ALIGNANT gliomas are the most common tumor in the central nervous system. Despite recent advances in treatment, their prognosis remains poor. The 5-year survival rate in patients harboring a GBM, the most common and malignant glioma subtype, is less than 3%, whereas the 5-year survival rate for patients with a lower grade astrocytoma is 30%. Therefore, the identification of novel therapeutic strategies for malignant gliomas merits a high priority. In this regard, gene therapy with adenoviral vectors is a promising new modality for the treatment of glioma.

Adenoviral vectors may be genetically designed to express therapeutic genes in cancer cells. To this end, tumor-selective transgene expression is critical and can be obtained by placing the transgene under the control of a TSP. Specifically, to achieve a high therapeutic index with adenoviral vectors in vivo, repression of TSPs in normal tissues, primarily those of the liver, emerges as the most critical predictor of a high tumor/normal tissue ratio.

**Object.** Malignant brain tumors have been proved to be resistant to standard treatments and therefore require new therapeutic strategies. Survivin, a recently described member of the inhibitor of apoptosis protein family, is overexpressed in several human brain tumors, primarily gliomas, but is downregulated in normal tissues. The authors hypothesized that the expression of tumor-specific survivin could be exploited for treatment of gliomas by targeting the tumors with gene therapy vectors.

**Methods.** Following confirmation of survivin expression in glioma cell lines, an adenoviral vector containing the survivin promoter and the reporter gene luciferase was tested in established and primary glioma cells, normal astrocytic cells, and normal human brain tissues. High levels of reporter gene expression were observed in established tumor and primary tumor cell lines and low levels of expression in astrocytes and normal human brain tissue. To test oncolytic potency, the authors constructed survivin promoter–based conditionally replicative adenoviruses (CRAds), composed of survivin promoter–regulated E1 gene expression and an RGD-4C capsid modification. These CRAds could efficiently replicate within and kill a variety of established glioma tumor cells, but were inactive in a normal human liver organ culture. Finally, survivin promoter–based CRAds significantly inhibited the growth of glioma xenografts in vivo.

**Conclusions.** Together these data indicate that the survivin promoter is a promising tumor-specific promoter for transcriptional targeting of adenovirus-based vectors and CRAds for malignant gliomas. The strategy of using survivin–CRAds may thus translate into an experimental therapeutic approach that can be used in human clinical trials.

**Key Words** • survivin gene • tumor-specific promoter • transcriptional targeting • adenoviral vector • conditionally replicative adenovirus
ing do not fully meet these criteria, and therefore novel TSPs are required for transcriptional targeting of gliomas.

The recently discovered survivin gene encodes the survivin protein, a member of the IAP protein family, which plays an important role in the survival of cancer cells and the progression of malignancy. Members of the IAP family directly inhibit terminal effector caspases through baculovirus IAP repeat–dependent recognition, thereby preventing apoptosis. Survivin is normally expressed during embryogenesis and is undetectable in fully differentiated adult tissues. In contrast, survivin is repeatedly expressed in a broad spectrum of human cancers including brain, breast, pancreas, esophageal, and ovarian tumors.

Survivin-mediated suppression of apoptosis and growth factor–independent cell survival is implicated in the resistance of the tumor to standard therapy. In this regard, 80% of GBM cells demonstrate abundant survivin expression, and a clear correlation is seen between the histological grade of a glioma and the fraction of survivin-positive tumor samples. In glioma tumors, survivin expression is also correlated with resistance to chemo- and radiotherapy and with poor prognosis.

Given this promising role for survivin, we developed a survivin promoter–based adenoviral vector for the purpose of transcriptional targeting of gliomas. We hypothesized that this strategy of tumor-specific regulation of gene expression could provide a novel transcriptional targeting approach for human gliomas. Furthermore, we used this survivin TSP approach in the context of CRAds use for tumor-selective viral replication and oncolysis. To achieve this goal, a TSP is required to control E1 gene expression in the adenovirus. Although other TSPs have been previously suggested in the context of glioma, such as hTERT, oncostatin-M promoter (a hematopoietic cytokine), the vascular endothelial growth factor promoter, the c-Myc promoter activated in medulloblastoma, and gas 1, in this study we identified the survivin promoter as a uniquely transcriptional targeting strategy for human glioma.

Materials and Methods

Cells, Tissues, and Animals

Samples of the human glioma tumor cell lines U251MG, U87MG, U373MG, D54MG, and D65MG were kind gifts from Dr. G. Y. Gillespie (Department of Neurosurgery, University of Alabama at Birmingham). Samples of the M59K and U118MG cell lines were obtained from the American Type Culture Collection (Manassas, VA). Cells of the U87MG line were cultured in minimal essential medium (Mediatech, Herndon, VA) containing 10% FBS, glutamine, penicillin (100 IU/ml), and streptomycin (100 μg/ml). The other cell lines—U251MG, D54MG, U118MG, M59K, and D65MG—were cultured in DMEM–Ham F12 50:50 (Mediatech) containing 15% FBS and supplemented with l-glutamine, penicillin, and streptomycin as previously described. In addition, 911 cells (a kind gift from Dr. Van Der Eb, Leiden University, The Netherlands) were maintained in DMEM. Each medium was also supplemented with fetal calf serum, penicillin, and streptomycin. Astrocyte cells, a kind gift from Dr. Charles Bonus, were cultured in DMEM and supplemented with 10% FBS, penicillin, and streptomycin. The three primary glioma tumors, VU84, VU119, and VU78, were cultured in DMEM with 10% FBS, l-glutamine, and anti-biotic agents. The cells were incubated at 37°C in a humidified atmosphere of 95% air/5% CO₂. Three tissue slices were examined per group.

Analysis of Survivin Promoter Activity in Glioma Cells

A recombinant adenoviral vector, AdSurvivin, was in use in this study as reported previously. To determine the transcriptional activity of the survivin promoter, 5 × 10⁴ cells of each established glioma cell line (U251MG, U87MG, U373MG, M59K, U118MG, D54MG, and D65MG), primary glioma cells, and normal controls (primary mesothelial cells, derived in our department from ascites fluid, and keratinocytes), were plated into 24-well tissue plates and infected with Ad-CMV, Ad-Cox2, Ad-Mk, or Ad-S at an MOI of 100 (100 vp/cell) in 200 μl of growth medium containing 2% FBS (infection medium). The infection medium was replaced by fresh medium containing 10% FBS after 2 hours. Twenty-four hours postinfection, luciferase activity was determined using the Reporter Lysis Buffer and Luciferase Assay System (Promega, Madison, WI) following the manufacturer’s protocol. Experiments were performed in triplicate, and luciferase activities are presented as relative light units normalized to the CMV promoter activity.

Quantitative Real-Time PCR for Detection of the Human Survivin Gene and Ad5 E4 Gene Expression

Total cellular RNA or DNA was extracted from cell cultures in 6-well plates by using the RNAeasy mini RNA extraction kit or the blood DNA kit (Qiagen, Valencia, CA). Both RNA and DNA samples were treated with RNase-free DNase and DNase-free RNase, respectively, to remove possible contamination. The survivin gene transcripts were detected in RNA samples by using an oligo pair (forward primer 5’ TGGAGGCTGCGGCAGCA and reverse primer 5’ GAGTGGCAGACACCCCATG) and the probe (ORF6TGCGCTATGACAG)

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TACGACCAACACGATCT). The oligos were designed using Primer Express (version 1.0; Perkin-Elmer, Foster City, CA) and synthesized by Applied Biosystems. Specifics of the real-time PCR reaction have been described by us elsewhere. Negative controls without templates were included in each reaction series, and an internal control (human GAPDH or β-actin) was used to normalize the copy number for the survivin and E4 genes.

Survivin Promoter Activity in Normal Human Brain Tissue

Specimens of normal human brain were obtained following approval from our internal review board and consisted of 200-μm slices of gray matter from the temporal lobe, which were acquired during surgery for epilepsy. The brain slices were prepared as previously described by Verwer, et al. Experiments were performed on normal brain slices excised from two patients with epilepsy. On Day 2, the brain specimens were infected in quadruplicate with 2 × 10⁵ vp of Ad-S or Ad-CMV. On Day 5, the brain specimens were harvested and the luciferase assays were performed.

Analysis of CRAd Replication in Tumor Cell Lines

Glioma cells (10⁵/well) were cultured in the manner described earlier, infected with 100 vp/cell of CRAd-S-S, CRAd-S-L, Adwt, or Ad-S in infection medium containing 2% FBS, and incubated at 37°C in a 95% air/5% CO₂ environment. After 2 hours in incubation, the infection medium was removed, the cells were washed three times with PBS, and the cells were placed in fresh culture medium with 10% FBS. Media from triplicate wells were collected 1, 3, and 9 days postinfection; DNA was extracted from 200 μl of media by using the DNeasy Tissue Kit (Qiagen), and quantitative real-time PCR was performed in the manner described earlier. The adenovirus E4 gene copy numbers were detected.

Replication of CRAd in the Human Liver

Human liver organ cultures, described earlier, were infected with CRAd-S-S, CRAd-S-L, Adwt, or Ad-S (10⁵ vp/slice, ~ 500 vp/cell) and incubated at 37°C in a humidified atmosphere of 95% air/5% CO₂. Three tissue slices were included per group. After a 2-day incubation, total DNA was extracted from the human liver organ cultures by using the DNeasy Tissue Kit (Qiagen). The DNA samples were treated with DNase-free RNase to remove possible RNA contamination and were stored at −80°C until use. The AdS E4 gene copy numbers were detected and normalized by referral to human GAPDH.

Analysis of In Vitro Glioma Cell Killing

The in vitro cytotoxic effects of CRAd-S-S and CRAd-S-L were analyzed by performing crystal violet staining. Briefly, 25,000 cells (including D65MG, U251MG, U373MG, U87MG, U118MG, or OV4, an ovarian cancer cell line used as a control) per well were plated onto a 24-well plate, and the cells were infected at 100, 10, 1, and 0 vp/cell with CRAd-S-S, CRAd-S-L, or Ad-S in infection medium. Two hours later, the infection medium was replaced with complete medium. After 10 more days of culture, the cells were fixed with 10% buffered formalin for 10 minutes and stained with 1% crystal violet in 70% ethanol for 20 minutes, followed by washing three times with tap water and air drying. Trypan blue exclusion experiments also were performed.

Analysis of CRAd Replication in Glioma Xenografts

The U118MG glioma cells were injected into the flanks of nude mice (5 × 10⁵ cells in 200 μl PBS per tumor, five animals/group). When the resulting tumors reached 5 mm in diameter, 5 × 10⁵ vp of CRAd-S-S or Adwt diluted in 50 μl PBS were injected into each tumor. On Days 1 and 7 postinjection, the tumors were collected and snap-frozen with dry ice/ethanol. The DNA was extracted, and the relative E4 copy numbers were determined.

Antitumor Effect of CRAd on Mouse Xenografts

Five BALB/c nude mice in each group were inoculated in the flanks with 5 × 10⁵ U118MG cells. The tumor cells were verified to have 95% viability by Trypan blue exclusion. When the tumor reached 5 mm in its widest diameter, 10⁴ vp of viral vector (CRAd-S-S, CRAd-S-L, or Ad-S) or PBS was injected intratumorally. The vector Ad-S was used as a nonreplicative control virus with an identical viral backbone. The same dose was repeated after 1 week. Tumor volumes were monitored twice a week, and the volumes were calculated by using the formula 1/2 xy², where x is the longest diameter and y the shortest diameter of the tumor. Based on the determined volume, the following equations were applied.

Tumor volume ratio = tumor volume at Day 30/tumor volume at Day 1 × 100%.

Tumor growth inhibition rate = 100% − (tumor volume at Day 30/ tumor volume at Day 1 × 100%).

Statistical Analysis

The luciferase activities measured in this study were analyzed by performing the Student t-test using commercial software (Statistics Analysis Software version 8.2; SAS Inc., Cary, NC). A probability value less than 0.05 was considered statistically significant.

Results

Survivin Promoter is Active in Both Established and Primary Glioma Cells

Four recombinant adenoviral vectors, Ad-Cox2, Ad-Mk, Ad-S, and Ad-CMV, were used in these experiments. All recombinant adenoviruses are isogenic, thereby allowing the determination of promoter activity via reporter gene expression. The CMV promoter in Ad-CMV is constitutively active, thereby serving as a positive control to allow normalization for each given cell line. The data shown in Fig. 1 represent promoter activity in established and primary low-passage glioma cells. Of all three TSPs, the survivin promoter exhibited the highest activity in the established glioma cell lines, with a mean of 11.2% of the activity of the CMV promoter. In contrast, both the Cox-2 and midkine promoters were relatively inactive, showing less than 4% of the activity of the CMV promoter. The differences were significant (p < 0.01). All three promoters were inactive in both normal primary mesothelial cells and the normal keratinocyte cell line. Because established cancer cell lines may not reflect the true nature of cancer cells, we repeated these experiments in primary low-passage glioma cells. Again, we observed that the survivin promoter was the most active of all three promoters in three primary glioma cells.

The mean survivin promoter activity was 20% of the activity of the CMV promoter in three primary cells, but with wide variation among the three cell types (5, 12.5, and 42.5% of the activity of the CMV promoter in VU84, VU119, and VU78 cells, respectively). The mean Cox-2 and midkine promoter activities were less than 5% of the CMV promoter. Thus, the survivin promoter is repressed in normal cells and is active in both established and primary glioma cells.

Survivin Promoter is Less Active in Normal Astrocytes and Normal Human Brain

A critical determinant of any candidate transcriptional targeting approach for glioma is tumor specificity. In the previous section, we described our study of survivin promoter activity in glioma cells. Next, we tested whether this promoter may be applicable to glioma gene therapy, that is, whether it is inactive in the normal brain. To this end, we
evaluated survivin promoter activity in normal astrocytes and normal brain tissue. First, D65MG glioma cells and astrocytes were infected with either Ad-S or Ad-CMV (Fig. 2 upper right). The survivin promoter activity in the D65MG glioma cell line (12.6% of the activity of the CMV promoter) was 12-fold higher than that in astrocytes (1% of the activity of the CMV promoter). This pattern correlates well with survivin gene expression (Fig. 2 upper left). The transcript levels of the survivin gene were sixfold higher in the D65MG glioma cell line (310 copies/ng RNA) than in primary astrocytes (48 copies/ng RNA). Thus, high levels of survivin gene expression and survivin promoter activity were observed in glioma cells but not in normal astrocytes.

To examine the tumor selectivity of the survivin promot-
er more fully, normal human cerebral cortex tissues, which were excised during epilepsy surgeries performed in two patients, were evaluated for survivin promoter activity (Fig. 2 lower). Normal human cerebral cortex displayed less activity, showing only 0.26% of the activity of the CMV promoter (p < 0.01). Thus, the survivin promoter is down-regulated in the normal human brain.

**Survivin–CRAds Induce Cytotoxicity in Glioma Cell Lines**

Encouraged by the glioma-specific activity of the survivin promoter, we used two CRAds that had been constructed by this group and described previously as oncolytic antiglioma agents. The short segment of the survivin promoter, CRAd-S-S, and the long segment of the survivin promoter, CRAd-S-L, were evaluated to determine their cell-killing effect in a variety of glioma cell lines. Cytotoxicity was evaluated after 10 days of incubation by staining the tissue with crystal violet (Fig. 3). Although the replication-incompetent Ad-S vector had no cytotoxic effect even at 100 vp/cell, the survivin-based CRAds induced cytotoxicity in all glioma cancer cell lines. Oncolytic efficiency, however, varied in the glioma cell lines tested. While survivin-based CRAds were highly efficient even at 1 vp/cell in D65MG cells, cytotoxicity was observed at 10 vp/cell for CRAd-S-S and 100 vp/cell for CRAd-S-L in U251MG, U87MG, and U118MG cells; and cytotoxicity was observed for both CRAds only at 100 vp/cell in U373MG cells. In contrast, the OV4 ovarian cancer cells were resistant to the survivin-based CRAds. Thus, both

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Fig. 2. Expression of the survivin gene in glioma cells. **Upper Left:** Basal survivin gene expression is increased in glioma D65MG cells and is decreased in astrocytes. The results were obtained by performing quantitative real-time PCR for survivin transcripts. Total RNA was extracted from 10⁶ cells. Real-time PCR reactions for transcripts of the human survivin gene or the control housekeeping gene GAPDH or β-actin were performed under conditions described in Materials and Methods. The survivin transcriptional levels in both the D65MG cells and astrocytes were normalized to GAPDH levels and expressed as survivin copies per nanogram of RNA. *p < 0.05 and **p < 0.01 for a comparison of survivin transcripts in normal astrocytes and D65MG glioma cells. **Upper Right:** Specific survivin promoter activity correlates with basal survivin gene expression. Glioma D65MG cells and normal astrocytes were infected with Ad-S or Ad-CMV, respectively, at an MOI of 100. Luciferase activity was analyzed after 2 days of infection and is presented as percentages of CMV promoter activity. The values represent mean values of triplicate assays, and each point represents the mean and SD from three determinations. *p < 0.05 and **p < 0.01 for a comparison of survivin promoter activities in normal astrocytes and D65MG glioma cells. **Lower:** Survivin promoter activity is decreased in normal human brain. Survivin promoter activity was measured in human brain specimens excised from two patients during epilepsy surgeries. Normal brain specimens were infected in quadruplicate with either Ad-S or Ad-CMV. After a 72-hour incubation, luciferase activity was measured. Each point represents the mean and the SD from four determinations. **p < 0.01 for a comparison of survivin and CMV promoter activities.
CRAd-S-S and CRAd-S-L show substantial oncolytic activity in established glioma cell lines.

**Survivin Promoter–Regulated CRAds are Inactive in the Human Liver**

Due to the fact that adenoviral replication is species specific and because the major adenoviral toxicity in gene therapy trials is hepatic, we tested the replication of survivin–CRAds in human liver specimens in organ cultures. To evaluate viral replication indirectly, we measured \( E4 \) gene copy numbers with quantitative real-time PCR, performed on DNA isolated from human liver slices 2 days after infection with CRAd-S-S, CRAd-S-L, or Adwt (Fig. 4). The \( E4 \) copy numbers of the CRAd-S-S and CRAd-S-L agents were 1.5 and 2.5 orders of magnitude fewer than that of Adwt, respectively. Thus, survivin promoter–regulated CRAds exhibit a “liver-off” profile, especially CRAd-S-L.

**Replication of Survivin–CRAds In Vitro and In Vivo**

Next, CRAd-S-S and CRAd-S-L replications were compared in vitro to Adwt replication in D65MG glioma cells. Viral replication was determined indirectly by examining the \( E4 \) gene copy numbers on Days 1, 3, and 9 postinfection (Fig. 5 upper). The \( E4 \) copy numbers of CRAd-S-S (3.2 million copies on Day 3 and 12.8 million copies on Day 9) were higher than both CRAd-S-L (1.1 million copies on Day 3 and 2.3 million copies on Day 9) and Adwt (0.34 and 2.86 million copies on Days 3 and 9, respectively). On a time scale, the replication rates increased twofold, fourfold, and 8.3-fold from Day 3 to Day 9 for CRAd-S-L, CRAd-S-S, and Adwt, respectively. Thus, despite their decreased replication in the human liver, CRAd-S-L and CRAd-S-S maintain their replicative potential in glioma cells.

Because no established glioma xenograft animal models involve the use of D65MG cells, we used U118MG glioma cells. Xenografts of U118MG cells were injected with either Adwt or CRAd-S-S, and harvested either on Day 1 or Day 7 after injection. Next, DNA was extracted from tumor samples and the \( E4 \) gene copy numbers were measured using quantitative real-time PCR (Fig. 5 lower). The \( E4 \) copy numbers increased to 815 copies/ng DNA in tumors injected with Adwt and 2574 copies/ng DNA in tumors injected with CRAd-S-S; these increases constitute a 50-fold and a 160-fold increase in the replication rate on Day 7 for cells.
treated with Adwt and CRAd-S-S, respectively. Thus, although both Adwt and CRAd replicate well in vivo in a glioma xenograft model, survivin promoter–regulated CRAd replication was superior to that of Adwt.

**Antitumor Effects of Survivin–CRAds**

The antitumor effects of CRAd-S-S and CRAd-S-L were analyzed in vivo in the U118MG xenograft model, survivin promoter–regulated CRAd replication was superior to that of Adwt.

**Discussion**

Malignant gliomas (GBM and anaplastic astrocytoma) are the most common types of primary central nervous system tumors and together have an incidence of 5 to 8 per 100,000 persons. The median survival of patients with malignant glioma varies from 14 weeks among those who receive conservative treatment to 40 to 59 weeks for those who receive aggressive therapy. Because survival in patients with malignant glioma remains poor, novel strategies are urgently required. In this regard, oncolytic virotherapy with CRAd agents is a promising and emerging approach that is already used in other types of cancer. The hallmark of oncolytic virotherapy is the potential for tumor-
specific replication and cell killing. To this end, transcriptional targeting is essential. In our previous reports we identified the survivin promoter as an excellent TSP that exhibits a “tumor-on” and “liver-off” profile. In this study, we focused on the survivin promoter as a key regulatory TSP for glioma. First, we demonstrated, in a recombinant adenoviral vector background, that the survivin promoter is active in a variety of established and primary glioma cells. This promoter activity correlated well with the native survivin gene expression pattern in these glioma cells. Encouraged by these findings, we used survivin promoter–regulated CRAds, in which the E1 gene was driven by the human survivin promoter, to achieve tumor-specific oncolysis. Although these survivin–CRAds demonstrated a glioma cell–killing capacity in vitro as well as in vivo, we detected only minimal viral transcription in normal human cells including astrocytes. Consequently, survivin-based promoter transcriptional regulation emerges as a potentially applicable antitumor approach for glioma.

Other promoters have been proposed for glioma-specific therapy, such as glial fibrillary acidic protein (a brain-specific expression but not a TSP) and the hTERT TSP. In this study, the Cox-2 and midkine promoters were compared with the survivin promoter on the basis of their proven potential for glioma. Although all three TSPs were repressed in normal cells, the survivin promoter demonstrated superior activity in both established glioma cell lines (with a mean 11.2% of the activity of the CMV promoter compared with a mean of 4% activity of the CMV promoter for the Cox-2 and midkine promoters; Fig. 1 upper). More importantly, the activity in primary glioma cells was 20% of the activity of the CMV promoter compared with a mean of less than 5% activity of the CMV promoter for the Cox-2 and midkine promoters (Fig. 1 lower).

Although decreased expression of survivin gene in normal tissues is expected, it is encouraging that the promoter is expressed in glioma cells, especially in view of the correlation between survivin expression levels and the prognosis.
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of glioma. Moreover, the increasing expression of survivin in progressive grades of astrocytic tumors has important clinical application, as survivin-based therapies can be implemented across a spectrum of malignant brain tumors.

Given that replication-incompetent vectors have proved to be inefficient as antitumor agents, replication-selective viruses have emerged as potentially powerful anticancer agents. Based on their capacity for gene amplification and intratumoral production of viral progeny, the intrinsic cell killing of adenoviruses has been diverted into CRAd oncolysis. These attributes of CRAds have recently been applied in the context of glioma. In this study, we constructed two novel CRAd agents, CRAd-S-S (nucleotides 230 to +30 of the human survivin gene) and CRAd-S-L (nucleotides −1430 to +30 of the human survivin gene), in which the survivin promoter regulated Ad5 E1 gene expression and viral infectivity was enhanced by capsid modification, that is, genetic insertion of the RGD-4C ligand. Of note, the E3 gene was retained in these CRAds for improved cell killing. Nevertheless, despite the cell killing attributes of our survivin-based CRAds, they maintained safe profiles in both normal brain and liver. Because the liver manifests a 95% uptake rate of adenovirus when delivered systemically, thereby potentially compromising liver function, the safety profile of survivin-based CRAds in liver tissue (1.5 and 2.5 orders of magnitude fewer E4 copies than those found in Adwt, CRAd-S-S, and CRAd-S-L, respectively) is especially encouraging. Furthermore, because malignant gliomas do not metastasize, local CRAd injection may be applicable on the basis of dramatic repression of the survivin promoter in normal human astrocytes and brain tissue.

The in vitro oncolytic effects of CRAd-S-S and CRAd-S-L were translated into antitumor effects in a glioma xenograft model and correlated with intratumoral replication rates. The difference we observed between the short and long versions of the survivin promoter in the CRAd context was that the long version of the survivin promoter was less hepatotoxic than the short version. These data are reminiscent of the Cox-2 promoter versions previously described. Thus, CRAd-S-L maintains an antitumor effect, while minimizing hepatotoxicity to increase the therapeutic index, an important parameter in cancer gene therapy.

In this study, the viral replication of CRAds was regulated by a tumor-specific promoter, the survivin promoter. Thus, viral replication was limited to tumor cells and minimized in normal host cells. However, adenoviruses are able to kill normal cells as well as cancer cells by leaking vectors, leading to host toxicity. The potential of killing normal cells relates to the promoter activity in normal cells. For this reason, we tested the promoter activity in human brain tissue. The data shown in Fig. 2 lower revealed only 0.26% of the activity of the CMV promoter, indicating that the toxicity of leakage of these agents to brain tissue is low because of the TSP.

Conclusions

We identified the human survivin promoter as a tumor-specific regulatory element for glioma. It is activated in established and primary glioma cells and is downregulated in normal astrocytes and human brain and liver tissues. The survivin promoter therefore represents a promising trans-


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