subcutaneously or in various organs, including the liver, peritoneum, sciatic nerve, urinary bladder, and cheek pouch *(18)*.

In addition to direct intratumoral inoculation, G207 has been successfully administered intravenously *(20–22)*, via portal vein *(23)*, intraarterially *(24)*, and intraperitoneally *(25,26)*.

2.2. *Safety*

Because HSV-1 is the most common viral cause of fatal encephalitis *(27)* and G207 was the first replication-competent HSV-1 vector, along with 1716 *(28)*, to be used in human brains, it was extensively evaluated for its toxicity in the brain. In BALB/c mice, the highest dose of G207 (10^7 plaque forming units [pfu]) caused no symptoms for over 20 weeks when inoculated intracerebrally or intraventricularly *(29)*. In A/J mice, one of the most susceptible mouse strains to HSV-1 infection *(30)*, intracerebral inoculation of clinical-grade G207 at 2×10^6 pfu caused only a temporary and slight hunching in 2/8 mice *(31)*. Furthermore, in BALB/c mice that survived an intracerebral inoculation of wild-type HSV-1 (strain KOS) at an LD_{50} dose (~10^3 pfu), a subsequent challenge with an intracerebral inoculation of G207 (10^7 pfu) at the same stereotactic coordinates did not result in reactivation of latent HSV-1 *(29)*.

Aotus nancymae (New World owl monkeys) are among the most sensitive nonhuman primates to HSV-1 infection *(32,33)*. A total of 22 *Aotus* primates have been used for safety evaluation of G207 (intracerebral and/or intraprostatic inoculation) *(34–36)*. In *Aotus*, a single intracerebral inoculation of G207, up to 10^9 pfu or repeat inoculations of 10^7 pfu, caused neither virus-related disease nor detectable changes in the brain as assessed by magnetic resonance imaging (MRI) and pathological studies *(34)*.

In contrast, an intracerebral inoculation of 10^3 pfu of wild-type HSV-1 (strain F) caused acute viral encephalitis, with the animal becoming moribund within 5 days of inoculation. Four *Aotus* were used to evaluate the shedding and biodistribution of G207 after intracerebral inoculation of clinical-grade, column-purified G207 (3×10^7 pfu) *(35)*. Using polymerase chain reaction analyses and viral culture, neither infectious virus nor viral DNA was detected from tear, saliva, vaginal secretion, blood, or urine samples at any time-point up to 1 month postinoculation. Analyses of tissues obtained at necropsy at 1 month showed G207 DNA distribution restricted to the brain, with no infectious

virus isolated. Histopathology revealed normal brain tissues, including the sites of inoculation *(35)*. All *Aotus* receiving an intracerebral G207 inoculation showed an increase in serum anti-HSV-1 antibody titers as early as 21 days postinoculation *(34,35)*.

2.3. Clinical Trial

A phase I clinical trial of G207 for recurrent malignant glioma was performed in 21 patients at two institutions in the United States *(37)*. This dose escalation study started at 10^6 pfu and increased to 3×10^9 pfu, with three patients at each dose. G207 was inoculated stereotactically into an enhancing region of the tumor, visualized by computerized tomographic scan with contrast enhancement. No acute, moderate-to-severe, adverse events attributable to G207 were observed *(37)*. Minor adverse events included seizure (2 cases) and brain edema (1 case). Among 7 biopsied or resected tumors analyzed, specimens from 2 patients were positive for G207 DNA by polymerase chain reaction analysis (56 and 157 days postinoculation). Of 19 patients, 5 were negative for serum anti-HSV-1 antibody prior to G207 treatment, and despite corticosteroid treatment of these patients, 1 patient seroconverted after G207 inoculation *(37)*.

The tools to evaluate efficacy included Karnofsky performance score and serial MRI *(37)*. An improvement in Karnofsky score was observed in 6 of 21 patients (29%) at some time after G207 inoculation. Of 20 patients that had serial MRI evaluations, 8 had a decrease in tumor volume (enhancing area) between 4 days and 1 month postinoculation. All patients, except 1 who died from cerebral infarction 10 months after G207 treatment, eventually showed tumor progression. Interestingly, this glioblastoma patient had no evidence of residual tumor at autopsy. Autopsy was performed in 5 cases, and histology of the brains showed no evidence of encephalitis, white matter degeneration, or inflammatory changes, and all were negative for HSV-1 immunoreactivity. In 3 cases, the tumor was localized to one region of the brain without significant tumor cell invasion into the surrounding brain tissue as usually observed with typical glioblastoma cases.

Overall, the phase I clinical trial confirmed the safety of G207 inoculated into the brain at doses up to 3×10^9 pfu. Currently, a phase Ib clinical trial for recurrent malignant glioma was performed [NIH 481 (2001-07)], and a phase II trial is planned. Similar results were obtained in phase I trials for glioma with 1716 in the United Kingdom *(28,38)*; 1716, which only contains deletions of *γ34.5 (15)*, was tested at a lower dose range (up to 10^5 pfu) *(28,38)*.

3. USE OF ONCOLYTIC HSV VECTORS FOR IMMUNE THERAPY

Although G207 proved safe in glioma patients and efficacious in animal tumor models, G207 is considerably attenuated, not only for pathogenicity, but also in its tumor cell-killing capability compared to wild-type HSV-1. One way to improve the efficacy of oncolytic HSV therapy would be to harness antitumor immune responses induced in the course of the oncolytic activity of HSV vectors.

3.1. Antitumor Immune Responses

A difficulty in investigating the immune effects of oncolytic HSV therapy has been the lack of suitable animal tumor models susceptible to HSV-1 infection. Many mouse strains and a majority of murine cell lines are relatively resistant to HSV-1 *(18,30)*. It was not recognized until development of immunocompetent mouse tumor models suitable for HSV-1 evaluation that the host immune response plays an important role in the antitumor activity of oncolytic HSV-1 vectors both in the brain and in the periphery *(39,40)*. Initially, murine N18 neuroblastoma cells, one of the more susceptible murine cell lines tested for G207 susceptibility, were used in syngeneic A/J mice. In A/J mice harboring established N18 tumors subcutaneously or in the brain, intraneoplastic inoculation with G207 caused a significant reduction in tumor growth or prolongation of survival *(39)*. Moreover, in A/J mice bearing bilateral subcutaneous N18 tumors, intraneoplastic G207 inoculation into one tumor alone caused growth reduction and/or regression of both the inoculated and the noninoculated contralateral

tumor, indicating induction of systemic antitumor immunity *(39)*. This inhibition of noninoculated tumor growth was also seen in animals bearing intracerebral brain tumors after subcutaneous tumor inoculation. Animals that were cured of their subcutaneous tumors by G207 were protected against tumor rechallenge, in either the periphery or the brain. Antitumor immunity was associated with cytotoxic T lymphocyte (CTL) activity that was specific to N18 tumor cells and persisted for at least 13 months.

G207-induced, systemic antitumor immunity was also observed in BALB/c mice bearing subcutaneous CT26 (colon carcinoma) tumors and DBA/2 mice bearing subcutaneous M3 (melanoma) tumors *(40)*. In the CT26 model, intraneoplastic inoculation of G207 induced CTL activity that recognized a dominant, tumor-specific, major histocompatibility complex (MHC) class I-restricted epitope (AH1) from CT26 cells. Similar systemic antitumor immunity induction by G207 was observed in Syrian hamsters bearing subcutaneous KIGB-5 (gallbladder carcinoma) tumors *(41)* and BALB/c mice bearing CT26 liver metastases *(42)*. Thus, in an immunocompetent condition, the oncolytic activity of G207 can be augmented by induction of specific and systemic antitumor immunity effective both in the periphery and in the brain.

When high-dose dexamethasone was given to A/J mice bearing subcutaneous N18 tumors for an extensive period (16 days), G207 retained antitumor activity and caused a significant suppression of tumor growth when inoculated into the tumors *(43)*. However, all immunosuppressed (dexamethasone-treated) mice treated with G207 displayed tumor regrowth despite initial shrinkage, whereas 50% of the G207-treated mice not immunosuppressed were cured. Dexamethasone administration significantly reduced neutralizing serum antibodies against G207 after intraneoplastic G207 inoculation, but this did not affect the amount of infectious G207 isolated from tumors. The most striking effect of dexamethasone administration was the abolition of G207-induced CTL activity against N18 cells *(43)*. These results further support the importance of tumor-specific CTL induction in the course of oncolytic HSV-1 antitumor activity.

The effect of circulating anti-HSV-1 antibodies on the efficacy of oncolytic HSV-1 therapy has been investigated because the majority of the population is HSV-1 seropositive *(44,45)*. A/J and BALB/c mice were immunized by repeated intraperitoneal inoculations of wild-type HSV-1 (strain KOS) and then the antitumor efficacy of G207 on established subcutaneous N18 and CT26 tumors was determined *(46)*. In both tumor models, the antitumor efficacy of G207 was the same whether the mice were immunized or not for HSV-1.

In a study using intraocular immunization, treatment of M3 melanoma tumors in DBA/2 mice with HSV-1 1716 was actually more effective than in nonimmunized mice *(47)*. Because HSV-1 predominantly spreads cell to cell, circulating antibodies known to neutralize free virus may have little effect on HSV-1 directly inoculated into tumors. When NV1020, at a low dose (10^6 pfu), was administered intravenously to immunized BALB/c mice with CT26 tumors in the liver, there was a detectable decrease in efficacy *(48)*. This efficacy attenuation with intravenous delivery was overcome by administering a higher dose (10^7 pfu) of NV1020.

3.2. *Third-Generation Oncolytic HSV-1 Vector*

The therapeutic benefits of oncolytic HSV-1 vectors depend on the extent of both intratumoral viral replication and induction of host antitumor immune responses. We are developing new generations of HSV-1 vectors by enhancing these properties and retaining the safety features of G207. G47Δ is one such vector created from G207 by introducing another genetic alteration, deletion of the *α47* gene and the overlapping *US11* promoter region *(31)* (Fig. 1). Because the *α47* gene product (ICP47) inhibits transporter associated with antigen presentation, which translocates peptides across the endoplasmic reticulum, the downregulation of MHC class I that normally occurs in human cells after infection with HSV-1 does not occur *(49)*. G47Δ-infected human cells in fact presented higher levels of MHC class I than cells infected with other HSV-1 vectors *(31)*. Further, human melanoma

cells infected with G47Δ were better at stimulating their matched tumor-infiltrating lymphocytes in vitro than those infected with G207. Unfortunately, the interaction of ICP47 with transporter associated with antigen presentation is species specific and is exceedingly inefficient in rodent cells *(50)*. Therefore, it is not possible to test the immune effects in vivo in mouse tumor models.

The deletion also places the late *US11* gene under control of the immediate-early *α47* promoter, which results in suppression of the reduced growth phenotype of *γ34.5*-deficient HSV-1 mutants *(51)*, including G207. In the majority of cell lines tested in vitro, G47Δ replicated better than G207, resulting in the generation of higher virus titers, and exhibited greater cytopathic effect *(31)*. In athymic mice bearing subcutaneous U87MG human glioma tumors and A/J mice bearing subcutaneous Neuro2a neuroblastoma tumors, G47Δ was significantly more efficacious than G207 at inhibiting tumor growth when inoculated intraneoplastically *(31)*.

Improved antitumor efficacy of G47Δ has also been shown in other immunocompetent mouse tumor models, including prostate and breast cancer *(65)*. Nevertheless, this deletion does not suppress the attenuated pathogenicity of *γ34.5* deletion mutants *(52)*, and the safety of G47Δ remained unchanged from G207 following injection into the brains of HSV-1-sensitive A/J mice *(31)*.

Thus, compared with the parental virus G207, G47Δ demonstrated (1) better induction of human antitumor immune cells; (2) better growth properties, leading to higher virus yields and increased cytopathic effect in vitro; (3) better antitumor efficacy in both immunocompetent and immunoincompetent animals; and (4) preserved safety. These features make G47Δ highly attractive for clinical application.

3.3. Combination With Immune Gene Therapy

Our experience using various HSV-1 vectors to treat tumors, including wild-type HSV-1, indicates that there is a limit to improving the antitumor efficacy of oncolytic vectors by simply bringing the replication capability closer to that of wild-type viruses, putting aside the difficulty of doing so without increasing pathogenicity. In developing new vectors, therefore, more emphasis is currently placed on enhancing the ability to induce antitumor immunity. The combination of oncolytic HSV-1 vectors with defective vectors expressing immunostimulatory molecules can improve therapeutic efficacy significantly (Fig. 2) *(53–55)*. In this approach, the oncolytic HSV-1 vector acts as a helper virus for the propagation of plasmid-based defective vectors *(56)*. An advantage of this approach is that different defective vectors can be generated with different oncolytic helper viruses for a multiplicity of combinations without creating new vectors.

We have developed an immune gene therapy strategy that would work for brain tumors as well as other cancers. The brain is considered an immune-privileged site, and patients with brain tumors are often under an immune-suppressed condition because of immunosuppressive factors secreted by the brain tumor and/or corticosteroid administration. On the other hand, a robust, nonspecific inflammatory response in the brain can cause undesirable brain edema.

To meet these requirements, we created a defective HSV vector (dvB7Ig) expressing a soluble form of B7-1, one of the most potent costimulatory molecules, and used it in combination with G207 *(54)*. Soluble B7-1 was designed as a fusion protein of the extracellular domain of B7-1 and the Fc portion of immunoglobulin G, so that it is secreted by tumor cells rather than expressed on the cell surface. Secreted soluble B7-1 should provide antigen-presenting cells increased T-cell stimulatory activity, activate T cells in an anergic state, and because it is in a dimeric form, provide a strong stimulation to T cells by crosslinking neighboring CD28.

The in vivo efficacy was tested in the poorly immunogenic murine neuroblastoma Neuro2a in A/J mice. Intraneoplastic inoculation of dvB7Ig/G207 at a low titer successfully inhibited the growth of established subcutaneous tumors, despite the expression of B7-1-immunoglobulin detected in only 1% or fewer tumor cells at the inoculation site, and prolonged the survival of mice bearing intracerebral tumors *(54)*. Inoculation of dvB7Ig/G207 induced a significant influx of $CD4^+$ and $CD8^+$ T cells in the tumor. In vivo depletion of immune cell subsets further revealed that the antitumor effect

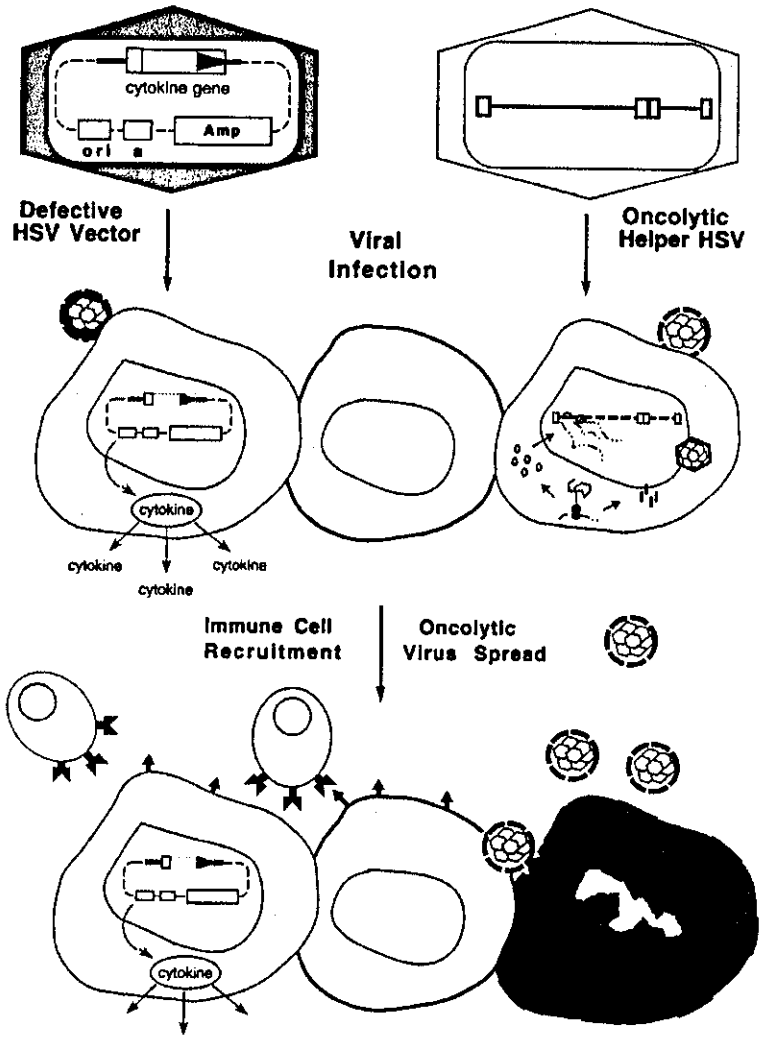

Fig. 2. Schematic diagram of immunomodulatory gene therapy using oncolytic HSV-1 vectors (like G207) as helper virus in combination with a defective HSV-1 vector expressing an immunomodulatory molecule. Defective HSV vector stocks are a mixture of defective particles (upper left) containing tandem repeats of an amplicon plasmid and HSV helper viruses (upper right) *(81)*. The amplicon plasmid consists of the cytokine/immunomodulatory gene, an HSV origin of replication (ori), and an HSV cleavage/packaging signal (a), but no viral coding sequences, and is packaged as a full viral genome length (~150 kb). Any conditional-lethal or replication-competent HSV mutant can be used as helper virus. When a mixture of helper and cytokine-expressing defective vector is inoculated into a tumor, the helper virus replicates, kills the infected cell, and spreads to other tumor cells (right side). On the other hand, tumor cells infected with the defective vector produce the cytokine and recruit immune cells (left side) that augment the antitumor immune response elicited by the oncolytic helper virus.

required $CD8^+$ T cells, but not $CD4^+$ T cells *(54)*. DvB7Ig/G207 treatment conferred tumor-specific protective immunity on cured animals. Thus, this approach proved to be a potent and clinically applicable means of treating brain tumors and other cancers.

A defective HSV vector expressing murine interleukin 12 (IL-12) in combination with G207 was very effective in treating subcutaneous CT26 tumors in BALB/c mice and inducing a tumor cell-specific $CD8^+$ CTL response *(53)*. An IL-2-expressing defective vector in combination with G207 had enhanced efficacy in murine squamous cell carcinoma and rat hepatocellular carcinoma models compared to G207 alone *(55,57)*. However, granulocyte-macrophage colony-stimulating factor (GM-CSF) expression from a defective vector did not have any increased efficacy over G207 alone in treating CT26 tumors (M. Toda and S. D. Rabkin, unpublished results, 1998). Whereas GM-CSF expression from replication-deficient HSV vectors did significantly enhance antitumor activity *(58, 59)*, as a tumor vaccine, GM-CSF-transduced cells have been found to be among the most effective *(60)*. This suggests that HSV infection may be inducing an immune response similar to that of GM-CSF expression, and that the spectrum of cytokines that will be effective in combination with oncolytic HSV vectors will be different from those used in tumor vaccines.

Replication-competent HSV-1 vectors that contain immunostimulatory molecule transgenes (i.e., IL-4, IL-10, IL-12, GM-CSF) have been created *(61–63)*. In particular, replication-competent HSV-1 vectors that express IL-12 have been shown in several animal tumor models to manifest direct oncolytic activity and express sufficient amounts of IL-12, which significantly augments antitumor activity without increasing toxicity, compared with the parental HSV-1 vectors *(62–65)*.

4. FUTURE DIRECTIONS

Now that it has been demonstrated in several clinical trials that oncolytic HSV-1 vectors can be administered safely in humans *(28,37,38,66)*, further development of oncolytic HSV-1 vectors will be directed toward improving antitumor efficacy. Doing so without compromising the safety of the vectors is the key to prevailing in this type of therapy. G47Δ is a good example of providing such an improvement in efficacy yet retaining safety features. A syncytial mutant (Fu-10) generated from G207, which forms tumor cell syncytium, was more efficacious in a lung metastases model than the parent, G207 *(22)*.

Expression of foreign transgenes, for example, "suicide" or immunostimulatory molecules, is another promising method to augment the activity of oncolytic HSV-1 vectors. A number of suicide genes, cytochrome P450 (CYP2B1) and cytosine deaminase (CD), have been incorporated into oncolytic HSV-1 vectors, and treatment with prodrugs significantly improved efficacy *(67,68)*. With the addition of foreign transgenes, it is important to be aware that they may increase the toxicity of the vector, decrease safety, and/or interfere with viral replication and decrease efficacy.

A practical method for improving the efficacy of oncolytic HSV-1 vectors is to combine them with conventional therapies. For example, a combination with cisplatin was shown to enhance the antitumor effect of G207 against human head and neck cancer *(69)*, and mitomycin C with 1716 was more effective than either treatment alone against human non-small cell lung cancer *(70)*.

Others have shown that ionizing radiation amplifies the replication of HSV-1 R3616 *(71)*, leading to improved survival of athymic mice bearing intracerebral U87MG tumors *(72)* and NV1020 (R7020) in some hepatoma tumor cell lines *(73)*. Although we did not observe such an enhancing effect of ionizing radiation with G207 in prostate cancer *(74)*, others have shown such an effect with G207 and cervical cancer *(75)*. Systemic delivery to brain tumors after intracarotid artery infusion can be enhanced by disruption of the blood–brain barrier using mannitol, bradykinin, or RMP-7 *(76–78)*. The replication and spread of oncolytic HSV-1 vector hrR3 in brain tumors after RMP-7 can be further enhanced by intraperitoneal administration of cyclophosphamide *(79)*. The combination of oncolytic HSV-1 vectors with established therapies should be rapidly translatable to the clinic.

5. CONCLUSION

Oncolytic virus therapy is an attractive treatment strategy because it is based on a new concept that the antitumor agent can amplify specifically at the tumor site after administration. This strategy

also has features that make it attractive for clinical application: (1) tumor cells are targeted irrespective of their genetic makeup; (2) it can be combined with conventional therapies such as surgery, radiation therapy, and chemotherapy; (3) combination with immunotherapy has potential synergistic effects; and (4) it can act as a vehicle for gene delivery in vivo. An increasing number of clinical trials using oncolytic viruses have been initiated or planned in recent years. We anticipate that oncolytic virus therapy will be established as an important modality of cancer treatment in the near future.

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Inhibition of angiogenesis in human glioma cell lines by antisense RNA from the soluble guanylate cyclase genes, *GUCY1A3* and *GUCY1B3*

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Abstract. Malignant gliomas, most of which show an elevated level of vascular endothelial cell growth factor (VEGF) expression, are well known for their hyper-vascularity. One of the possible inducers of VEGF in tumor cells is nitric oxide (NO), which is synthesized by NO synthase and stimulates soluble guanylate cyclase (GC) in tumor cells. Here, we report that 2 of 9 human glioma cell lines, CCF-STTG1 and U-87MG, overproduced cyclic GMP (cGMP) and showed increased expression of both or either subunits of soluble GC1, GUCY1A3 and GUCY1B3. Transfection of antisense GUCY1A3 or GUCY1B3 into these two glioma cell lines markedly reduced the content of cGMP and expression of VEGF. The angiogenic activity *in vitro* was subsequently inhibited, which was determined by induction of HUVEC cell growth. Furthermore, subcutaneous tumor formation by U-87MG cells in nude mice was dramatically suppressed to less than 0.05% in volume by transfection of either antisense GUCY1A3 or antisense GUCY1B3, which was accompanied by the significant decrease in vascular index to about 10%. These findings demonstrate that cGMP is an upstream mediator of VEGF expression in glioma cells and that soluble guanylate cyclases could be the target molecules for controlling neo-vascularization in a subset of human malignant gliomas.

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Abbreviations: VEGF, vascular endothelial cell growth factor; bFGF, basic fibroblast growth factor; PDGF, platelet derived growth factor; HGF, hepatocyte growth factor; NO, nitric oxide; GC, guanylate cyclase; cGMP, cyclic GMP; GUCY1A3, soluble guanylate cyclase 1 α 3; GUCY1B3, soluble guanylate cyclase 1 ß 3; RT, reverse transcription; PCR, polymerase chain reaction; bp, base pairs; SDS, sodium dodecyl sulphate; EIA, enzyme immuno-assay; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; PBS, phosphate-buffered saline; HUVEC, human umbilical vascular endothelial cells

Key words: angiogenesis, glioma, vascular endothelial cell growth factor, soluble guanylate cyclases

Introduction

Malignant gliomas, the most common type of adult human brain tumors, are well known for their hypervascular features (1). Since the growth of solid tumors, including malignant gliomas, requires neo-vascularization, one of the most promising approaches to the control of malignant gliomas is the blockade of oxygen or nutrition to tumor cells by inhibiting neo-vascularization (2,3). The vascular endothelial cell growth factor (VEGF), an endothelial cell-specific mitogen, is one of the main factors involved in neo-vascularization in tumor tissues (4). In fact, the expression of VEGF is highly up-regulated in malignant gliomas (5,6). A hypoxic state or several cytokines, including basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and hepatocyte growth factor (HGF), are widely accepted inducers of VEGF expression in tumor cells (7-11). In addition, some studies suggest that nitric oxide (NO) induces VEGF expression in tumor cells (12,13). Since several studies have demonstrated that VEGF up-regulates NO synthase expression in endothelial cells through their VEGF receptors (14-17), the paracrine cascades of VEGF and NO between a tumor and the surrounding endothelial cells would be activated autonomously in terms of tumor angiogenesis.

We are interested in the possible involvement of the cascade downstream of NO in autonomously activated angiogenesis in malignant glioma cells. Chin *et al* have reported that the induction of VEGF by NO is abrogated by guanylate cyclase (GC) inhibitors in hepatocellular carcinoma and glioma cells (12). Gallo *et al* also demonstrated positive correlation between NO synthase activity, cyclic GMP (cGMP) level, and tumor angiogenesis (18). NO is known to activate soluble GCs (19). Three types of soluble GCs have been reported so far, all of which are hetero-dimeric enzymes

consisting of an α and a ß subunit that catalyze the conversion of GTP to cGMP (20). Among them, the type 3 GC is expressed in the human brain, and both subunits [soluble guanylate cyclase 1α 3 (GUCY1A3) and soluble guanylate cyclase 1ß 3 (GUCY1B3)] have been previously cloned (21). In the present study, we investigated the possible involvement of the soluble GCs/cGMP cascade in VEGF-mediated angiogenesis in glioma cells using the antisense RNA of a pair of subunits of soluble GCs, GUCY1A3 or GUCY1B3.

Meterials and methods

Cell lines. Eight human glioma cell lines (Hs683, SW1088, SW1783, DBTRG-05MG, CCF-STTG1, U-87MG, U-118MG, and U-373MG) were obtained from the American Type Culture Collection (Rockville, MD). One human glioma cell line (T98G) was obtained from the Human Science Research Resources Bank (Osaka, Japan). Cells were cultured according to the supplier's recommendation.

Northern blot analysis. Poly(A) RNAs were extracted from cells using a Fast Track mRNA isolation kit (Invitrogen, Carlsbad, CA). RNA (2 µg) was subjected to electrophoresis in 1% agarose gels containing 2.1 M formamide and transferred onto nylon membranes, Hybond-N+ (Amersham Pharmacia, Cleveland, OH). The probe for VEGF was the reverse transcription (RT)-polymerase chain reaction (PCR) product of 563 base pairs (bp) amplified from human brain poly-A RNA using a pair of primers, 5'-ATG AAC TTT CTG CTG TCT TGG -3' and 5'-CTT GTC ACA TCT GCA AGT AC-3'. A cDNA probe for human ß-actin was purchased from Clontech (Palo Alto, CA). These probes were labeled with [α-^{32}P]dCTP using a Multiprime DNA labeling system (Amersham Pharmacia). Hybridization was carried out at 42°C for 18 h in 5X SSC, 20 mM sodium phosphate, 1x Denhardt's solution, 0.2% sodium dodecyl sulfate (SDS), 100 µg/ml denatured salmon testis DNA, 10% dextran sulfate, and 50% formamide. Membranes were then washed with 2X SSC/0.1% SDS at 42°C for 15 min followed by exposure for 1-4 days at -70°C to BioMax film (Kodak, Rochester, NY) with an intensifying screen.

Enzyme immunoassay (EIA) for cGMP and enzyme-linked immunosorbent assay (ELISA) for human VEGF. The content of cGMP and VEGF in a cultured medium was quantified using EIA for cGMP (Assay design, Ann Arbor, MI) and ELISA for human VEGF (R&D systems, Minneapolis, MN) according to the manufacturer's recommendation. Cells (10^6) were seeded on 6-well plates and cultured with a medium containing 10% fetal bovine serum (FBS) for the detection of cGMP or 0.2% FBS for the detection of VEGF. Twenty-four hours before the measurement, the medium was aspirated. The cells were washed twice with phosphate-buffered saline (PBS) and then incubated with 2 ml of a fresh medium containing 10% FBS for EIA for cGMP or 0.2% FBS for ELISA for VEGF.

Transfection of antisense GUCY1A3 and antisense GUCY1B3 expression vectors. Antisense-oriented DNA fragments were obtained by RT-PCR from human brain poly(A) RNA (Clontech). The pairs of primers used are as follows: 5'-ATA AGAATGCGGCCGCGCAGCAGGGTAAGAGACACC-3' and 5'-GGGGTACCAACCACCCCAAGGATGTTTTC ATC-3' for a 491-bp fragment complementary to the 5' portion of GUCY1A3 cDNA; 5'-ATAAGAATGCGGCCGC GCTGGGGGATTACACAAAGA-3' and 5'-GGGGTACCC AATGAGTCAAACCCCAAAGAC-3' for a 515-bp fragment complementary to the 3' portion of GUCY1A3 cDNA; 5'-ATA AGAATGCGGCCGCGCAGACACCATGTACGGATT-3' and 5'-GGGGTACCTGGATTTGTTGTGCCACTGTT-3' for a 482-bp fragment complementary to the 5' portion of GUCY1B3 cDNA; and 5'-ATAAGAATGCGGCCGC GGTGGAGACTGTTGGTGACA-3' and 5'-GGGGTA CCCTCCTCTTCACCCCATAATCC-3' for a 473-bp fragment complementary to the 3' portion of GUCY1B3 cDNA. Each fragment was cloned into the NotI/KpnI restriction site of a plasmid pcDNA3.1/Hygro(+) to obtain pASGUCY1A3-5', pASGUCY1A3-3', pASGUCY1B3-5', and pASGUCY1B3-3', respectively. These plasmids were transfected into cells using Lipofectamine (Gibco/BRL, Rockville, MD), and stable clones were obtained by selection against hygromycin (100 µg/ml for CCF-STTG1 cells or 150 µg/ml for U-87MG cells). CCF-STTG1-derived cells carrying pcDNA3.1/Hygro(+), pASGUCY1A3-3', pASGUCY1B3-5', and pASGUCY1B3-3' are named STTG1cp, STTG1as GUCY1A3-3', STTG1as GUCY1B3-5', and STTG1as GUCY1B3-3', respectively, while U-87MG-derived cells carrying pcDNA3.1/Hygro(+), pASGUCY1B3-5', and pASGUCY1B3-3' are named U-87cp, U-87asGUCY1B3-5', and U-87asGUCY1B3-3', respectively. Three independent clones for each plasmid were analyzed.

Angiogenic assay in vitro. Glioma cells ($2.5x10^4$) of mock or antisense transfectants were seeded onto Millicell (Millipore, Bedford, MA), whereas $5x10^3$ of human umbilical vascular endothelial cells (HUVEC) (Kurabo, Tokyo, Japan) were seeded on a 24-well plate. Twenty-four hours later, the cells were washed twice with PBS and co-cultured with HU media (Kurabo) containing 0.2% FBS for 24-72 h. The number of HUVEC was counted at each time point as an indicator of angiogenic activity *in vitro*.

Tumor angiogenesis assay in vivo. Glioma cells ($1x10^5$) of mock or antisense transfectants was inoculated into the flanks of 5- to 6-week old female BALB/c athymic nu/nu mice. Tumor growth was monitored by measuring the xenografts in three dimensions. Tumors were removed at 32 days after injection, fixed in 10% formalin, blocked, cut into sections of 4-6 µm in thickness, and stained with hematoxylin and eosin or a monoclonal antibody against CD-31 (Dako; Carpinteria, CA). The vascular index in each sample was counted in x200 fields. All animal experiments were performed in accordance with institutional guidelines.

Results

Increased expression of VEGF in human glioma cell lines. We first examined the expression of VEGF in 9 human malignant glioma cell lines. Northern blot analysis revealed that VEGF

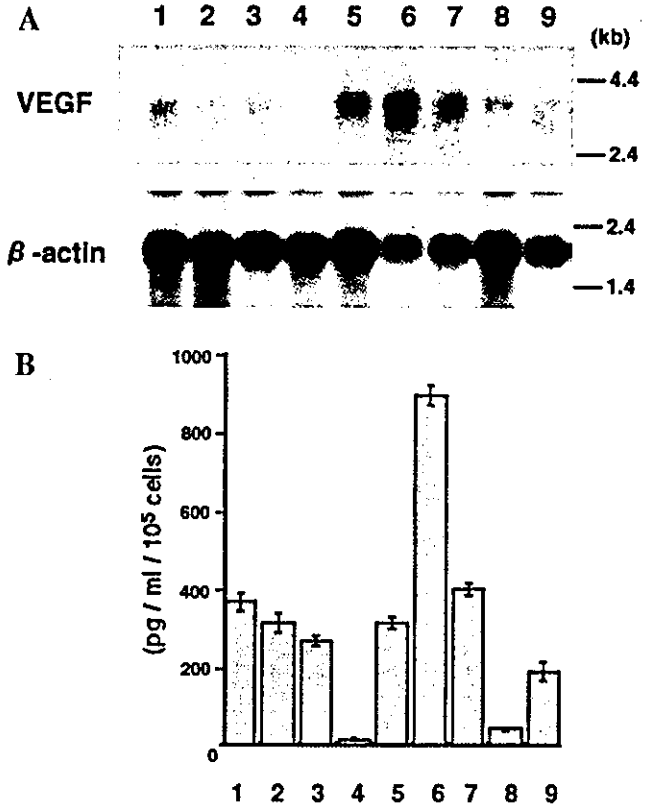

Figure 1. Increased expression of VEGF in human malignant glioma cell lines. A, Northern blot analysis of the VEGF (upper) and ß-actin (lower); B, ELISA of VEGF protein in a cultured medium of glioma cells. The content of VEGF was adjusted by the volume of medium and the number of cells. Lane 1, Hs683; 2, SW1088; 3, SW1783; 4, DBTRG-05MG; 5, CCF-STTG1; 6, U-87MG; 7, U-118MG; 8, U-373MG; 9, T98G.

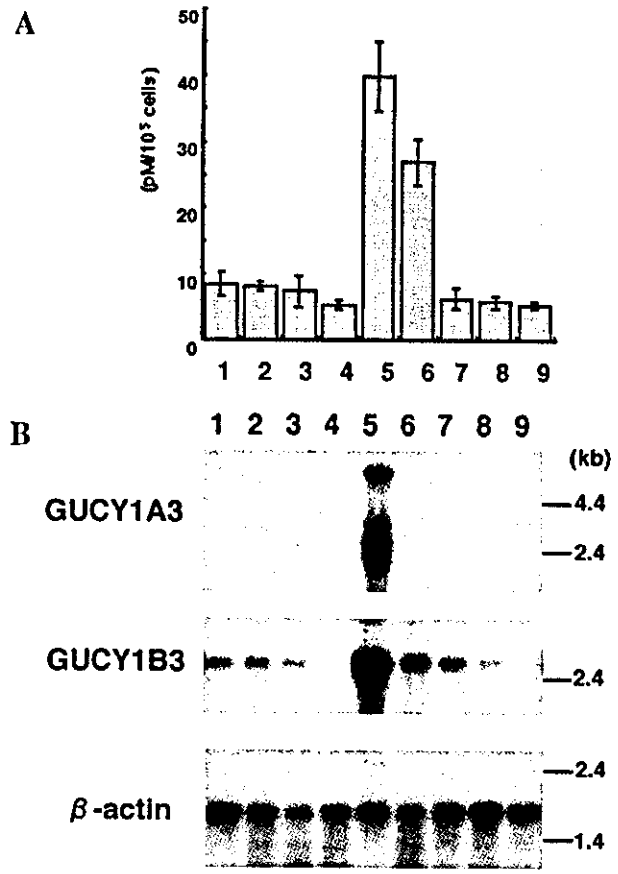

Figure 2. Activation of the guanylate cyclases/cGMP cascade in human malignant glioma cell lines. A, Increased amount of cGMP in the cell lysate from two human malignant glioma cell lines. cGMP was detected by an EIA and adjusted for the number of cells. B, Northern blot analysis of the two subunits of soluble guanylate cyclase, GUCY1A3 (upper) and GUCY1B3 (middle), and ß-actin (lower). Lane 1, Hs683; 2, SW1088; 3, SW1783; 4, DBTRG-05MG; 5, CCF-STTG1; 6, U-87MG; 7, U-118MG; 8, U-373MG; 9, T98G.

was significantly expressed in all cell lines except DBTRG-05MG cells, in which overexpression of VEGF was detected in 3 cell lines, CCF-STTG1, U-87MG, and U-118MG (Fig. 1A). Secretion of VEGF proteins into the cultured medium was subsequently measured in the same series of glioma cell lines using an ELISA. Significant amounts of VEGF proteins were detected in the cultured medium of 7 cell lines except DBTRG-05MG and U-373MG, in which U-87MG cells showed the highest content of VEGF (Fig. 1B).

Activation of GCs in a subset of human glioma cell lines. In order to understand the possible role of GCs/cGMP cascade in VEGF expression, we examined the level of cGMP in the same set of glioma cell lines. An EIA against cGMP revealed that the content of cGMP in the conditioned medium was significantly increased in two glioma cell lines, CCF-STTG1 and U-87MG (Fig. 2A), both of which were shown to express a high level of VEGF. We next examined the expression of two subunits of the soluble GCs, GUCY1A3 and GUCY1B3, in the same series of glioma cell lines. CCF-STTG1 cells showed overexpression of both genes, while U-87MG cells expressed relatively high amount of GUCY1B3 (Fig. 2B). A low amount of GUCY1A3 expression was also detected by long exposure in U-87MG cells (data not shown). These results suggest that the elevated level of cGMP in these cells would be due to the increased expression of GUCY1A3 or GUCY1B3, although possible reduced activity of phosphodiesterases might also participate in a high content of the cGMP.

Inhibition of angiogenic activity in vitro by antisense RNA of soluble GCs, GUCY1A3 or GUCY1B3. Next, for the assessment of the direct involvement of the GCs/cGMP cascade in the angiogenic activity of glioma cells, we tried to inhibit either GUCY1A3 or GUCY1B3 in CCF-STTG1 cells and GUCY1B3 in U-87MG cells by transfecting antisense RNA. All cell lines expressing antisense RNA showed a marked reduction in the content of cGMP in comparison with those in CCF-STTG1 or U-87MG cells transfected with vectors alone, suggesting that the activity of soluble GCs was inhibited by antisense RNA (Fig. 3A and B). Correspondingly, Northern blot analysis revealed that VEGF expression in the cell lines expressing antisense RNA was prominently suppressed (Fig. 3C and D). Concentration of the VEGF proteins in a cultured medium was also significantly reduced in both CCF-STTG1-derived and U-87MG-derived cell lines expressing antisense RNA (Fig. 3E and F). However, a small portion of VEGF was still present in the cultured medium, suggesting that additional mediators are involved in VEGF expression in these cell lines. Alternatively, a small amount of cGMP not inhibited by the antisense RNA of GUCY1A3 and GUCY1B3 may induce VEGF expression.

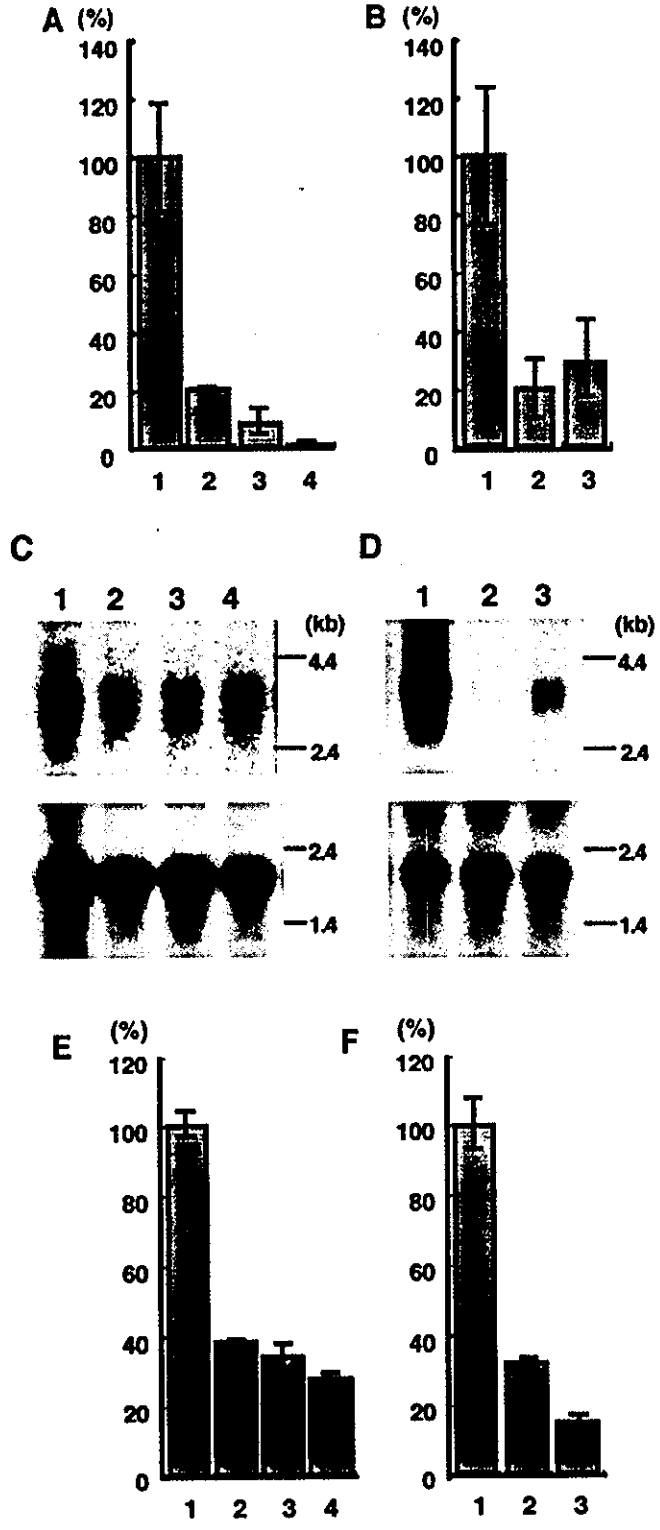

Figure 3. Reduction of cGMP content and VEGF expression by the antisense RNA of GUCY1A3 or GUCY1B3. The results in CCF-STTG1 (A, C, E) and U-87MG (B, D, F) cells are shown. A and B, Reduction of cGMP content by the antisense RNA of GUCY1A3 or GUCY1B3. The content of cGMP was detected by EIA and adjusted by the number of cells; it was then presented as a relative amount in comparison with that of cells transfected with the control plasmid. C and D, Reduced expression of the *VEGF* gene by the antisense RNA of GUCY1A3 or GUCY1B3. E and F, Reduction of VEGF protein in a cultured medium of the cells transfected with the antisense RNA of GUCY1A3 or GUCY1B3. VEGF protein was detected by ELISA and adjusted by the volume of cell medium and the number of cells; it was then presented as a relative amount. A, C and E: Lane 1, STTG1cp; 2, STTG1asGUCY1A3-3'; 3, STTG1asGUCY1B3-5'; 4, STTG1asGUCY1B3-3'. B, D and F: Lane 1, U87cp; 2, U87aspGUCY1B3-5'; 3, U87aspGUCY1B3-3'.

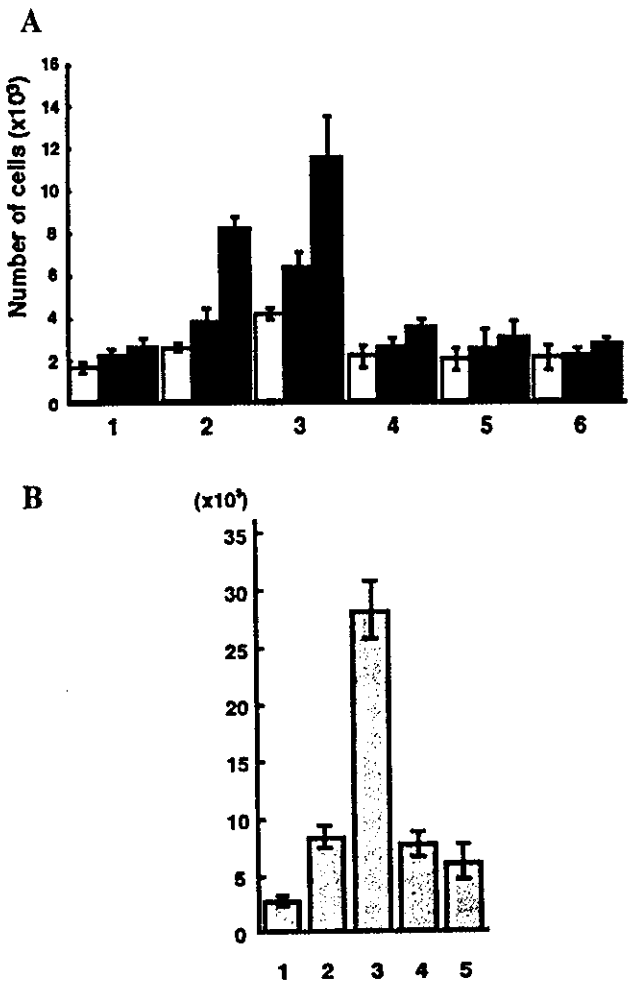

Figure 4. Inhibition of the angiogenic activity *in vitro* by antisense RNA of GUCY1A3 or GUCY1B3. A, Mitogenic activity of HUVEC cells by cultured media derived from the CCF-STTG1 cell line and its derivatives. The number of HUVEC cells was counted at 24, 48, and 72 h after incubation with relevant media and is indicated with open, gray, and closed bars, respectively. Cultured media with 0.2% of FBS (lane 1), with 10 ng/ml of VEGF (lane 2), and derived from STTG1cp (lane 3), STTG1asGUCY1A3-3' (lane 4), STTG1asGUCY1B3-5' (lane 5), and STTG1asGUCY1B3-3' (lane 6) were examined. B, Mitogenic activity of HUVEC cells by cultured media derived from the U-87MG cell line and its derivatives. The number of HUVEC cells at 72 h after incubation with relevant media was counted. Culture media with 0.2% of FBS (lane 1), with 10 ng/ml of VEGF (lane 2), and derived from U87cp (lane 3), U87asGUCY1B3-5' (lane 4), and U87asGUCY1B3-3' (lane 5).

We then examined the angiogenic activity of the secreted VEGF from these cell lines *in vitro* using human umbilical vein endothelial cells (HUVEC) as indicators. CCF-STTG1 cells with a vector alone, as well as three CCF-STTG1-derived cell lines expressing the antisense RNA of GUCY1A3 or GUCY1B3, were cultured with HUVEC cells so that only the cultured medium and not the cells, interacted with each other. HUVEC cells grew rapidly in the cultured medium from CCF-STTG1 cells with a vector alone, where the growth rate was almost equivalent to that treated with 10 ng/ml of VEGF in the medium. In contrast, the cultured medium from CCF-STTG1-derived cell lines expressing the antisense RNA of GUCY1A3 or GUCY1B3 did not show significant angiogenic activity because the growth rates of HUVEC cells in these

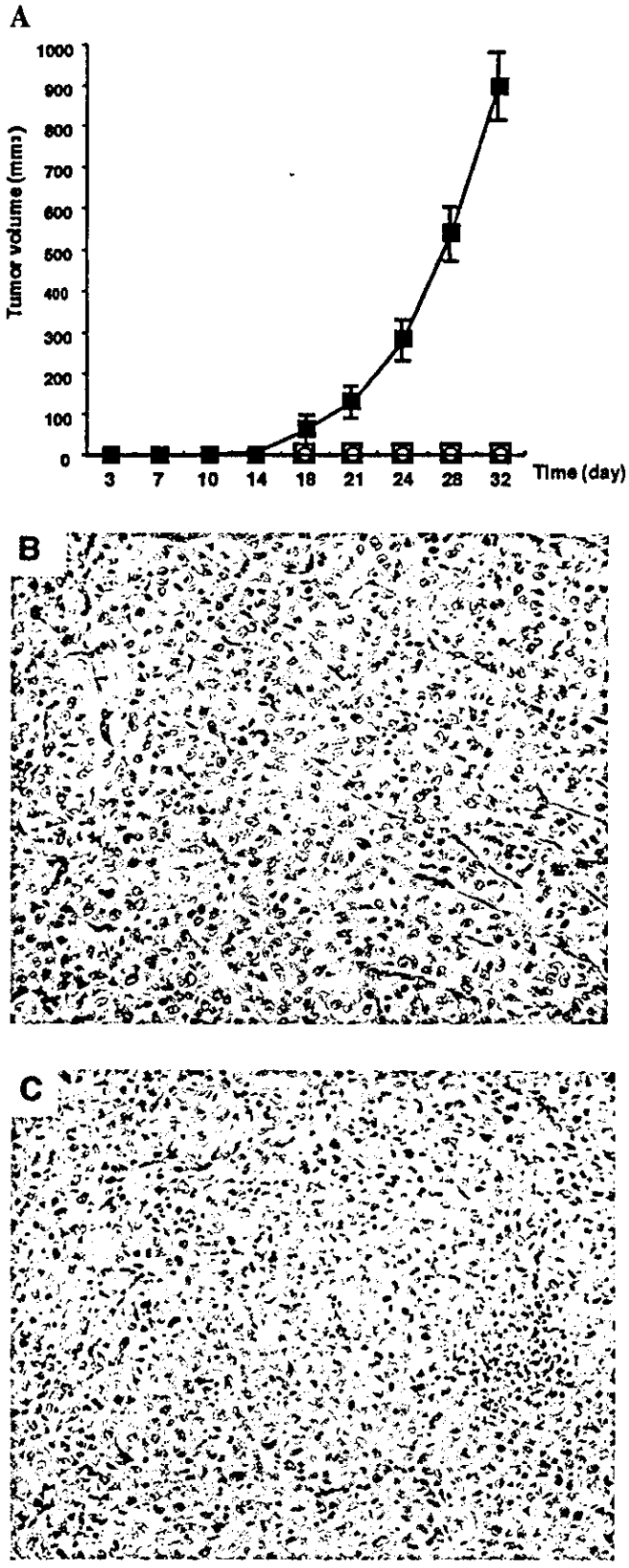

Figure 5. Inhibition of angiogenesis and tumor formation in nude mice by antisense RNA of GUCY1B3. A, Tumor formation in nude mice. The average volume of tumors formed at eight sites was determined at the indicated times after injection of 10^5 cells from the following U-87MG derivatives: U87cp (■), U87asGUCY1B3-5' (○) and U87asGUCY1B3-3' (⊏). The average volumes of tumors in autopsy at day 32 were 910 mm^3, 0.4 mm^3, and 0.4 mm^3, respectively. B and C, Microscopic examination of the tumors derived from U87cp (B) and U87asGUCY1B3-5' (C) with hematoxylin and eosin staining. Microvessels per field: 21.9±8.9 (U87cp), 2.25±0.95 (U87asGUCY1B3-5'), and 2.33±1.15 (U-87asGUCY1B3-3').

media were essentially the same as that with 0.2% FBS alone (Fig. 4A). Similar results were obtained in a series of experiments using U-87MG cells with a vector alone and U-87MG-derived cells expressing the antisense RNA of GUCY1B3. Mitogenic activity towards HUVEC cells was much stronger in the media of a 72-h culture with U-87MG cells carrying a vector alone than in the medium with 10 ng/ml of VEGF. Since 10 ng/ml of VEGF generates almost full mitogenic activity to HUVEC cells, U-87MG cells appear to secrete other mitogenic factors in addition to VEGF (Fig. 4B).

Inhibition of angiogenesis and tumor formation in nude mice by the antisense RNA of GUCY1B3. We finally examined the angiogenic activity and its inhibition *in vivo* by the antisense RNA of GUCY1B3. Because only U-87MG cells, and not CCF-STTG1 cells, formed subcutaneous tumors when injected into nude mice, we tested the tumorigenicity of U-87MG cells with a vector alone as well as that of two U-87MG-derived cell lines expressing the antisense RNA of GUCY1B3. U-87MG cells with a vector developed tumors, which continued to grow rapidly and aggressively, at all injection sites (8/8) (Fig. 5A). The average volume of the tumors was 910 mm^3 when the mice were sacrificed at day 32. Tumors were soft and round; they were also rich in veins, some of which could be observed macroscopically on the surface of the tumors (data not shown). In sharp contrast, the incidence and size of the tumors were dramatically decreased in two U-87MG-derived cell lines expressing the antisense RNA of GUCY1B3 (Fig. 5A). Palpable tumors could not be detected until the mice were sacrificed at day 32, when only one very small tumor with a volume of 0.4 mm^3 could be recognized in each cell line at autopsy. A microscopic examination demonstrated that the vascular index of tumors derived from U-87MG cells with a control plasmid (average 21.9 per field) was significantly higher than that of the small tumors derived from cells expressing the antisense RNA of GUCY1B3 (average 2.3 per field) (Fig. 5B and C).

Discussion

Numerous studies have demonstrated that hypoxia and several cytokines are the major inducers of VEGF expression in various cells, including those of gliomas (7-11). In addition, some experiments have suggested that NO can also induce VEGF through activation of the GC, although this pathway might overlap, at least in part, with the signals from hypoxia or several cytokines (12,13). In the present study, we have newly demonstrated that the soluble GCs/cGMP cascade is autonomously activated in a subset of human malignant glioma cells. Furthermore, blocking this cascade by the antisense RNA of either subunit of soluble GCs type 3 reduced cGMP content and VEGF expression, inhibited angiogenic activity both *in vitro* and *in vivo*, and suppressed tumor formation in glioma cells. It is promising that the inhibition of either subunit of GCs is sufficient to inhibit angiogenesis. These findings strongly suggest that a pair of subunits of soluble GCs, GUCY1A3 and GUCY1B3, is an upstream mediator of VEGF expression in glioma cells and provides a possible molecular target for anti-angiogenic therapy in a subset of human gliomas.

Downstream cascades from cGMP to VEGF in tumor cells, including some cGMP-dependent kinases, would also be important for intervening in tumor neo-vascularization. Such molecules might regulate VEGF expression either by transcriptional control or through some specific cytokines (13,22,23). Several studies have demonstrated that the hypoxia inducible factor 1 complex is involved in the transcription of the *VEGF* gene at the downstream of hypoxia stimulation as well as NO generation. In this connection, it is noteworthy that U-87MG cells showed very strong mitogenic activity to HUVEC cells that cannot be explained by VEGF activity alone. Nonetheless, the inhibition of the GCs suppressed most of the angiogenic activity of U-87MG both *in vitro* and *in vivo*, suggesting that additional cytokines regulated by cGMP could be involved in the elevated activity of angiogenesis and tumorgenesis in U-87MG cells. Furthermore, drastic suppression of tumorigenicity observed in U-87MG cells by antisense GUCY1B3 suggests that GC/cGMP might also be involved in some pathways regulating tumor formation other than the angiogenesis. Modification of soluble GC activity by some specific inhibitors could provide a novel strategy against human malignant gliomas.

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Clinical Article

Multimodal strategy for managing meningiomas in the elderly

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Summary

Background. The incidence of brain tumors in elderly patients is increasing. It has become possible to treat meningiomas in the elderly by several modalities. We developed a successful multimodal strategy to treat these patients.

Methods. We registered 35 patients with meningiomas. Symptomatic meningiomas were treated surgically at the time of diagnosis (n = 19). Of the 16 asymptomatic meningiomas, 5 were removed at the time of diagnosis. The other asymptomatic meningiomas (n = 11) were treated conservatively and when the tumors increased in size, surgical treatment was considered. "Operated" patients with residual or recurrent tumors underwent radiosurgery with a gamma knife.

Findings. Surgical mortality and morbidity were 4% and 16%, respectively. Of the 25 "operated" patients, 21 (84.0%) had a good Karnofsky scale (≥80%) at discharge. In all but two of the 11 patients with asymptomatic, conservatively treated meningiomas, the tumors did not increase during the follow-up period. Gamma knife radiosurgery, performed to treat 3 residual and 1 recurrent tumor, resulted in very good tumor control and none of the tumors increased after gamma knife surgery.

Conclusions. Meningiomas in elderly patients require a multimodal approach. Our strategy, which includes surgery, radiosurgery, and conservative treatment, resulted in good tumor control and made it possible for patients to pursue their activities of daily life.

Keywords: Meningioma; elderly patients; gamma knife; asymptomatic.

Introduction

The apparent increase in the incidence of brain tumors in the elderly is due in part to improved diagnostics such as computed tomography (CT) scanning and magnetic resonance imaging (MRI), and an increase in life expectancy [15]. Meningiomas are benign tumors which can be removed surgically and they are often curable. However, in elderly patients, surgical intervention is associated with significant morbidity and mortality [1, 2, 5, 9, 14, 16] although advances in microsurgical techniques and anesthetics have led to improved results [3, 4, 8, 12]. Niiro *et al.* [11] recommended surgical removal even in elderly patients with symptomatic meningiomas, however, the surgical indication for asymptomatic meningiomas remains unclear.

We monitor patients with asymptomatic meningiomas and obtain follow-up MRI at 3–6 month intervals. If the tumors increase in size, surgical resection is considered. The introduction of gamma knife surgery has resulted in the good control of residual and recurrent tumors [7] and it is now possible to manage meningiomas in the elderly multimodally. We now report the results of our strategy for treating elderly patients with meningiomas.

Materials and methods

Treatment strategy

In this study, patients over 70 years old are defined as elderly patients. Figure 1 is a flowchart of our treatment strategy. In principle, patients with symptomatic meningiomas are operated on. In patients with asymptomatic meningiomas we usually perform MRI at 3–6 month intervals. If the tumor size increases or the patient becomes symptomatic, surgical resection is considered. In cases where the tumor measures ≥3 cm, is located around eloquent areas, or is surrounded by severe peritumoral edema, surgery is performed at the time of diagnosis. The surgical risk in all patients is carefully evaluated by a neuro-anesthesiologist and/or other experts. If the tumor adheres to or shows evidence of invasion into important structures, we intentionally do not resect the adhering or invading portion(s) but deal with them radiosurgically using a gamma knife. Meningiomas that recur after initial resection are also treated by gamma knife.

Patients

Between 1994 and 2003, 131 intracranial meningiomas were diagnosed in our Department of Neurosurgery. Of these, 35 (26.7%) were