Journal of NeuroVirology (1998) 4, 204–216
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Principles of treatment of malignant gliomas in adults: An overview

S Ausim Azizi[1] and Curtis Miyamoto[2]

Departments of [1]Neurology and [2]Radiation Oncology and [1,2]The Brain Tumor Center, Allegheny University of the Health Sciences, Broad and Vine, MS 423, Philadelphia, PA 19102, USA

The cornerstone of conventional treatments of malignant gliomas in adults has been surgical debulking, radiation therapy and chemotherapy. Almost always a combination of these treatments is used. With these conventional treatments the outcome, as measured by survival and quality of life, has remained universally dismal. Novel treatments, which are at different stages of laboratory and clinical trials, may offer a ray of hope for treatment of malignant gliomas. Development of these methods are directly related to the discoveries, over the past two decades, of cellular and molecular mechanisms involved in the genesis of brain tumors. Understanding of the mechanisms of tumor genesis may open new avenues of effective treatments for this devastating cancer.

Keywords: glioma; glioblastoma; radiotherapy; chemotherapy; gene therapy; TGF-beta

Introduction

Because of extremely short post-diagnostic survival time and universal fatality, malignant brain tumors are considered among the most devastating cancers that afflict human beings. Among the 10 000 to 15 000 newly diagnosed malignant brain tumors, less than 10% survive 2 years and less than 5% live to 5 years (Levine *et al*, 1989; Shoenberg, 1983). Tumors of the central nervous system are a common cause of cancer death in young adults. Although, aggressive treatment modalities have extended the median survival from 4 months to 1 year, the survival is often associated with significant impairment in the quality of life. Furthermore, it appears that the incidence of primary brain tumors is increasing, at least in the elderly (Boring *et al*, 1994). The pathogenesis of high grade primary brain tumors is characterized by local recurrence, though multifocal gliomas do occur. Current treatment of malignant gliomas can be divided into two broad categories: (1) Conventional treatments, and (2) investigational treatments. This division is somewhat artificial, as many experimental treatments are in fact innovations in conventional treatments. Furthermore, the 'experimental treatments' often rapidly move to the mainstay of the management of brain tumors.

Correspondence: SA Azizi
Received 3 November 1997; revised 23 January 1998; accepted 26 January 1998

Conventional treatments

Surgical resection, radiation therapy, and to a lesser extent, chemotherapy have been the cornerstone of treatment for high grade gliomas. Almost always a combination of these treatments is used. Improvements in surgical techniques including intra-operative mapping of the eloquent areas of brain (Black, 1997), and most recently the use of magnetic resonance theater (MRT) (Gould and Darzi, 1997; Fried *et al*, 1996) may offer some benefits for these patients in prolonging survival by a few months. However, even with aggressive surgical treatment the rate of recurrence for malignant gliomas is greater than 90%, and interestingly, the site of recurrence is often in the resected tumor bed, i.e., the recurrence remains local (Hochberg and Pruitt, 1980). In general, post-surgical quality of life remains poor.

Although the clinical benefits from radiotherapy, as measured by length of survival, appear to be modest, it is more effective than chemotherapeutic agents tested thus far, and with relatively low short term complications (Blomgren, 1996). Recent technical modifications in radiotherapy, such as hyperfractionation, quality of radiation, stereotaxic treatment with sparing of normal tissue, and addition of radiosensitizing substances have marginally improved clinical efficacy of radiotherapy for highly malignant brain tumors. The rationale behind the current methods of radiotherapy is to confine the radiation effects to the tumor area and to take advantage of the rapid division of malignant

cells and inflict damage to malignant cell nuclei at vulnerable times in the cell cycle. The damage can be at molecular or even at karyotypic levels (Lai *et al*, 1997).

Several chemotherapeutic agents, tried for the treatment of recurrent malignant gliomas, have shown only minor clinically meaningful activity against primary malignant brain tumors. With regard to systemic chemotherapy, many issues need to be considered including penetration of the blood-brain barrier and dose limiting toxicity. Therefore, there is no consensus, as yet regarding the most effective chemotherapeutic regimen. Furthermore, the logistics of administration and undesirable peripheral side effects often prove to be prohibitive.

Radiotherapy

Radiation therapy is one of the most effective treatments for anaplastic astrocytomas and glioblastoma multiforme. These tumors are theoretically ideal for radiation therapy as they are almost always confined to one location. Different methods for treatment delivery and types of irradiation have been developed.

Mechanisms of radiation effects
Ionizing radiation is thought to produce secondary electrons, which result in damage to the cellular DNA and the cell membrane, ultimately causing cell death. Many forms of DNA damage can occur. These include double and single strand breaks, base damage and DNA-protein cross-links. The double strand breaks have the greatest correlation with human tumor cell line death (Kelland *et al*, 1988; Schwartz *et al*, 1988; Wlodek and Hittelman, 1987). The exact mechanism by which ionizing radiation destroys tumor cells more efficiently than normal cells is not clear. It is widely believed that the reason for the tumor cells' sensitivity to radiation and chemotherapy is that they replicate more quickly than the normal tissues. This is partially true, though not a full explanation, because in many normal tissues, e.g. endothelial, epithelial and hematopoetic tissues, self renewing cells replicate as quickly as tumor cells. It is likely that the anticancer agents, among other mechanisms, induce p53 dependent apoptosis, thereby leading to cell death (Gupta *et al*, 1996).

Standard fractionation
The most widely used form of treatment is external beam photon irradiation. Photons can be either X-rays (artificially produced) or gamma rays (produced from radioactive decay). Commonly radiation is delivered in multiple fractions in order to decrease toxicity to the normal tissue, while increasing tumor response.

The delivery of 180 to 200 centiGray (cGy) fractions of irradiation once a day, until a certain total dose is achieved, is termed 'standard fractionation.' The time interval between fractions allows the normal (and tumor) cells to undergo repair of sublethal radiation damage. This is the first of what has been termed the four 'R's of radiation biology. These are repair of sublethal damage, reassortment, reoxygenation, and repopulation. The mechanism of repair in sublethal radiation damage is not well understood. The damage repair is possibly related to the integrity of cell cycle check-points in normal tissue, where normal check-points, e.g. p21, arrests the cell cycle in radiation damaged tissue allowing time for repair. Given the prevalence of check-point defects in tumors (Waldman *et al*, 1997), radiation damaged malignant cells may proceed through cell cycle and likely into an apoptotic pathway.

Standard radiation therapy increases survival by 3–6 months (roughly doubling the survival from surgery alone). The Brain Tumor Study Group has shown an increase of median survival from 14 weeks for surgery alone, to 36 weeks with post-operative radiation treatment. The one year survivals were 3% and 24% respectively. Traditionally, total doses of 5000–7000 cGy have been given. There has been no proven survival benefit from increased total radiation doses, i.e., over 6000 cGy.

Hyperfractionation
This is the delivery of two doses of radiation daily, separated by 4–6 h. It has the theoretical advantage of delivering a higher total dose of radiation to the tumor, while minimizing damage to the normal tissue. The use of hyperfractionation may increase local control without increasing toxicity due to late effects, which are mediated by the radiation damage to slowly dividing normal tissue (Nelson *et al*, 1993; Beck-Bornholdt *et al*, 1997). The overall length of treatment is the same as for standard fractionation. Again, the rationale behind this method of treatment is to radiate the maximum number of tumor cells at vulnerable times in the cell cycle. In addition, the time between the radiation treatments allows for the repair of sublethal damage in the normal tissue. In clinical studies involving children and adults, utilization of this technique has resulted in improved local control and survival (Wara *et al*, 1986; Nelson *et al*, 1993). No dose-response correlation was noted between hyperfractionated RT doses of 48.0 and 54.4 Gy. The median survival for anaplastic astrocytoma patients at 72.0 Gy was 49.9 months. Slightly higher toxicity was noted with doses of 80 Gy and above (Nelson *et al*, 1993).

Accelerated fractionation
Another method of radiation delivery is accelerated fractionation, which is the use of standard sized fractions given twice daily. This reduces the overall number of treatment days by half (Thames

et al, 1983; Peters *et al*, 1982; Fowler, 1984). The potential late term effects are unchanged since the total number of fractions are unchanged. Theoretically, there is an increased probability of tumor control and possibly survival because of a reduction in repopulation in rapidly proliferating tumors however, this has yet to be tested in a prospective randomized trial. The acute effects of radiation are increased which may necessitate a break in treatment. In one clinical study it was noted that the rate of disease progression during the course of treatment was much lower than for standard therapy (Werner-Wasik *et al*, 1996), though in some cases contrast enhanced tumor-negative lesions mimicking a brain tumor, consisting of necrosis and reactive gliosis, were observed on Magnetic Resonance Imaging (Van Tassel *et al*, 1995).

Brachytherapy
This method, also known as interstitial implantation, involves the direct placement of one or multiple radioactive source(s) in the area of the tumor by a minimally invasive neurosurgical procedure. This allows the delivery of high doses of radiation to the target volume while minimizing radiation exposure to surrounding structures. The radiation sources can be implanted directly into the tumor cavity either for a short time or indefinitely, depending on the desired effect. A key factor in the success of brachytherapy is the ability to deliver adequate doses of radiation to the tumor tissue with a good margin of treatment. This in turn is limited by tumor size (the larger the tumor the more difficult they are to include completely and uniformly in the treatment volume), and location, i.e., accessibility to treatment (Schupak *et al*, 1995). Clinical trials with stereotactic placement of Iodine-125 and Iridium-192 combined with chemotherapy have shown improved survival for glioblastoma (Sneed *et al*, 1997). However, other studies have shown a significant rate of failure requiring re-operation (Hopkins *et al*, 1995).

Stereotactic radiosurgery
Stereotactic irradiation is the delivery of a single fraction of high dose irradiation to a limited volume of tissue in one setting, using multiple arcs or beams from different directions. There are two conventionally used methods: One is given using the 'Gamma Knife', and the other is using a linear accelerator. The gamma knife consists of multiple collimated helmets with 210 cobalt-60 sources, while the linear accelerator utilizes multiple sweeps or arcs. Both techniques are designed to deliver radiation to a targeted focus from multiple directions, thus sparing the surrounding normal tissue (Hall *et al*, 1995). Recently, a hypofractionated sterotactic radiotherapy method was tried on patients with high grade gliomas with satisfactory palliative results (Shepherd *et al*, 1997). Three dimensional reconstruction of target areas is employed in both techniques. Basic differences in techniques are presented in Table 1.

Chemotherapy

Unfortunately, despite valiant efforts, systemic chemotherapy – as measured by survival time – has been the least effective of conventional treatments for malignant gliomas. To achieve a cure for malignant glioma via chemotherapy, the drug or a combination of drugs must be effective against tumor cells, and must be given frequently and in sufficient quantities to reach the dividing malignant cells at a particular phase in the cell cycle, while sparing the normal brain tissue. The mechanism of action of commonly used anticancer treatments at a biochemical level are reasonably well understood. The chemotherapeutic agents used for treatment of malignant gliomas have been alkylating agents, BCNU (Carmustin), CCNU (Lomustin) and Procarbazine; DNA cross linking agents, Carboplatin and Cisplatin; mitosis inhibitors, Vincristine sulfate; and more recently, topisomerase inhibitors, which are derivatives of Campothecin, a plant alkaloid (Matsumoto *et al*, 1995; Weingart *et al*, 1995;

Table 1 General comparison of linac based versus gamma knife stereotactic irradiation.

	Linear accelerator	*Gamma knife*
Source	Linear accelerator	60-Cobolt
Field arrangement	Multiple arcs and static fields	Multiple static fields
Field blocking	Multileaf collimators, cerrobend, independent jaws	Not conventionally employed
Time to treat (average)	Longer	Short
Field shaping isocenter	Blocking, multiple isocenters, addition of static fields, differential weighting of fields	Multiple isocenters
Fractionation	Commonly done	Not commonly done
Availability	Commonly available	At select centers
Collimator sizes	5 – 50 mm	4 – 18 mm
Imaging for planning	CT is mandatory. Image fusion with angiography and MRI.	MRI has been the standard. Now CT and angiography compatible

Lamond *et al*, 1996; Nakatsu *et al*, 1997; Balmaceda *et al*, 1997). Generally, for treatment of malignant gliomas – similar to the treatment of other cancers – a combination of the above drugs are administered. Furthermore, chemotherapy is almost always used as an adjunct to surgical and radiation treatments. It is known that radiation of the central nervous system disrupts the blood-brain barrier (Rubin *et al*, 1994). Therefore, combination treatments may have added advantage. Thus, radiation plus chemotherapy protocols, in a variety of configurations have been studied or are currently under study (Elliott *et al*, 1996a; Warnick, 1994; Prados *et al*, 1996; Kyritsis *et al*, 1996; Ameri *et al*, 1997; Kiu *et al*, 1995; Brandes *et al*, 1996; Fountzilas *et al*, 1997; Boiardi *et al*, 1997). Although a number of protocols have failed, studies comparing different chemotherapeutic regimens have not shown superiority of one set over the others (Fujiwara *et al*, 1995).

Systemic chemotherapy for brain tumors has the added disadvantage of inability to cross the blood-brain barrier. Only a handful of chemotherapeutic agents are capable of doing so. Studies are under way to evaluate breaching of the blood brain barrier for further penetration of chemotherapeutic agents (Elliott *et al*, 1996b). In addition, the feasibility and kinetics of superselective intraarterial application of chemotherapeutic agents, i.e., infusion into the arteries that feed the tumor, is under study (Nakagawa *et al*, 1994; Fujiwara *et al*, 1995).

A recently approved modality of treatment is the placement of chemotherapeutic agents directly into the tumor cavity after resection. This treatment involves the application of BCNU (carmustin) impregnated biodegradable wafers in the tumor bed at the time of surgical resection (Sipos *et al*, 1997). Clinical studies have shown some improvement in increased post-operative survival (Valtonen *et al*, 1997).

New and experimental treatments

A major limiting factor in conventional therapies for malignant gliomas is their non-specific nature, which causes dose limiting toxicity to normal brain tissue. Despite a recent pessimistic report on the rate of success in treatment of cancer (Bailar and Gornik, 1997), giant strides in understanding the cellular and molecular biology of cancer have been made, opening the way for newer therapeutic approaches. The experimental treatments are at different stages of laboratory and clinical trials and are too numerous to exhaustively review in this article; thus we concentrate on a few of the methods, promising for the future treatment of brain tumors. These treatments are primarily biological, and take advantage of the specific molecular and immunological properties of malignant gliomas. Most of these treatments are used in conjunction with conventional therapies in different centers, though their feasibility, safety, and efficacy remain largely unclear.

New methods of radiotherapy

Boron-neutron capture therapy
Neutrons differ from most other forms of external beam irradiation in that they have a high linear energy transfer, i.e., they deposit larger amounts of energy per length of tissue penetrated. High Linear Energy Transfer radiations are less affected by sublethal damage repair (the repair that occurs between fractions of radiation), hypoxia, and cell cycle effects, which makes neutrons extremely effective at killing both the normal and tumor cells. This fact has greatly reduced the therapeutic ratio of neutron radiation. One method of increasing the efficacy of neutron beam capture therapy is to concentrate the radiation dose to the tumor *versus* the normal brain. To accomplish this, boron is administered to the patients prior to irradiation. Boron-a stable isotope ^{10}B-captures slow neutrons and is converted to lithium and helium atoms, releasing energy for the tumor kill. Of course, sufficient quantities of boron must accumulate in the tumor tissue to allow for greater differentiation between the tumor and normal tissue. To accomplish this, a variety of boron-containing compounds with selectivity for neoplastic cells compared to normal cells have been devised (Barth and Soloway, 1997). Some of these compounds include the use of monoclonal antibodies against epidermal growth factor receptors, bispecific antibodies (Liu *et al*, 1995) and sodium borocaptate (Yang *et al*, 1997). These have been employed in clinical studies with moderate success (Barth *et al*, 1997; Nakagawa and Hatanaka 1997).

Proton beam irradiation
Protons are positively charged particles produced by a cyclotron. Proton beam irradiation has some potential advantages due to what is termed as the Bragg peak effect. The Bragg peak effect denotes that the proton beam has a very specific range. This allows the proton beam to be used for the irradiation of tumors adjacent to critical normal structures (i.e. the brain stem or optic chiasm). The biologic effect of protons is the same as for photons (X-rays and gamma rays) or electrons, which are low Linear Energy Transfer radiations. Several institutions are utilizing this technique for treatment of high grade astrocytomas (Shrieve and Loeffler, 1995).

Immune therapy

The advent of specific monoclonal antibodies directed against cell surface molecules has allowed for the definition of a number of glial epitopes

associated with gliomas, and has opened a new era in the treatment of cancers in general, and brain tumors in particular. Antibodies raised against the neural cell adhesion molecules (Patel *et al*, 1989), epidermal-growth factor receptors (Brady *et al*, 1990) and tenascin-C (Natali *et al*, 1991) are of interest for treatment of malignant gliomas. The rationale for this treatment is that the antibodies bind to the tumor tissue and cause disruption of neoplastic cell function by blocking receptors to trophic factors and/or other epitopes. Furthermore, these monoclonal antibodies can be coupled with toxins (Press *et al*, 1986) or radioactive sources (Emrich *et al*, 1996), causing further selective destruction of tumor cells.

Monoclonal antibodies against epidermal growth factor receptors (EGFR) for treatment of brain tumors have been studied extensively and have progressed to phase II clinical trials. These receptors are overexpressed in malignant gliomas (Reifenberger *et al*, 1989), whereas their expression is low in normal brain. EGFR may have a role in oncogenesis and tumor growth (Libermann *et al*, 1985). Theoretically, blocking of these receptors could inhibit proliferation of tumor cells (Weiner, 1995). Preliminary clinical studies have shown substantial *in vivo* tumor binding and concentration of one type of these antibodies, (EMD55900), after intravenous administration of a single 200 mg dose (Faillot *et al*, 1996). In a study in this institution (Miyamoto *et al*, 1995), 60 patients with clinical and radiologic diagnosis of glioblastoma were preoperatively treated with an average of three intravenous or intra arterial infusions of iodine 125-labeled murine anti-EGFR monoclonal antibodies. The study revealed that repeated administration of these antibodies is safe and may have some benefit in the management of primary glioblastomas, especially for those patients who do not qualify for other forms of more aggressive management (Miyamoto *et al*, 1995). A tumor-specific variant of epidermal growth factor receptor has been identified (Tsugu *et al*, 1997; Wikstrand *et al*, 1997). These aberrant receptors may be present in up to 50% of gliomas. Monoclonal antibodies against this group of receptors can be another avenue of immune therapy (Okamoto *et al*, 1996).

Interleukin-2, because of its ability to mobilize the organism's immune defenses against malignant cells, is another immunotherapeutic agent. It has been demonstrated that this cytokine stimulates the proliferation of tumor infiltrating lymphocytes *in vitro* (Lorruso *et al*, 1994). In a preliminary study of long-term survival in 19 patients after intra-cavitary introduction of interleukin-2 and lymphokine activated T-cells, it was shown that this agent may increase the post-diagnostic survival time of patients with malignant gliomas (Hayes *et al*, 1995).

Interferon beta can inhibit proliferation of glioma cells *in vitro* without a similar effect on the growth of normal astrocytes (Harada *et al*, 1995; Nehashi *et al*, 1995; Yokoyama *et al*, 1997). Furthermore, it was demonstrated that its antiproliferative effect occurs by arresting the cell cycle specifically at the S-phase (Garrison *et al*, 1996). The implications are that interferon must be available to tumor cells during the S-phase to be effective. Preliminary studies with beta interferon in children and adults with gliomas, have indicated that this form of immunotherapy may be safe and feasible (Packer, 1996).

Another approach to the immune therapy is vaccination against tumor cells, along with the boosting of the tumor specific immunity with granulocyte-macrophage colony-stimulating-factor. A recent study (Yu *et al*, 1997) demonstrated that subcutaneous inoculation of rats with irradiated GM–CSF producing tumor cells protected the animals against subsequent tumor implantation. GM–CSF has been used in the past to reconstitute immunity subsequent to aggressive chemotherapy and bone marrow transplantation (Rampling *et al*, 1994).

Trophic factors and treatment of malignant gliomas

With the increasing understanding of the role of growth factors and their receptors in the genesis of gliomas, other avenues of treatment for this cancer may open up. One such factor is Insulin Like Growth Factor-I (IGF-I) (Sandberg-Nordqvist *et al*, 1993). This factor is secreted by neoplastic cells and may, through a feedback loop, contribute to their growth, proliferation and maintenance. It has been reported that IGF-1 modulates the epidermal growth factor mediated glial cell growth in culture (Chernausek, 1993). As discussed above, EGF receptors are overexpressed in gliomas (Tuzi *et al*, 1991). It is conceivable that IGF-1 and EGF work in concert to maintain and nourish gliomas. Trojan *et al* (1993) indicated that treatment of established brain glioblastoma with antisense IGF-1 complementary DNA that blocks the synthesis of IGF-1 resulted in regression of these tumors. However, later it was noted that tumor regression may have been due to rejection of allogeneic C_6-glioma cells implanted in the rat brain (Beutler *et al*, 1997).

Other important factors, essential for maintenance and progression of tumors, therefore a target for therapy, are a variety of angiogenic molecules that are elaborated by the tumor cells (Weidner, 1996). A number of angiogenic factors have been described and several mechanisms for their activation have been proposed (Liotta *et al*, 1991; Hanahan and Folkman, 1996). Recently, it was reported that basic fibroblast growth factor (BFGF) might be involved in the initiation of angiogenesis (Czubayko *et al*, 1997). A number of angiogenesis-inhibiting drugs have progressed to clinical trial

stages (Fine, 1995). These include Marimastat, a metalloproteinase inhibitor (Uhm *et al*, 1997), Thalidomide and the calcium channel blocker CA1. Paradoxically, an important source of angiogenesis inhibitor molecules may be the neoplastic tissue itself (Good *et al*, 1990).

Transforming growth factors

Another class of trophic factors which are important in possible regression and/or maintenance of brain tumors are the Transforming Growth Factors. These families of factors act through a serine/threonin kinase membrane receptor group. Intracellular transduction is accomplished via a group of proteins (SMADS), which are immediately translocated to the cell nucleus. A number of studies have indicated that Transforming Growth Factors may contribute to apoptotic death in certain types of cells (Ohta *et al*, 1997; Tachibana *et al*, 1997). TGF-β was one of the first factors identified from malignant gliomas to suppress the immune system (Bodmer *et al*, 1989).

Recently, we have carried out a preliminary set of experiments designed to study the localization and expression of Transforming Growth Factors (TGF-β) in normal glial cell and malignant glioma cells. We utilized immunocytochemistry to detect TGF-β in cultures of normal astrocytes, glioblastoma cells obtained from patients, and C6 glioma cells lines. A monoclonal antibody against TGF-β1 (R&D system, Minneapolis, MN) was used. The antibodies were visualized utilizing indirect immunofluorescence technique. It was noted that in normal glial cells, TGF-β was concentrated in the cytoplasm, whereas in both glioblastoma cells and in the C6 glioma cell lines, this factor showed reactivity in the nucleus (Figure 1). The implication of these findings is not clear at this point.

Gene therapy

Advances in molecular biology in the last decade have better illustrated the mechanisms involved in the genesis of malignant gliomas. It is now generally understood that, to some extent, tumor genesis occurs either by overexpression of oncogenes or inactivation of tumor suppressor genes. Understanding the genetic and molecular mechanisms of oncogenesis represents the ultimate challenge, and likely is the only route to cure and control brain tumors.

One method of gene therapy may be the correction of genetic defect(s) either by introducing the

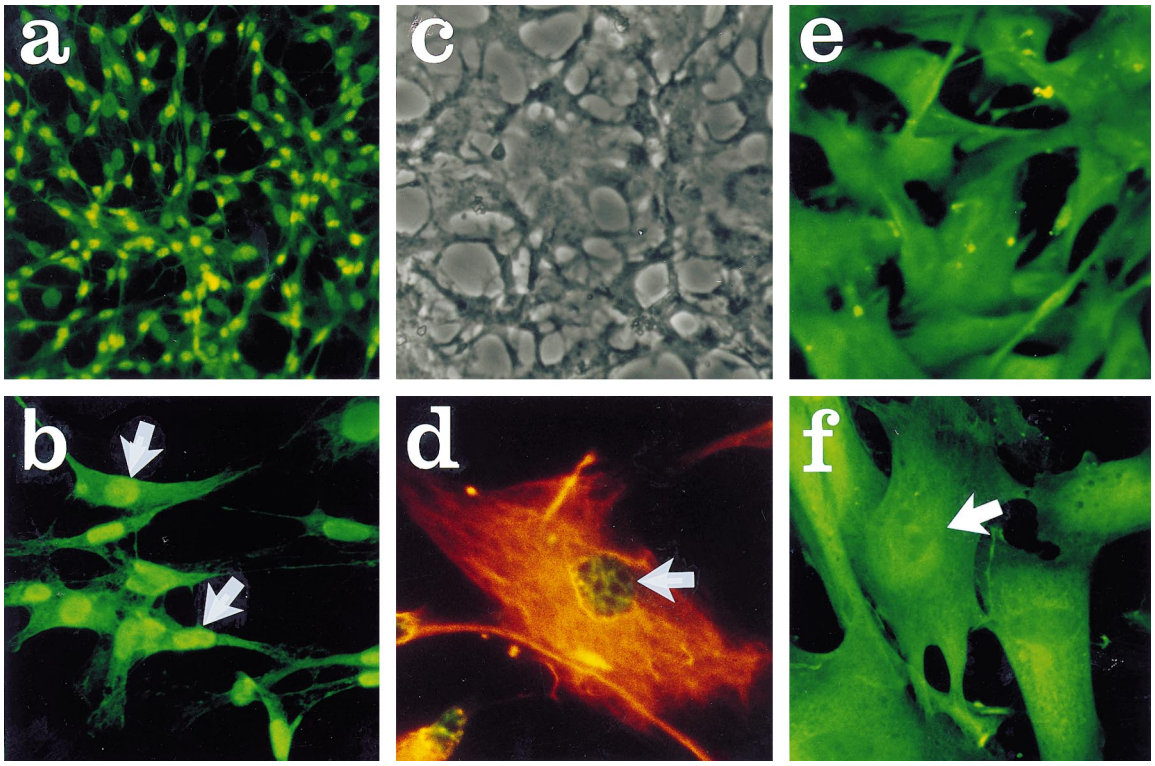

Figure 1 Photomicrographs demonstrate the localization of antibodies to TGF-β in cultures of normal and malignant glial cells. (**a, b**) Low and high magnification photos demonstrate concentration of TGF-β antibodies (arrowheads) in the nucleus of C6-glioma cells. (**c, d**) Similar findings indicate concentration of fluorescent labeled antibodies to TGF-β in the nucleus (arrow) of a primary culture of human glioblastoma, obtained from a patient. The cells were also labeled with antibodies against glial fibrillary acidic protein (GFAP), shown in orange in this double labeled section. (**e, f**) Indirect fluorescence immunocytochemistry indicate concentration of antibodies mainly in the cytoplasm of normal astrocytes, obtained from normal adult human brain.

missing tumor suppressor gene or by blocking the overexpression of oncogene(s). However lack of appropriate methods of gene engineering, as well as sparse understanding of the location and sequences of the gene(s) and the exact mechanisms of activation and translation, have thus far limited this approach.

A second gene therapy approach for the treatment of malignant gliomas involves the use of suicide genes such as thymidine kinase or cytosine deaminase from herpes simplex virus and *E. coli*, respectively (Ezzedin *et al*, 1991; Oldfield *et al*, 1993; Izquierdo *et al*, 1996; Kruse *et al*, 1997). This strategy can be effective either alone or by conferring chemosensitivity and radiosensitivity (Kim *et al*, 1997) to the malignant cells. Thymidine Kinase, once expressed in the replicating tumor cells, rapidly phosphorylates the antiviral agents, acyclovir and ganciclovir (pyrimidine derivatives) and causes the accumulation of monophosphate derivates of these drugs in the transduced malignant cells, which will ultimately inhibit DNA polymerase and causes cell death. Indeed, studies in animal models have shown a significant increase in survival time with intrathecal application of thymidine kinase containing herpes vectors (Kramm *et al*, 1996). Experimental models have shown that a number of non-transduced neighboring tumor cells are also destroyed. One explanation for this phenomenon, called the 'bystander effect,' may be the existence of gap junctions between tumor cells, allowing for the exchange of tumorcidal factors (Dilber *et al*, 1997). Other mechanisms including immune mediated destruction and inflammatory process may be involved. Similarly, cytosine deaminase, a bacterial and fungal enzyme, can deaminate 5-fluorocytosine to 5-fluorouracil (5-FU), which is a commonly used chemotherapeutic agent in cancer treatment (Ge *et al*, 1997). Introduction of a cytosine deaminase gene into the glioma tumor cells will cause the transduced cells to accumulate 5-FU, which inhibits thymidylate synthesis causing subsequent cell death.

Another experimental approach has been the introduction of genes into the tumor cells that code for cytokines, e.g., interleukin-2 (Breder *et al*, 1996), or antisense molecules against growth factors. The rationale for the use of IL-2 is that this molecule can mobilize a cytotoxic immune response against tumor cells given that the patient is sensitized to autologous tumor cells. In fact, applied in this manner, the treatment caused substantial tumor shrinkage as was shown by imaging studies (Sobol *et al*, 1995).

Gene transfer methods for treatment of malignant gliomas

The technology to effectively deliver the 'suicide' genes into the tumor cells, while sparing the normal brain tissue, is at its infancy. It suffers from the same drawbacks as chemotherapy. Much like other therapies, for a cure to occur, all malignant cells must be effectively destroyed. A variety of viral vectors including adeno-associated (Mizuno *et al*, 1996), adenoviral (Boviatsis *et al*, 1994; Okada *et al*, 1996; Maron *et al*, 1996; Fueyo *et al*, 1996), retroviral (Takamyia *et al*, 1993; Short *et al*, 1990; Culver *et al*, 1992) as well as non-viral methods (Zhu *et al*, 1996) have been devised to carry the given genes into the cells. However, in the case of gliomas, the seemingly simple mechanics of effectively reaching a proportion of tumor cells to effect a cure has proven to be surprisingly difficult. Currently, little information is available regarding the efficacy of gene therapy for brain tumors both in terms of the number of tumor cells transduced by a given dose of genetic material, and the distribution of the transduced cells; i.e., the quantitative kinetics of gene therapy in general has not been worked out. Direct application of small and large molecules, e.g., nucleotides and active vectors into the tumor extracellular space by an infusion process, called convection enhanced delivery system (Kroll *et al*, 1996; Levy *et al*, 1997), is currently under evaluation (Neuwelt *et al*, 1994; Nilaver *et al*, 1995; Muldoon *et al*, 1995). This method circumvents the blood brain barrier, and may allow therapeutic molecules to reach not only the tumor core but also the advancing edge. Bradykinin-like drugs have been used to breach the blood brain barrier (Nilaver *et al*, 1995; Bartus *et al*, 1996; Elliot *et al*, 1996b) prior to selective intra arterial infusion of therapeutic agents including gene carrying vectors or modified viral particles. Modified viral particles can be used not only as vectors but as therapeutic agents. One such virus, a genetically engineered herpes simplex virus (gamma 34.5), capable of replicating in the dividing tumor cells but avirulent to the surrounding terminally differentiated normal tissue has been studied (Mineta *et al*, 1995; Boviatsis *et al*, 1994; Andreansky *et al*, 1996). Application of these viral particles to a xenogeneic mouse glioma model improved survival (Andreansky *et al*, 1996). Use of this type of viral therapy in human trials must be carefully considered because of their potential for producing lethal encephalitis.

Another approach is *ex vivo* gene therapy. This involves the *in vitro* engineering of cells capable of continuously producing anti-tumor factors. These factors would include, among others, suicide genes, cytokines (Lichtor *et al*, 1995) and antisense molecules (Saleh *et al*, 1996). The engineered cells are implanted in the tumor bed and presumably the tumor killing effects would persist *in vivo*. The search for suitable donor cells and appropriate genes are under intense study. Allogeneic fibroblasts, lymphocytes, syngeneic normal and malignant glial cells, as well as xenogeneic cells, are being evaluated (Isacson and Breakfield, 1997).

Recently, we have been investigating the feasibility of using progenitor cells with astrocytic characteristics from adult brains, and marrow stromal cells (Prockop, 1997) as donor cells, and for cell mediated gene transfer into the nervous sytem (Azizi, 1997; Azizi *et al*, 1998). Astrocytes and bone marrow stromal cells, because of their post graft properties of migration (Anderson *et al*, 1993; Emmett *et al*, 1988; Lund *et al*, 1993), integration into the CNS (DelBigio *et al*, 1995) and elaboration of growth factors may be the ideal cells for *ex vivo* gene transfer.

Current molecular approaches

In recent years a wealth of information has emerged regarding the molecular mechanisms of regulation of the cell cycle and its role in the genesis of gliomas. One commonly known suppressor gene is p53, which is located in chromosome 17 and is involved in several aspects of cell cycle control as well as suppression of malignant transformation (Asai *et al*, 1994; Bogler *et al*, 1995). This may be accomplished either by inducing apoptosis or arrest in the cell cycle (Gomez-Manzuno *et al*, 1996). Aberrant expression of the p53 gene is thought to be an early event in malignant transformation of many human astrocytic tumors (Haapasalo *et al*, 1993). It has been suggested that the loss of wild type p53 function is associated with genomic instability, accelerated growth and malignant transformation of cultured embryonic astrocytes (Yahanada *et al*, 1995). However, other studies have shown that the expression of wild type p53 may be increased in some malignant gliomas (Alderson *et al*, 1995; Bogler *et al*, 1995), thus indicating a complex molecular mechanism. One path for the involvement of p53 in the genesis of brain tumors may be enhancing the expression of p21, which in turn increases the phosphorylation activity of cyclin D1. A substrate for cyclin D1 is the retinoblastoma protein (pRB) (Mayol *et al*, 1995). This protein forms a suppressor complex with E2F. *E2F-1* is a ubiquitously expressed gene, and its product E2F-1 attaches to E2F promoter which augments the transcription of S-phase specific genes and orchestrates the transition from G1 to S-phase in the cell cycle (Parr *et al*, 1997). Thus, hyperphosphorylation of pRB releases the E2F1 transcription factors, and accelerates the cell's progression to S-phase. It is possible that cells with 'defective' DNA, i.e., neoplastic cells, would accelerate through the S-phase and undergo apoptosis, whereas the malignant cells with defect(s) in this pathway would remain in G-phase for repair, become viable, proceed through the normal cell cycle and ultimately form brain tumors. Indeed, it has been reported that tumors that are resistant to radiotherapy have a faster repair mechanism of DNA breaks (Schwartz *et al*, 1988). It has been reported previously that a gene defect or loss of expression of pRB gene product in human malignant gliomas is associated with advanced disease (Hamel *et al*, 1993; Paggi *et al*, 1994; Dynlacht *et al*, 1997). Although the above pathway may be only one of the possibly many molecular mechanisms by which the genesis of brain tumors can occur, understanding of the steps in this pathway affords us a rich opportunity for therapeutic interventions at many points.

Another gene associated with gliomas is p16, located in 9p21 chromosomal region. Deletion of this region is one of the common chromosomal alterations during the evolution and genesis of gliomas. It has been observed that aberrations or deletions in p16 gene is associated with malignant transformation (Arap *et al*, 1997), whereas introduction/reintroduction of this gene into malignant glioma cells inhibit glioma proliferation (Fueyo *et al*, 1996; Hama *et al*, 1997) and induce the malignant cells into senescence (Uhrbom *et al*, 1997). Future studies on molecular therapies will be concentrated on harnessing the power of selectively targeted molecular therapeutics.

Conclusions

Despite a recent pessimistic report that the treatment of cancer over the past two decades has been a failure (Bailar and Gornik, 1997), major advances in understanding of the mechanisms of genesis of tumors has been made. These continued discoveries have inevitably brought us on the verge of launching new effective treatments for malignant gliomas.

(Supported by the Department of Neurology, In memory of Eileen S Kelberg).

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