

Past, present and future perspectives of genetic therapy in gliomas

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High-grade gliomas are relatively frequent in adults and consist in the most malignant form of primary brain tumor. They are resistant to standard treatment modalities such as surgery, radiation, and chemotherapy, being fatal within 1 to 2 years of onset of symptoms. Owing to the promising practical clinical benefits that can be expected for the near future, an exposition of the basic issues in genetic therapy of gliomas seems timely. In this article we intend to provide a general review that covers the most important genes already studied as possible agents for genetic therapy in gliomas. In a critical analysis we intend to expose and discuss anti-tumoral mechanisms and therapeutical results of studies with the following class of genes: prodrug activation systems, apoptosis-related genes, anti-angiogenic factors genes, radiosensitization genes, chemosensitization genes, apoptosis-related genes and immunogenes. Finally we discuss the historical importance, actual role and further developments that can be expected from each of these class of agents for the future of genetic therapy of gliomas.

Key Words: Brain tumors, genetic therapy, gene transfer, high-grade gliomas

Introduction

Gliomas in adults are devastating diseases, with very poor survival despite their lack of distant metastases. The best available treatments, such as surgical resection and radiotherapy have been only temporarily successful, once recidives are almost always present, being fatal within 1 to 2 years of onset of first symptoms. Two factors promoting the use of gene therapy for gliomas are the failure and toxicity of conventional thera-

pies and the identification of the genetic abnormalities that contribute to the malignancy of gliomas.

Uncontrolled cellular proliferation, lack of apoptosis, invasion, and angiogenesis are among the biologic processes that render these tumors both aggressive and difficult to treat. Until recently, our knowledge of this type of tumor has mainly been based on chromosomal and molecular studies performed in the last two decades. This has increased tremendously with the advent of new technologies, in particular expression arrays for simultaneous analysis of thousands of genes.

During the malignant progression of gliomas several tumor suppressor genes are inactivated, and numerous growth factors and oncogenes are overexpressed progressively. Consequently, gene therapy of gliomas may aim at molecular interference with 'gain of function' genes (oncogenes) or replacement of 'loss of function' genes (tumor suppressor genes). Such approaches require transgene expression in whole tumor cell populations (if not other mechanisms come into play) which cannot be achieved with current vector systems. Hence other strategies have been pursued which may be independent of genes actually involved in tumorigenesis. Microbial genes (e.g. herpes simplex virus thymidine kinase) may be transferred into the tumors allowing for prodrug activation (e.g. ganciclovir). Furthermore, that frequently involves ex vivo transfection of autologous tumor cells with such genes.^[1]

Malignant gliomas have been chosen for the first clini-

cal studies on novel gene therapy approaches because these tumors are non-metastatic and develop on the largely postmitotic background of normal glial and neuronal tissue. Several molecular strategies have been tested, either in animal models or clinical trials: prodrug activating systems, introduction of tumor suppressor or cell-cycle-related genes, inhibition of growth factors and/or their receptors, inhibition of neovascularization, immunomodulatory maneuvers, oncolytic viruses, inhibition of matrix metalloproteinases, induction of local expression of toxic agents and sensitization of tumors to chemotherapeutic agents and radiotherapy.^[2,3]

Critical evaluation of gene transfer and therapy studies has led to the conclusion that, even using identical vectors, the anatomical route of vector can dramatically affect both the efficiency of tumor transduction and its spatial distribution, as well as the extent of intratumoral and intracerebral transgene expression. The safety and efficiency of these therapeutic systems in humans have been availed by several controlled preclinical and clinical therapeutic trials.^[4-6]

Genes

Prodrug Activation Systems HSV-TK/Ganciclovir

Tumor cell transduction with herpes simplex virus thymidine kinase (HSV-TK) gene and treatment with ganciclovir (GCV) is the more widely studied cancer gene therapy.^[7-11] HSV-TK converts the pro-drug ganciclovir (GCV) into a toxic nucleotide analogue, the incorporation of which into cellular DNA blocks cell proliferation. Following repetitive ganciclovir (GCV) intraperitoneal or intravenous injection, effective killing of glioma cells in mouse brain is observed [Figure 1]. In vivo studies relate a significant regression of the tumors in 50-80% of the rats treated with this therapy modality as compared with control animals. In some animals tumors completely disappeared after this treatment protocol and mice were considered are completely cured.^[12] [Figure 2]

Posterior analysis of the inoculated tissue showed that transduced glioma cells are found far from the implantation site, potentiating the induction of the therapeutic effect, what was explained as result of what was called “bystander effect”. The “bystander effect” is a paradigm

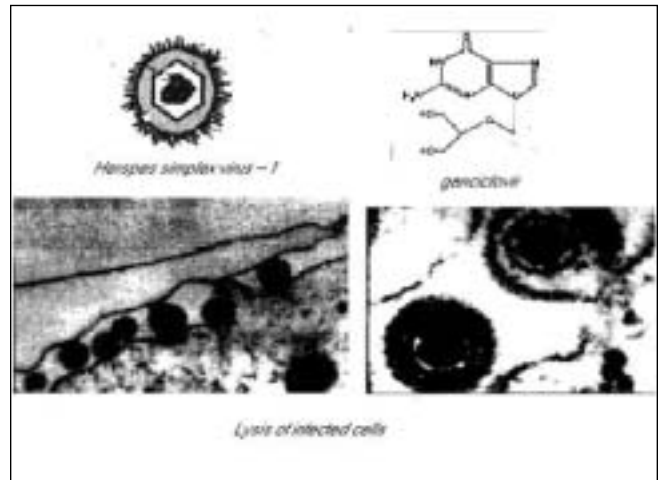


Figure 1: Therapy with HSV-tk oncolytic virus and further ganciclovir injection

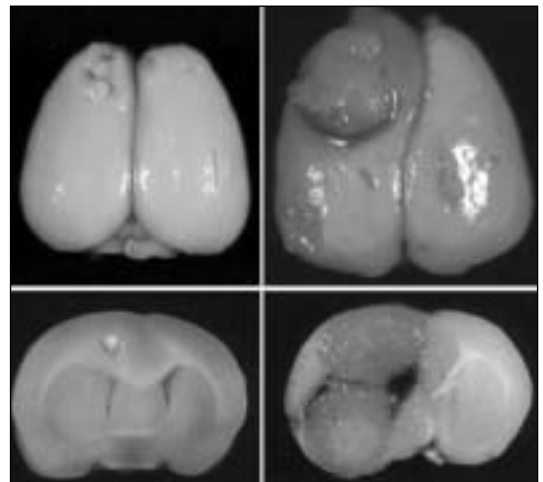


Figure 2: Brains of rats injected in with 9L cells of gliosarcoma. Left - “HSV/tk ganciclovir Group” -Only an empty cavity is observed at the site where before there was the tumour. Right - “Control Group” - The growing tumour occupies almost all the left frontal lobe of the rat

of the suicide gene therapy and consists in a phenomenon of bystander cell killing, that is to say, the death of adjacent tumor cells not transduced with the HSV-TK gene after treatment with the antiviral drug, ganciclovir (GCV). Evidence from quantitative in vitro assays of glioma cell lines suggest that both murine and human gliomas are similar in expressing high sensitivity to the bystander effect. In vitro studies revealed that the presence of only 5% of HSV-TK-expressing transduced cells in the culture results in more than 90% tumor cell death/stasis after addition of GCV.^[13,14]

Several lines of evidence support gap junction intercellular communication (GJIC) as important in the bystander effect. In vitro metabolic assays, performed with GCV in the medium, indicate that tumor are more re-

duced when culture conditions support cell-cell contact of parental and HSV-TK-transduced cells. Additionally, a double dye transfer assay showed that cell communication through the gap junction is greatest for glioma, less for melanoma, and much less for colorectal carcinoma cell lines. In vitro metabolic assays with mixtures of TK+/TK- homologous tumor cells confirmed that glioma cell lines are more susceptible to bystander killing than melanomas.

Finally, the importance of a gap junction protein, such as connexin-43, in facilitating the bystander effect was demonstrated in a HT29 low-GJIC cell line study. When the TK-nontransduced cell population expressed connexin-43, a better bystander kill was achieved compared to the parental counterpart.^[15]

Thymidine kinase gene therapy modifications

Connexin

As previously exposed, Connexin (Cx)-dependent gap junctions between cells facilitate the intercellular spread of TK-activated GCV, thereby creating a bystander effect that improves tumor cell killing. However, tumor cells often have reduced connexin expression, thus thwarting bystander killing and the effectiveness of TK/GCV gene therapy. To improve the effectiveness of this therapy, some authors studied an HSV vector (TOCX) expressing Cx43 in addition to TK in U-87 MG human glioblastoma cells in vitro and in vivo.^[8,13] In vivo experiments in which U-87 MG tumoral cells were preinfected with HSV vector (TOCX) expressing Cx43 in addition to TK injected into the flanks of nude mice showed complete cures of all animals following GCV treatment, whereas animals with non-infected cells (control group) uniformly died from the tumor. The TOCX injection into U-87 MG intracranial tumors resulted in prolonged survival of the host animals when compared with the animals treated only with HSV-TK/GCV therapy. Together, these results suggest that the combination of TK and Cx may be beneficial for the treatment of high-grade gliomas.

Sustained release silicone formulation

In order to enhance the cytotoxic effects of herpes simplex virus thymidine kinase suicide gene therapy, some researchers developed a silicone formulation of ganciclovir (GCV-pellet) of 1 mm in length and in diam-

eter containing a total amount of 0.15 mg of GCV. In vitro experiments demonstrated that GCV was gradually released over a period of 7 days. The results confirmed that sustained release of a low and effective GCV dose with the silicone formulation significantly prolonged survival in combinations with HSV-TK expression if compared to intraperitoneal administration of GCV. Histological examination suggested that the treatment appears to be safe [Figure 3].^[16]

P450/Cyclophosphamide

Cyclophosphamide is an inactive prodrug which is converted by hepatic cytochrome P450 2B1 to cytotoxic metabolites which produce interstrand DNA cross-linking in a cell cycle-independent fashion. The limited ability of these metabolites to cross the blood-brain barrier contributes to the poor activity of cyclophosphamide against brain tumors.

It had been suggested that in situ transduction of tumor cells with the P450 2B1 gene -responsible for bioconverting the chemotherapeutic cyclophosphamide (CPA) into its active metabolites 4-hydroxyCPA/aldophosphamide (AP) - using retroviral and adenoviral vectors could increase the sensitivity of 9L gliomas cells

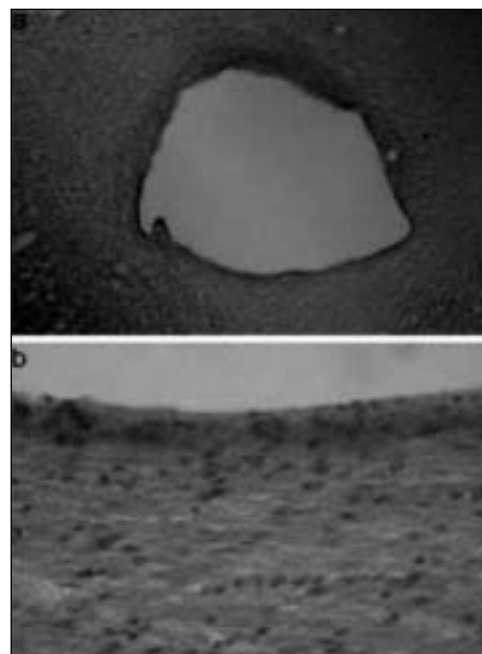


Figure 3: Histopathological sections of area adjacent to tumor that was injected with HSV/tk viral therapy with sustained release silicone formulation. (a) H/E, x10, (b) H/E, x40). No immunological or toxic reaction is observed in the normal areas adjacent to the cavity left by the tumour

to cyclophosphamide and have a potential role in the therapy of malignant gliomas. In vivo studies showed that P450 2B1 gene transduction prolongs the survival of animals bearing intracerebral 9L tumors treated with cyclophosphamide.^[17] More prolonged and higher concentrations of activated metabolites are generated by intraneoplastic injection compared with systemic administration of prodrug.^[18]

Na⁺/I⁻ symporter

Radioiodide concentrating activity in the thyroid, mediated by human Na⁺/I⁻ symporter (hNIS), provides a mechanism for effective radioiodide treatment for patients who have invasive, recurrent, and metastatic thyroid cancers after total thyroidectomy.

Some attempts to develop hNIS gene transfer for radioiodide therapy in patients with malignant glioma have been tried. Although more in vivo research is necessary, tumoral hNIS transduction through adenovirus vector followed by radioiodide therapy is a modality of genetic therapy that promises to have great progress in the next years.^[19]

Folypolyglutamyl synthetase

Although antifolates are popular agents for use in chemotherapy, they display minimal toxicity against slow-growing tumors and are toxic to actively replicating cells in normal tissues. These drugs are converted intracellularly into polyglutamate derivatives by the enzyme folypolyglutamyl synthetase (FPGS). Because tumors with high expression of FPGS often respond to nontoxic antifolate doses, it has been investigated whether augmenting tumoral FPGS activity by gene delivery would enhance tumoral antifolate sensitivity. The results suggest that FPGS gene delivery enhances the antifolate sensitivity of some glioma cell lines. However this approach still need further evaluation as a therapeutic strategy.^[20]

Anti-angiogenic factors

Inhibition of angiogenesis has been considered among the most promising approaches to treat highly vascularized solid tumors such as high-grade gliomas. However chronic systemic delivery of therapeutic pro-

teins, such as inhibitors of angiogenesis, present a number of difficult pharmacological challenges. The concept that targeted antiangiogenesis, using virally mediated gene transfer, represents a promising strategy for delivering this antiangiogenic factors.^[21]

Angiostatin, Endostatin, and IFN-alpha(1)

Some researchers evaluated the effects of local production of three endogenous inhibitors of angiogenesis: angiostatin, endostatin, and interferon (IFN)-alpha(1), using stably transfected rat (9L) and human (GL15) glioblastoma cells on tumor vascularization and growth in an in vitro assay system based on the implantation of tumor cells into organotypic brain slice cultures.

Although all the three genes showed angiogenesis inhibitory effect, IFN-alpha demonstrated the most potent antiangiogenic effect in organotypic brain slice cultures.^[20] In vivo, after intracerebral implantation of such genetically modified high-grade glioma cells, IFN-alpha(1) caused a dramatic decrease in tumor volume revealed by magnetic resonance imaging and by post-mortem histology. Mechanisms of this antitumor effect were most likely caused by the major antiangiogenic action of the cytokine, once IFN-alpha 1) expression provoked a pronounced decrease in blood vessel density, which was accompanied by extensive necrosis in the body mass of the tumors.^[22]

Platelet factor 4 (sPF4)

Retroviral and adenoviral vectors that express a novel, secretable form of the antiangiogenic protein, platelet factor 4 (sPF4), have been constructed. It was showed that vector-mediated sPF4 transduction selectively inhibits endothelial cell proliferation in vitro, and results in hypovascular tumors that grow slowly in vivo. Additionally, tumor-associated angiogenesis is inhibited and animal survival is prolonged, following transduction of established intracerebral gliomas by an sPF4-expressing adenoviral vector.^[21]

p16 gene

Recent studies have indicated that the loss of p16 is a frequent event in the progression of malignant gliomas. The loss of p16 promotes the acquisition of malignant characteristics in gliomas, which are among the

most angiogenic of all human tumors. High-grade gliomas can be distinguished from low-grade gliomas by the intense angiogenesis in addition to their frequent loss of p16.

Infection with a recombinant replication-defective adenovirus vector containing cDNA of wild-type p16 significantly reduced the expression of vascular endothelial growth factor, which is thought to be a pivotal mediator of tumor angiogenesis in p16-deleted glioma cells. Restoring wild-type p16 expression into p16-deleted glioma cells markedly inhibited angiogenesis induced by tumor cells in vivo. Furthermore, wild-type p16 inhibited neovascularization more potently than did wild-type p53 transfer.^[23]

Apoptosis-related genes

p-53 gene

P-53 gene therapy is in clinical development for the treatment of various cancers. P-53 gene is thought to function abnormally in the majority of malignant gliomas, although it has been demonstrated to be mutated in only approximately 30%. This fact has encouraged studies in which adenoviral transduction with wild-type human p53 has been investigated in an attempt to slow tumor cell growth. Some authors showed that multiple gene replacements with the simultaneous exposure to adenovirus containing p53 gene can produce additive effects in the treatment of glioma cell lines.^[24]

Rb gene

The development of high-grade gliomas involves progressive inactivation of several tumor suppressor genes. Abnormalities of the retinoblastoma tumor suppressor gene are found in the majority of cancers, including at least 30% of malignant gliomas. In face of the evidence that restoration of the retinoblastoma protein function may have therapeutic application for gliomas it was evaluated the effect of the adenovirus transfer of the 3.2-kb cDNA of the Rb gene to human glioma xenografts implanted subcutaneously in nude mice. The findings provide direct evidence that inactivation of the retinoblastoma protein is a critical event in gliomas, and suggest that the restoration of wild-type retinoblastoma activity in these tumors through vector delivery gene

therapy may have great therapeutic utility.^[25]

TRAIL

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces apoptosis in a variety of transformed cell lines, but generally spares most normal cells. Transduction with an adenoviral vector expressing human TRAIL cDNA resulted in both direct glioma cell killing as well as a potent bystander effect through presentation of TRAIL by transduced normal cells. Administration of adenovirus containing TRAIL gene significantly prolonged survival of mice harboring intracerebral glioblastomas. Taken together, these data suggest that vector-mediated transduction of TRAIL may represent an effective strategy for high-grade glioma gene therapy.^[26]

FADD gene

Fas/APO-1 (CD95), a cell surface cytokine receptor, triggers apoptotic cell death by specific agonist antibody, suggesting that Fas/APO-1 may be a promising target for treatment of tumors. It has been showed that treatment with anti-Fas antibody (FADD) effectively induced apoptosis in malignant glioma cell lines with high expression of Fas/APO-1. The retroviral transfer of FADD gene significantly enhanced the transduction efficiency and effectively inhibited both in vitro and in vivo survival of malignant glioma cells through induction of apoptosis. These findings suggest that the FADD gene is a novel and useful tool for the treatment of malignant gliomas.^[27]

Other apoptosis-related genes

Some studies suggest that adenoviral vector-mediated delivery of other apoptosis-related genes may be also potentially useful in the gene therapy approach toward the treatment of human brain gliomas. Apoptosis-related genes already studied are caspase-8, p33ING1, p73alpha, Bax, Apaf-1, caspase-9, IkappaBdN, NF-kappaB, caspase-3, gas-1, Bcl-2, and Bcl-X(L).^[28,29]

Immunogenes

Cancer immunogene therapy is based on vaccination with radiated, autologous tumor cells transduced with immunostimulatory genes. Several cytokines have been studied whether as vaccine ex vivo transfection

models or the classic in situ inoculation of the vector modified to carry the cytokine gene.^[30-32]

The vaccination treatment using tumor cells genetically modified to express certain cytokines consist in the following steps. First glioma cells are cultured primarily from surgically resected tumor tissues of patients. Posteriorly, in vitro infection with a recombinant virus vector containing the gene of the cytokine is proceeded, and lately, transduced cells are re-injected in the patient. Vaccination therapy induces specific activation of cytotoxic T lymphocytes measured by cell-mediated cytotoxicity assay, suggesting the generation of a specific anti-tumor response and the potential for systemic immunity. This immunization results in regression of implanted cells as well as the original brain tumour. Mice cured of their intracerebral tumors by these vaccination therapy also demonstrated protective immunity upon rechallenge, showing that these modality of therapy may have promising results in preventing the important problem of tumour recidives, almost always present in high grade gliomas.^[33]

Several cytokines have been studied: IL-12, IL-6, IL-4, IL-18, IFN-gamma, TGF-beta, TNF-alpha, GM-CSF, B7-2, TNF-alpha, LIF and LT.^[34-37] In conclusion, genetic immunotheapy has shown to be a promising potential both for treatment of established intracranial glioma through potentiating of antitumor immunity of the patient and for vaccination against subsequent intracranial glioma cell implantation.

Chemosensitization genes

Cell cycle regulator WAF1/Cip1

Studies have shown that the negative cell cycle regulator WAF1/Cip1 is often overexpressed in human gliomas and that WAF1/Cip1 overexpression renders glioma cells resistant to chemotherapy agents. It has been investigated whether down-regulation of WAF1/Cip1 could sensitize gliomas to chemotherapy. The results showed that the attenuation of WAF1/Cip1 expression initiated glioma cell death and sensitized glioma cells to apoptosis induced by 1,3-bis(2-chloroethyl)-1-nitrosourea and cisplatin. Thus, blocking WAF1/Cip1 production may serve as a useful chemosensitization regimen for treating glioma.^[38]

Cytosine deaminase/5-fluorocytosine

5-Fluorouracil (5-FU) is a potent antimetabolite used for chemotherapy of gastrointestinal (GI), breast, and head and neck malignancies. Despite initial excitement, therapeutic index of 5-FU has showed to be very poor. It is unclear whether this lack of utility is due to problems with drug delivery or inherent insensitivity. Adenovirus (Ad) vector-mediated cytosine deaminase (CD)/5-fluorocytosine (5-FC) gene therapy has been proposed as a potential technique to overcome pharmacokinetic issues associated with systemic 5-FU and is particularly well suited to use with tumors in which local control is paramount, such as malignant gliomas. Results of clinical studies in human patients with high-grade gliomas confirm the previous findings in rat models, demonstrating the potential clinical utility of adenovirus 5-FC gene therapy for gliomas.^[39]

Radiosensitization genes

Bax gene

It has been proposed that adenovirus-mediated transfection of proapoptotic Bax gene could enhance the cytotoxicity of radiotherapy (RT) in RT-refractory glioma cells. Results of in vivo experiments showed that apoptotic death may be enhanced by the combination of the treatment with adenovirus containing Bax gene (Ad-Bax) and RT. Remarkably, combined treatment induce regression of tumors in mice. In animals injected with subcutaneous tumors (D54 MG line), levels of apoptosis after RT alone, Ad/Bax alone, or the combination were, respectively, 12.3%, 32.1%, and 78.5%. These results confirms that Ad/Bax synergistically radiosensitizes glioma, with a seemingly favorable therapeutic index.^[40]

p53 gene

As exposed before there are studies, even in advanced clinical trial stage, in which adenoviral transduction with wild-type human p53 demonstrated to slow tumor cell growth. Investigations with p53 gene have also suggested that adenoviral vector-mediated expression of human wildtype p53 could not only slow tumor cell growth but also enhance the radiosensitivity of malignant glioma cells that express native wild-type p53.

It was demonstrated that RT2 tumour cells express native rat wild-type p53 before the transduction and markedly overexpressed human p53 following adenoviral p53 transduction. P53 transduction followed by radiation resulted in marked decreases in RT2 cell survival and an increase in apoptosis at radiation doses from 2 to 6 Gy. Animals receiving cranial radiation after intracerebral implantation with RT2 cells previously transduced with p53 survived significantly longer than control animals.^[41] These results support a new perspective in the p53 genetic therapy, showing the ability to enhance the radiosensitivity of malignant glioma cells that express wild-type p53 by using adenoviral transduction to induce overexpression of p53. This offers new hope for the p53 viral mediated genetic therapy as a successful therapeutic strategy, not only in human gliomas that express mutant p53, but also in those that express wild-type p53.

Cytokines

Some cytokine vaccination (GM-CSF, IL-4, IL-12) therapies have shown to have not only primary immunity generation against the tumour, but also an important radiosensitization effect. In some studies with tumors treated with vaccination therapy and posteriorly irradiation, about 80-100% of the glioma-bearing mice was cured.^[42-44]

Other genes

Carcinoembryonic antigen (CEA)

CEA has potent antitumor activity against gliomas *in vitro*, as well as in orthotopic U87 animal models. Retrovirus mediated infection of a variety of glioblastoma cell lines including U87, U118, and U251 with CEA gene resulted in significant cytopathic effect consisting of excessive syncycial formation and massive cell death at 72-96 h from infection.

Mice with established xenografts tumors treated with a total dose of 8×10^7 plaque forming units of Measles Virus-CEA (MV-CEA) had statistically significant regression of tumors and prolongation of survival in MV-CEA treated animals compared with the control groups. Histological examination of brains of MV-CEA-treated animals revealed complete regression of the tumor with

the presence of a residual glial scar and reactive changes, mainly presence of hemosiderin-laden macrophages.^[45]

Urokinase-type plasminogen activator

Urokinase-type plasminogen activator (uPA) and its receptor (uPAR) play an important role in the invasiveness of gliomas and other infiltrative tumors. In glioma cell lines and tumors, high grade correlates with increased expression of uPAR and uPA. Downregulation of uPAR and uPA by delivery of antisense sequences of uPAR and uPA in a single adenoviral vector (Ad), Ad-uPAR-uPA have been proposed as a method for genetic therapy in gliomas. Subcutaneous injections of bicistronic antisense constructs into established tumors (U87 MG) caused marked regression of these tumors.^[46] These results support the therapeutic potential of targeting the individual components of the uPAR-uPA system by using a single adenovirus construct for the treatment of malignant gliomas.

Linamarase

A new strategy for cancer gene therapy has been developed using a plant gene that encodes the enzyme, linamarase. This enzyme hydrolyzes cyanogenic glucoside substrate, linamarin, into glucose, acetone and cyanide. Retroviral vectors that carry linamarase as a potential killer-suicide gene caused a marked sensitization to the innocuous substrate, linamarin, followed by cell death. A study has demonstrated that this system can eradicate very large intracerebral gliomas *in vivo* helped by a cyanide bystander effect. Animals showed total regression of the glioma by magnetic resonance imaging (MRI) and do not show other appreciable toxic effects.^[47]

Clinical Trials

The most studied candidates for gene therapy, which are in advanced stages of clinical trials are: the prodrug activating system herpes simplex thymidine kinase (HSVtk)/ganciclovir (GCV), utilizing either retrovirus vector producer cells or adenovirus vectors, adenovirus mediated p53 gene transfer, adenovirus mediated IFN-beta gene transfer and

studies with oncolytic therapy with herpes virus or adenovirus vectors. Other vectors and genes previously discussed are still in cell or animal protocols investigation stage.

The therapeutic potential of a designed bicistronic Moloney vector (pLIL-2-TK), combining the expression of a suicide gene (thymidine kinase, tk) with an immunomodulatory gene (human interleukin 2, IL-2) has also been tested in a small pilot study in humans. Results showed evidence of transgene activity in the treated tumours and good results in regression of the tumours is presented.^[48]

The most advanced study with p53 gene is a phase I clinical trial of p53 gene therapy using an adenovirus vector (Ad-p53) has been undertaken. Fifty-eight patients underwent a two-stage approach. In stage 1, Ad-p53 was stereotactically injected intratumorally via an implanted catheter. In stage 2, the tumor-catheter was resected en bloc, and the postresection cavity was treated with Ad-p53. In all 15 patients, exogenous p53 protein was detected within the nuclei of astrocytic tumor cells. Exogenous p53 transactivated p21CIP/WAF and induced apoptosis. However, transduced cells resided on average within 5 mm of the injection site. The clinical toxicity was minimal and a maximum-tolerated dose was not reached. Although anti-adenovirus type 5 (Ad5) titers increased in most patients, there was no evidence of systemic viral dissemination.^[49] The results of this clinical trial showed that although p53 adenoviral transfection is a promise therapy, at the dose and schedule evaluated, transduced cells were only found within a short distance of the injection site, revealing that widespread distribution of this agent remains a significant goal.

There is a ongoing phase I/II clinical study in adult patients with recurrent high-grade glioma which aimed to evaluate biological safety, maximum tolerated dose and antitumor efficacy of a cytokine vaccination model, using a genetically modified replication-disabled Semliki forest virus vector (SFV) that carries the human interleukin 12 (IL-12) gene.^[50]

Several other Phase I and II clinical studies in patients with recurrent malignant glioma have shown a favorable safety profile and some efficacy of retroviruses

(RV) mediated gene therapy.^[51]

More than 300 patients with glioma have been already treated in clinical trials with oncolytic viruses, and in most cases virus was administered directly into the tumor. Total intratumoral doses of up to 2×10^{12} virus particles were well tolerated, and Phase I clinical studies have demonstrated low to moderate toxicity from the clinical use of oncolytic adenovirus or HSV-1 virus, either in the brain or in the rest of the body. Variable levels of gene transfer and in some cases anti-tumour effect have been observed.^[52]

Conclusions

From the results until the present it is clear that gene therapy strategies for gliomas are quite promising but more critical research is required, mainly in the vector field. Although much effort has been put into this area of research, neuro-oncologists are still awaiting a vector system that allows selective and efficient tumor cell transduction. Further major advancements in virus designs, application modalities and understanding of the interactions of the host's immune system with the virus are clearly needed before oncolytic virus therapy of malignant brain tumours can be introduced to clinical practice.

Major steps to improve gene transfer into central nervous system and efficacy of gene therapy for malignant brain tumors include: 1) design of more effective vector systems; 2) development of new or improved prodrug/suicide systems, gene replacement approaches, or strategies targeting the immune response or tumor angiogenesis; 3) study of new techniques to enhance delivery of genetic vectors into brain tumors and for monitoring gene delivery into tumors.

Furthermore, clinical studies did not address main limiting factors for in vivo gene therapy, such as insufficient gene transfer rates to the tumor with used local delivery modalities, and resulting inability of a particular transgene-prodrug system to confer permanently eradicating cytotoxicity to the whole neoplasm. The ultimate molecular therapy will probably involve the application of multiple simultaneous (combinatorial) therapeutic modalities.

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