Use of the Glucose Starvation-inducible Glucose-regulated Protein 78 Promoter in Suicide Gene Therapy of Murine Fibrosarcoma

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ABSTRACT

A new strategy in anticancer gene therapy uses stress-responsive cellular promoters that offer the advantage of enhanced gene expression in a variety of tumors. Although the feasibility of their selective expression has been demonstrated, functional evidence of their ability to activate therapeutic agents within the tumor environment leading to tumor eradication has not been established. Glucose deprivation, chronic anoxia, and acidic pH known to persist in poorly vascularized solid tumors strongly induce the transcription of the glucose-regulated protein 78 (grp78) gene, which encodes an M₆, 78,000 stress-inducible protein. In this report, we tested directly the efficacy of the grp78 promoter in a retroviral system to drive the expression of the herpes simplex virus-thymidine kinase (HSVtk) suicide gene, using a murine fibrosarcoma model, in the context of their syngeneic, immunocompetent hosts. Our results showed that under glucose starvation conditions, the expression of HSVTK was enhanced in tumor cells where the HSVtk gene was driven by the internal grp78 promoter, in contrast to the Moloney murine leukemia virus long terminal repeat, where suppression was observed. We further demonstrated that in vivo, HSVTK expression was elevated to much higher levels inside tumors when driven by the internal grp78 promoter, resulting in complete eradication of sizable tumor mass, with no recurrence of tumor growth. Our study suggests that the glucose starvation-inducible grp78 promoter could be useful for enhanced expression of a variety of therapeutic agents within the solid tumor environment.

INTRODUCTION

Of the several strategies that have been developed to accomplish gene therapy of cancer, the suicide gene approach can be an effective method for cancer gene therapy (1, 2). In addition to achieving selective antitumor effects after administration of the prodrug, suicide gene therapy exhibits the desirable bystander effect such that when only a fraction of tumor mass is genetically modified, tumor regression can be achieved (3). Furthermore, in mice with a competent immune system, establishment of immunological memory specific for immunogenic tumors has been reported after suicide gene therapy (4–8). This raises the possibility of using tumors transduced with suicide genes as live antitumor vaccines. If successful, this could lead to elimination of metastasized tumors, a major challenge in cancer gene therapy. Although this approach is presently used in a variety of clinical trials to selectively eliminate cancer cells (9, 10), targeting gene expression is one of the most difficult and important goals in effective cancer gene therapy. There are two major strategies: targetable entry and tissue or cell-type-specific gene expression. Targetable entry involves vector engineering to change vector binding tropism, thus allowing cell-type-specific transduction. Alternatively, tumor cell-specific expression relies on restricting expression of the delivered gene exclusively to the tumor. For this purpose, tissue-specific or disease-specific promoters have been identified that allow targeted gene expression in vivo (11, 12). For example, the tyrosinase and fetal a-fetoprotein gene promoters have been exploited to direct expression of foreign genes in melanomas and liver cancers, respectively (13, 14). Retroviral vectors regulated through a tetracycline-responsive system have also been devised (15).

Successful application of the suicide gene approach in vivo requires maximization of expression of the suicide gene in the tumor environment, in addition to effective prodrug delivery. Presently, a majority of approved clinical protocols use retroviral vectors as the gene-delivery system (10). The most common promoter used to drive expression of the foreign gene has been a constitutive, general-purpose viral promoter such as the MuLV4 LTR also used in this study. These promoters, although effective in vitro, often fail to express the genes under their control once cells are transferred back into a syngeneic host (16). Furthermore, a previous study examining the effect of glucose availability on the HaMSV enhancer/promoter located in the retroviral LTR revealed that the activity of the HaMSV LTR was suppressed under glucose-free conditions (17). These results suggest that within the environment of a fast-growing solid tumor devoid of nutrient due to insufficient blood supply, the viral LTR may be suppressed and unable to sustain foreign gene expression. Other viral promoters such as SV40 and cytomegalovirus are also widely used as internal promoters of retroviral vectors to express the foreign gene, with the cytomegalovirus promoter being a highly potent, constitutive promoter in vitro (18). Nonetheless, mammalian cells appear to be able to recognize viral promoters as foreign and inactivate them by methylation or other mechanisms (10). In previous studies in the B/C10ME fibrosarcoma system, the expression of the marker gene driven by SV40 in tumors was considerably lower than that driven by the grp78 promoter (17). Furthermore, even if these viral promoters escape genomic silencing, the expression pattern of the foreign gene will be constitutive in normal as well as tumor cells. This unregulated expression could be highly problematic in cancer gene therapy.

To circumvent these difficulties, using the cell’s own cis-regulatory sequences may increase the chance of sustaining longer-term gene expression and also offer the prospect of inducible and regulated expression of the foreign gene. A novel approach toward anticancer therapy uses stress-responsive promoters that are active in a variety of tumors. Previous studies demonstrated the feasibility of intratumoral control of tumor necrosis factor-a gene expression using a radiation-inducible promoter (19). Recently, it has been shown that the hypoxic-responsive element from the mouse phosphoglycerate kinase-1 gene can be used to control expression of marker and therapeutic genes in response to hypoxia in vitro and within a solid tumor in vivo (20). Despite these significant advances, functional data on the ability of specific cellular promoter elements to induce the activation of non-toxic prodrugs that become tumorcidal in vivo have not yet been established.

For most solid human tumors, during tumor growth, angiogenesis

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The abbreviations used are: MuLV, murine leukemia virus; LTR, long terminal repeat; HaMSV, Harvey murine sarcoma virus; GRP, glucose-regulated protein; ERSE, endoplasmic reticulum stress element; GCV, ganciclovir; DAB, 3,3-diaminobenzidine.
leads to the formation of capillary sprouts with eventual development of a tumor microcapillary network (21). However, most fast-growing tumors have a heterogeneous distribution of blood supply; by having a high interstitial and a low intravascular pressure, a decrease in nutrient supply results, leading to necrosis in the center of the tumor. Glucose deprivation, chronic anoxia, and low pH known to persist in poorly vascularized solid tumors induce a class of stress proteins referred to as the GRPs (17, 22–26). The grp78 gene encodes for an endoplasmic reticulum-localized, $M_r$ 78,000 molecular chaperone, also known as BiP, which is the major stress-inducible protein in a variety of chemically, virally, or radiation-transformed cells (27).

Previously, we have demonstrated that a rat grp78 promoter can be used as a potent internal promoter in a retroviral vector to drive high level expression of the neomycin phosphotransferase (neo) reporter gene in a murine fibrosarcoma model system (17). As a proof-of-principle, we showed in vivo correlation between high level of expression of the grp78 mRNA and the neo mRNA driven by the internal grp78 promoter in tumor sections, through the use of in situ hybridization. This was in contrast to the low neo mRNA expression level driven by the internal SV40 promoter, in the context of a genetically identical construct. In this study, we test directly the efficacy of the grp78 promoter in a retroviral system to drive the expression of the HSVtk suicide gene using the fibrosarcoma model system described above. The model system used in this study, unlike other analyses using nude mouse models, takes advantage of a syngeneic immunocompetent host, the BALB/c mouse. Such model systems are useful because they more closely resemble a clinical situation. Furthermore, evidence has accumulated that complete eradication of tumors expressing suicide genes necessitates host immune response and induction of immunological memory (4, 5, 7).

Here we compare the efficacy of the grp78 promoter with the MuLV LTR presently used extensively to drive expression of heterologous genes in clinical trials. Our results show that under glucose starvation, the expression of HSVVTK as well as in vitro GCV killing is enhanced in tumor cells where the HSVtk gene is driven by the internal grp78 promoter, resulting in highly efficient GCV killing through bystander effects. In vivo with large tumors, after GCV treatment, the tumor mass persists when the HSVtk gene is driven by the retroviral LTR. In contrast, HSVVTK expression is elevated to much higher levels inside tumors transduced with the retroviral vector containing an internal grp78 promoter. Eradication of the tumor is observed, and mice remain tumor-free after GCV withdrawal.

**MATERIALS AND METHODS**

**Cell Culture Conditions.** The tumor cell line B/C10ME was cultured in high glucose DMEM containing 4.5 mg/ml glucose supplemented with 10% FCS, 2 mm glutamine, and 1% penicillin-streptomycin-neomycin antibiotics. Transduced B/C10ME cells were maintained in 2 ng/ml of G418, respectively.

To generate individual transduced clones, transduced cells were plated into 96-well plates by serial dilution to a final concentration of 0.3 cell/well. Individual clones were then isolated and expanded.

**Retroviral Vector Construction.** The G1TkSvNa retroviral construct (28) was obtained from Genetic Therapy, Inc. (Novartis, Summit, NJ). G1NaGrpTk (Fig. 1) was constructed by removing the 356-bp SV40 promoter region of a retroviral vector G1NaSvTk (29) by SalI and BglII and replaced with a 695-bp rat grp78 promoter spanning –520 to +175 (30). Retroviral vector plasmid DNA was prepared by Qiagen Maxi kit and transfected into ecotropic retroviral producer cell line PES01. The viral supernatant was harvested, an amphotropic retroviral producer cell line PA317 was transduced, and drug (G418)-resistant clones were selected. Retroviral vectors were collected and titered by NIH3T3 cells.

**Western Blot.** For the detection of HSVTK, GRP78, and $\beta$-actin, 20 µg of cell lysate was prepared as described previously (31), resolved on a denaturing SDS-8% polyacrylamide gel, and transferred onto Hybond nitrocellulose membrane (Amersham Life Science, Inc., Arlington Heights, IL). The membrane was blocked with 5% nonfat milk (Bio-Rad Laboratories, Hercules, CA) in TBS buffer (20 mM Tris-HCl, pH 7.5, 140 mM NaCl) for 1 h at room temperature prior to the incubation with polyclonal rabbit anti-HSVtk antibody (obtained from Dr. W. Summers, Yale University School of Medicine, New Haven, CT), or monoclonal mouse anti-GRP78 antibody (StressGen, British Columbia, Canada), or monoclonal mouse anti-$\beta$-actin antibody (Sigma Chemical Co.) 1 h at room temperature. For all of the primary antibodies, 1:1000 dilutions were used. The secondary antibodies used were: goat anti-rabbit IgG conjugated with horseradish peroxidase (Promega Corp., Madison, WI) and diluted 1:3000 in TBS buffer for detecting HSVTK; and goat antimouse IgG conjugated with horseradish peroxidase (Promega) and diluted 1:5000 in TBS buffer for detecting GRP78 and $\beta$-actin. The immunocomplexes were detected with the Enhanced Chemiluminescence (ECL) kit (Amersham Life Science Inc.).

**In Vitro GCV Sensitivity Assay.** Individual clones of B/C10ME cells transduced with either the G1TkSvNa or the G1NaGrpTk retroviral vector were seeded in duplicate at 5 x 10^3 cells/well in a six-well plate. On day three after seeding, the cells were incubated with either control medium or 0.1 µg/ml GCV. Fresh GCV was added daily to the cells, which were counted every 3 days using the trypan blue dye exclusion method. For glucose starvation treatment, on the second day after seeding, the cells were maintained on glucose-free DMEM supplemented with diazylated FCS for a period of 30 h. After 30 h, the cells were incubated with 0.1 µg/ml GCV. GCV was added daily, whereas the culture medium was changed every third day for all cell cultures. When the cells reached ~70% confluency, the cultures were transferred to 10-cm diameter dishes.

**Assay for In Vitro Bystander Effect.** To measure the GCV killing effect, nontransduced B/C10ME cells were cocultured with different ratios of B/C10ME clonal cell lines stably transfected with G1NaGrpTk. Typically, a total of 3000 cells with various ratios (90%-10%, 75%-25%, and 50%-50%) were plated in quadruplicate in 96-well plates and treated with 10 µg/ml GCV for 10 days. The number of remaining viable cells was measured by cell proliferation assay (Promega).

**Tumor Formation.** Confluent cultures of B/C10ME clones were harvested with trypsin-EDTA (Life Technologies, Inc.) and washed three times in PBS. Approximately 2 x 10^6 viable cells were resuspended in 200 µl of PBS.
BALB/c mice (6–8 weeks of age), obtained from The Jackson Laboratory, were s.c. injected with an 18-gauge needle in their right flank. Tumors were palpable within 12 days of inoculation, and biperpendicular measurements were taken of the progressively growing tumor daily. Tumor growth was monitored by measurement of the larger and smaller diameters. At the indicated times after injection, mice were injected with GCV daily at a dosage of 100 mg/kg of body weight for ~10 days. Tumors were judged to have regressed after losing both measurability and palpability. For each retroviral construct, multiple injections of two to three independently derived transduced clonal cell lines were performed.

**Immunohistochemistry.** Tumor tissues were removed, stored at ~80°C, and cut by cryostat to 4-μm sections. The frozen sections were fixed by 10% formalin solution for 15 min and treated with 3% hydrogen peroxide. A rabbit polyclonal antibody against HSVTK (supplied by Dr. W. Summers, Yale University Radiobiology Laboratory, New Haven, CT) was added to the sections for 1 h at room temperature. After washing three times with PBS, a horseradish peroxidase-labeled polymer conjugated to goat anti-rabbit antibody (Dako, Carpenteria, CA) was added and incubated for 30 min. After three washes with PBS, the slides were stained with DAB, counterstained with methyl-green, covered with regular Permount, and viewed under a Zeiss microscope.

**RESULTS**

**Unique Features of Stress-inducible grp78/BiP Promoter.** Under glucose starvation and anaerobic conditions, the grp78 promoter is highly induced. The mammalian grp78 promoter functionally redundant and contains multiple stress-inducible elements interacting with the CBF and YY1 transcription factors (32–34). The genetic code for endoplasmic reticulum stress signaling leading to grp gene induction consists of two units of a 19-bp sequence motif (CCAT)N6(CCACG) termed ERSE. This sequence contains a tricistronic structure, with a high affinity CBF/NF-Y binding site separated by precisely 9 bp of a GC-rich sequence motif to a low affinity YY1 binding site (35).

In the construction of the retroviral vector G1NaGrpTk, the rat grp78 promoter, spanning 520 bp upstream and 175 bp downstream of the site of initiation of transcription, serves as an internal promoter driving the expression of the HSVtk gene (Fig. 1). This 695-bp grp78 promoter subfragment contains three ERSEs, a TATA element, and an internal ribosome entry site, a unique and useful feature of the 5’ untranslated region of grp78 to allow internal initiation of translation (36). In the G1NaGrpTk vector, the MuLV LTR directs the expression of the neo gene, which is used as a selection marker. For comparison, instead of using a retroviral vector with another internal promoter such as SV40, which has previously been shown to be ineffective to drive a reporter gene in a tumor environment (17), the G1TkSvNa retroviral vector was used. In this vector, the viral LTR drives the expression of the HSVtk gene, whereas the SV40 promoter drives neo expression (Fig. 1). The rationale for choosing G1TkSvNa is that it represents an improved retroviral vector for suicide gene therapy (28) and is the vector of choice in current clinical protocols (10). Both vectors were transduced into B/C10ME, a murine fibrosarcoma cell line that is syngeneic with the BALB/c mice. The advantage of the B/C10ME as a model system is that it has been previously established that kinetics of tumor growth and subsequent regression can be readily monitored in the recipient mice (37).

**Glucose Deprivation Induces grp78-driven HSVtk Expression in Vitro.** To create clonal B/C10ME cell lines with stably integrated retroviral vectors, the cells infected with the retroviruses were selected with G418. Serial dilution plating was performed after selection to isolate individual clones. The individual clones were expanded and analyzed. Under standard culture conditions, B/C10ME cells transduced with either retroviral construct exhibited equivalent plating efficiencies and growth rates (see below). Thus, the basic growth properties of the transduced cells in vitro were similar.

To test for the efficacy of the LTR and the grp78 promoter to drive expression of the HSVTK protein, total cell lysates were prepared from individual clonal lines under normal culture and glucose-starved conditions. The proteins were separated by SDS-PAGE and subjected to Western blot analysis. The levels of HSVTK, GRP78, and β-actin in each sample were measured. As expected, there was no detectable HSVTK in the nontransduced B/C10ME cells (Fig. 2). In the clonal line with the HSVtk gene driven by the LTR, there was HSVTK expression under normal culture conditions. However, when the cells were subjected to glucose starvation for 24 h, whereas the level of GRP78 was induced as expected, the level of HSVTK was reduced, consistent with our previous observation that the transcription activity of viral LTR was suppressed in glucose-starved cells (17). In contrast, in the clonal cell lines with the HSVtk gene driven by the grp78 promoter, the level of HSVTK was up-regulated in glucose-starved cells (Fig. 2).

To analyze HSVtk activity under normal and glucose-starved conditions, clonal cell lines derived from B/C10ME transduced cells with each respective retroviral construct were analyzed using an in vitro GCV-sensitivity assay (Fig. 3). For this purpose, about 5000 cells were seeded in duplicates in six-well plates, and on the third day of seeding, either remained untreated or incubated with 0.1 μg/ml of GCV. One set of cells was cultured in normal culture medium containing 4.5 mg/ml of glucose, and an identical set of cells was maintained in glucose-free medium supplemented with dialyzed FCS for 30 h before the addition of GCV. An example of the GCV survival test for a typical B/C10ME-derived clone transduced with G1TkSvNa (G1TkSvNa/clone no. 3) is shown in Fig. 3A. Without the addition of GCV, the cells continued to grow exponentially, and by the end of the 12th day, the cell number had reached $4 \times 10^6$. The addition of GCV...
resulted in loss of live cells at a similar rate for both sets of cells. By the end of the 12th day, about 1000 cells survived (Fig. 3A). Thus, for the LTR-driven HSVtk, the sensitivity to GCV was similar in cells cultured in normal or glucose-free medium.

The results of the GCV survival assay for a typical clonal line (G1NaGrpTk/clone no. 3) derived from B/C10ME cells transduced with G1NaGrpTk are shown in Fig. 3B. Under normal culture conditions, the growth rate as well as sensitivity to GCV was similar to that driven by the LTR. However, in contrast to the LTR-driven HSVtk cells, when G1NaGrpTk transduced cells were pretreated with the glucose-free medium, the decrease in viable cells was much more pronounced. Thus, by day 9, there were no more surviving cells. Furthermore, to demonstrate that these cells exhibit a bystander effect, HSVTK-positive cells were cocultured with various ratios of non-transduced HSVTK-negative cells. Over 90% killing was observed when only 10% of G1NaGrpTk cells are present in the culture (Fig. 3C). Collectively, these in vitro studies show that the retroviral construct containing an internal grp78 promoter produces higher levels of HSVtk inducible by glucose deprivation, thereby enhancing the sensitivity of tumor cells to GCV.

Complete Eradication of Tumors in G1NaGrpTk Transduced Cells. To directly compare the therapeutic efficacy of the G1NaGrpTk vector with G1TkSvNa, B/C10ME clones transduced with the respective retroviral constructs were injected s.c. at a dose of $2 \times 10^7$ cells per BALB/c mouse. As controls, the parental, nontransduced cells were also injected. Tumors were palpable after 12 days of injection. At day 21, when the average tumor diameter reached $\sim$2 cm, GCV was administered. The rationale for starting the GCV treatment when the tumor had reached a sizable mass instead of just being palpable is that this will offer a more vigorous test for the potency of the retroviral vectors. For the parental B/C10ME cells, as expected, upon addition of GCV, the tumors continued to grow at various rates, and growth was arrested as tumors reached substantial mass (Fig. 4A). In the nine mice injected with three different G1TkSvNa clonal cell lines, the majority of tumor growth was arrested upon GCV treatment for 2–3 days, but subsequently, tumor growth continued (Fig. 4B). Thus, at this stage of tumor growth, the LTR-driven HSVtk was insufficient to mediate efficient GCV toxicity. In contrast, in mice injected with the G1NaGrpTk clonal cell lines containing the internal grp78 promoter driving HSVtk expression, tumor regression was observed in all four mice injected with two independently derived clonal lines after GCV treatment. By day 29, there were no visible tumors in any of the animals (Fig. 4C). Complete tumor eradication was also observed in mouse mammary tumor clonal cell lines transduced with G1NaGrpTk. All mice remained healthy and developed no tumors after withdrawal of the GCV treatment.

To confirm that higher efficacy of G1NaGrpTk is due to higher expression of HSVTK within the tumor, immunohistochemistry staining for the HSVTK protein was performed with the tumor tissues. Examples of the immunohistochemistry staining using antibody against HSVTK in B/C10ME tumors are shown in Fig. 5. As expected, the parental cells showed the absence of HSVTK protein staining (Fig. 5A). A much higher level of staining was detected in tumors derived from G1NaGrpTk transduced cells (Fig. 5C) as compared with that derived from G1TkSvNa (Fig. 5B). Notably, the HSVTK staining for the G1TkSvNa was in isolated patches, suggesting there were areas within the tumor unfavorable for LTR-driven

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**Figure 3.** In vitro GCV-sensitivity assay for B/C10ME cells. A, about $5 \times 10^3$ G1TkSvNa/clone #3 cells were seeded in duplicate into six-well plates and incubated without (X) or with 0.1 (●) or 0.01 (○) μg/ml GCV starting at day 3 as indicated. The cells were then incubated in normal medium (—) or pretreated in glucose-free medium (---), and the number of surviving cells was determined by the trypan blue exclusion method. B, same as A except that G1NaGrpTk/clone #3 cells were used. C, in vitro bystander effect, nontransduced B/C10ME cells (TK-) were cocultured with a different ratio of B/C10ME clonal cell lines stably transduced with G1NaGrpTk. A total of 3000 cells with various ratios were plated in quadruplicate in 96-well plates and treated with 10 μg/ml GCV for 10 days. The number of remaining viable cells was measured by cell proliferation assay. Bars, SD.

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5 X. Chen, D. Zhang, and A. S. Lee, data not shown.
gene expression. In contrast, the staining for G1NaGrpTk was much more enhanced across the tumor section, as observed previously with the endogenous grp78 transcript and the neo mRNA driven by the grp78 promoter (17). Thus, within the tumor environment, G1NaGrpTk containing an internal stress-inducible grp78 promoter is more effective in directing high-level HSVTK expression than the retroviral LTR.

**DISCUSSION**

In cancer gene therapy, a major technical difficulty is the lack of specificity in targeting suicide gene expression in the anatomical site of tumors. To address this issue, we used a novel approach to use a stress-inducible promoter from the grp78 gene to direct the expression of the HSVtk gene in solid tumors. Increased GRP78 expression is detected in chemical- and radiation-transformed cells (27), as well as in tumor cells that become drug resistant (38, 39). Within the tumor environment, glucose deprivation, chronic anoxia, and acidic pH induce the GRPs, in particular GRP78 (22, 23, 40). Thus, grp78 mRNA levels are elevated in a variety of tumors, correlating with tumor size (17, 41). These results strongly suggest that in regions of the tumors deprived of glucose and oxygen, the cells experience a stress response resulting in the specific activation of the grp78 promoter.

Here, we have shown that a truncated rat grp78 promoter (with most of the distal basal elements removed while retaining its array of stress-inducible elements; Fig. 1), when used as an internal promoter in a retroviral construct, can drive high level expression of HSVTK in vitro under glucose-starved conditions (Figs. 2 and 3). These in vitro studies confirm our previous observation that the internal grp78 promoter is capable of inducing a high level of marker gene transcript in glucose-deprived cells, in contrast to the HaMSV LTR which was repressed (17). The latter observation could be one explanation for our observation here that for tumors which had reached a sizable tumor mass, the G1TkSvNa retroviral vector was relatively ineffective in reducing tumor mass after GCV treatment (Fig. 4). In vivo, the G1NaGrpTk retroviral vector was highly effective in directing HSVTK expression within the tumor environment (Fig. 5), leading to
complete eradication of sizable tumors in their syngeneic host after GCV treatment. The potency of G1NaGrpTk, coupled with the known bystander effects of suicide gene approach, suggests that this type of vector could offer a distinct advantage in solid tumor cancer therapy.

Another potentially significant observation of our studies is that the mice with regresssed tumors remained tumor free after withdrawal of GCV treatment. One speculation is that protective immunity might have been induced in these mice, preventing regrowth of tumors. In support of this idea, there are several examples of long-lasting antitumor immunity in various tumor models in response to HSVtk transduction and GCV treatments (42–45). Furthermore, it has been reported recently that the immune response, elicited by mammary adenocarcinoma cells transduced with IFN-γ and suicide genes, may induce regression of lung metastases (8). This leads to the question of whether tumors transduced with suicide genes can be used as live antitumor vaccines (44). Although it could be argued that many human tumors are not immunogenic and hence may not qualify for this treatment approach, recently it has been discovered that induction of apoptosis in tumor cells leads to a dramatic change in antigen presentation that could lead to enhancement of the cell-mediated immune response to the tumor. Future investigation into the mechanism of immunogenic memory of the mice with regresssed tumors will address this important issue.

In conclusion, the application of the stress-inducible grp78 promoter in clinical trials to express suicide gene or in combination with cytokine genes (46) in retroviral vectors or other gene delivery systems in a variety of tumors warrants further investigation. Future investigation into the feasibility of coupling the grp78 promoter stress control element to other tumor-specific stress elements, such as the hypoxic response element (11, 20), will directly test whether a tumor-specific promoter with stringent yet sustained expression in a tumor environment is achievable.

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