Overall, malignant primary and metastatic brain tumors remain as lethal in the 1990s as they were in the 1970s. Just as the introduction of radiation therapy 2 decades ago added several months to survival time, numerous chemotherapeutic trials have demonstrated only limited efficacy. Fortunately, recent discoveries in molecular medicine have not only revolutionized our understanding of the events that lead to tumorigenesis, but they have created novel tools for targeting malignant cells. Novel treatment modalities are now being tested in pre–phase I animal models, while others are in different stages of clinical trials. The purpose of this review is to briefly summarize the emerging strategies of gene therapy, antiangiogenesis, immunotherapy, and targeting tumor cells.

GENE THERAPY

Gene therapy refers to the transfer of genetic material into mammalian cells with the aim of eliciting a therapeutic response. Virus-mediated delivery is primarily based on the concept of transferring a gene of interest, driven by an appropriate promoter, into target cells; this is accomplished by introducing recombinant viral particles designed to be replication-defective in mammalian cells but readily propagated in the laboratory in genetically engineered cells. Gene therapy is a technology with numerous applications, including antiangiogenesis, immunotherapy, replacement of defective genes, and suppression of harmful genes (see below). The current status and limitations and the future directions of adenoviral- and retroviral-mediated gene transfer have recently been discussed in the “Basic Science Seminars in Neurology” section of the ARCHIVES.¹,²

Suicide gene therapy for malignant brain tumors has been tested in humans. An example is the transfer into malignant cells of the herpes simplex thymidine kinase (HSV-tk) gene, which converts the nontoxic nucleotide analog ganciclovir into phosphorylated compounds that halt transcription of DNA in dividing cells. Because of the inability of retroviral vectors to infect quiescent cells, Culver et al³ hypothesized that recombinant retroviruses can target suicide gene delivery into rapidly multiplying tumor cells while sparing the background of nondividing neural tissue. Preclinical animal experiments showed that treating experimental rat gliomas by HSV-tk retrovirus–producing cells followed by ganciclovir produced complete regression. Furthermore, mixing experiments demonstrated a “bystander effect,” or the killing of wild-type tumor cells located in proximity to transduced cells expressing HSV-tk. This bystander effect is believed to be caused by cell-to-cell transfer of phosphorylated ganciclovir via gap junctions between HSV-tk–transduced cells and adjacent unmodified cells. Unfortunately, clinical trials have uncovered limiting drawbacks. Treatment of 15 patients with intratumoral implantation of xenogeneic HSV-tk retrovirus–producing cells showed limited antitumor activity; nonetheless, in situ hybridization for HSV-tk demonstrated survival of the vector-producing cells but limited gene transfer to the tumor. The findings suggest that techniques to improve gene delivery into the target cell need to be developed before clinical utility is achieved.⁴
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limitations imposed by diffusion of oxygen and nutrients prevent it from growing beyond a few millimeters in diameter. Before these experiments, it was widely believed that dilatation of pre-existing blood vessels was the main basis for tumor vascularization. This hypothesis has been supported by experimental findings showing that (1) tumors grow in vitro as spheroids and do not expand beyond a few millimeters in diameter, (2) tumor growth in vivo is directly related to its vascularization, (3) different classes of recently discovered antiangiogenic molecules have demonstrated no effect on tumor cells in vitro, but they retard or inhibit tumor growth in nude animals, and (4) the ability to induce neovascularization, or the acquisition of the angiogenic phenotype, is a crucial event in the progression of tumors into advanced stages of carcino-
genesis, which is linked in some tumors to mutations in the tumor suppressor gene p53 (the angiogenic switch). The angiogenic phenotype is produced by one of the fol-
lowing: (1) molecules secreted by the tumor itself; (2) a host cell recruited by the tumor; (3) molecules derived from the extracellular matrix; or (4) loss of physiological inhibition of endothelial cell proliferation. The first inducers of angiogenesis to be discovered are the basic fibroblast growth factor (FGF) and acidic FGF. Another molecule was isolated at around the same time and named vascular permeability factor, or vascular endothelial growth factor (VEGF), for eliciting vascular permeability and for being a potent inducer of angiogenesis. Recently, a family of FGF and VEGF molecules and their receptors have been identified, and a pattern has emerged in which FGF and VEGF are secreted by a wide variety of carcinomas and bind to receptors expressed on endo-
thelial cells leading to activation through phosphoryla-
tion of the cellular signal transduction pathway. Other angiogenic peptides include transforming growth factor α (TGF-α), and TGF-β.

The balance hypothesis of the angiogenic switch as proposed by Hanahan and Folkman7 stipulates that an-
angiogenesis is controlled by a relative balance of inducers and inhibitors. Thus, either reducing an inhibitor concent-
ration or inducing VEGF or FGF can alter the balance and activate the angiogenic switch leading to the growth of new blood vessels. Inhibition of angiogenesis was first discovered when interferon alfa and platelet factor 4 were found to inhibit endothelial cell chemotaxis and proliferation, respectively. Since then, a paradigm has emerged suggesting that a class of inhibitors is con-
tained within other endogenous proteins that are either weak or not themselves inhibitors. Examples include frag-
ments of thrombospondin 1, fibronectin, prolactin, epidermal growth factor, plasminogen (angiostatin), and col-
gen XVIII (endostatin). Angiostatin and endostatin specifically inhibit endothelial proliferation, and po-
tently inhibit angiogenesis and tumor growth. Tumors in animals treated with these molecules demonstrate regression into dormant microscopic lesions. Further-
more, while acquired drug resistance is a frequent prob-
lem in the treatment of cancer, drug resistance does not
develop after repeated cycles of tumor treatment with end-
ostatin.7,8-11 The media have recently reported on a con-
troversy that scientists at the National Cancer Institute in Bethesda, Md, have been unable to reproduce some of the antiangiogenesis experiments, and Folkman’s re-
sponse12 was that it was too soon for the institute’s sci-
entists, who began their studies only last year, to have mastered techniques to successfully isolate these com-
pounds.

Cytokines, including interleukin 12, interferon gamma, and the -C-X-C- chemokines, have also emerged as potent inhibitors of angiogenesis. In addition to en-
dogenous compounds, synthetic inhibitors have been iso-
lated, including the fungus-derived compound AGM1470 (TNP470), thalidomide, a number of metalloproteinase inhibitors, and others.2 Clinical trials of TNP470 are cur-
cently under way for the treatment of malignant brain tumors. Another class of antiangiogenic molecules is monoclonal antibodies that target the VEGF receptors (eg, Flk-1) or tumor endothelial cells. Sprouting capil-
aries express a specific type of cell-matrix interaction mol-
cule, the α,β, integrins, whose contact with the matrix
is crucial for the survival of the endothelial cells and thus neovascularization. Antibodies that target the α, β, integrins inhibit tumor angiogenesis by inducing programmed cell death of tumor endothelial cells.

Gene therapy is a useful tool to induce antiangiogenesis by transferring either antiangiogenic or angiogenic signal–blocking genes to brain tumor cells. Retroviral-mediated transduction of malignant gliomas to express a dominant-negative mutant of the Flk-1/VEGF receptor, truncated or inactive VEGF receptors, or antisense VEGF show suppression of tumor growth in vivo.13-15

IMMUNOTHERAPY

A 3-stage process is required for the generation of effector T cells after peripheral vaccination: (1) tumor-antigen uptake and processing at the site of injection by professional antigen-presenting cells, such as dendritic cells; (2) migration of antigen-presenting cells into regional draining lymph nodes, where naive T cells are primed; and (3) circulation of activated T cells that either perform or initiate effector mechanisms leading to tumor cell destruction.14 The idea that the mammalian brain is an immunologically privileged organ was initially based on the observations that (1) allografts of carcinomas and embryonic tissue are more successful in the brain than in the subcutaneous space27; (2) the brain lacks a defined lymphatic drainage16; (3) the expression of major histocompatibility complex class I and II molecules in the brain is low19; and (4) only activated T lymphocytes cross the blood-brain barrier.20 Medawar21 demonstrated that although skin homografts transplanted to a naive brain survive, they break down when implanted into the brains of animals that had previously rejected a cutaneous transplantation. The findings suggested the hypothesis that whereas the brain may be incapable of priming or initiating an immune response, T cells activated in the periphery carry effector functions into the central nervous system. Nonetheless, recent evidence shows that the immunological privileges of the brain are not complete; microglia have recently emerged as capable of presenting antigen and initiating an immune response when properly activated. New data have suggested that the mammalian brain can be stimulated to evolve into an “immunologically active” organ.22

Immunotherapy for malignant brain tumors is primarily based on 2 strategies: (1) inducing a systemic antitumor response that carries immune effector functions into the central nervous system; or (2) eliciting a primary immune response in the brain. Experimental approaches to achieving the former goal include (1) vaccination with tumor-derived peptides, (2) vaccination with dendritic cells loaded with tumor peptides, and (3) genetically modifying tumor cells to abolish their secretion of immunosuppressive molecules. Of 32 patients vaccinated with a synthetic peptide derived from the gp100 melanoma–associated antigen, 13 experienced objective regression of their tumors, including melanomas metastatic to the brain.23 Mice injected with bone marrow–derived dendritic cells pulsed with E7 peptide are effectively protected against a subsequent intracerebral challenge by C3 sarcoma that expresses the major histocompatibility complex class I–restricted peptide epitope E7. More importantly, this strategy is also effective against established experimental brain tumors.24 Some gliomas secrete TGF-β, which exerts potent immunosuppressive effects including the inhibition cytotoxic T lymphocytes. Antisense TGF-β gene therapy abolishes secretion of TGF-β and renders rat 9L glioma cells immunogenic when implanted subcutaneously.25 Successful induction of primary antitumor immunity in the brain is demonstrated by experiments in which malignant gliomas are genetically modified to secrete interferon gamma. In this model, intracerebrally implanted animals show prolonged survival times, tumor rejection, and specific intracerebral as well as systemic antitumor immunity when rechallenged by the parental line.22

TUMOR CELL–TARGETED THERAPY

Based on early microscopic observations, replicating cells were thought to progress through 4 major cell cycle states: M, mitosis; G1, the gap between mitosis and the onset of DNA replication; S, the period of DNA replication; and G2, the gap between the S and M phases. Recent advances have led to the current notion that DNA replication and mitosis are induced by activation of S phase– and M phase–specific kinases called cyclin-dependent kinases (CDKs). These proteins are only active when complexed with molecules called cyclins (because of their fluctuations in abundance during the cell cycle). For example, in animal cells, S phase is induced by CDK2 complexed with S-phase cyclins (E or A types), and M phase by CDK1 complexed with M-phase cyclins (A, B types). Mammalian cells also express CDKs that complex with G1-specific cyclins (D type) to promote synthesis of proteins needed for chromosomal duplication and trigger activation of S-phase CDKs.26

As cells progress through the cell cycle, they undergo discrete transitions that occur at precise times and in a defined order. Advancement is governed by regulatory circuits that control cell cycle checkpoints to ensure that DNA replication, chromosomal segregation, and earlier cell cycle events are complete to a high fidelity. When damage is detected, checkpoints either induce repair by activating the repair machinery and by arresting the cycle to provide additional time for repair, or eliminate the cell by activating the programmed cell death pathway (apoptosis). Loss of checkpoint control, resulting in the introduction of mutations and improper reproduction of the genome (genomic instability), is implicated in the evolution of normal cells into cancer cells. Cell elimination is arguably the most effective mechanism to prevent cancer; its goal is to ensure the survival of the organism.27

Of the various mammalian checkpoints, the G1 DNA damage checkpoint is the one studied in most detail. Oncogenic processes exert their greatest effect by targeting regulators of G1 phase progression. The most frequently disrupted tumor suppressor genes in cancer cells, retinoblastoma (RB) and p53, control the decision to divide at a checkpoint in late G1 phase, after which cells commit to an autonomous program that carries them
through cell division. In at least half of human cancers, p53 is mutated, and other indirect mechanisms also contribute to its inactivation. In response to DNA damage, p53 is activated; without it, cells are unable to arrest in G1 phase in response to γ irradiation and are less likely to go into apoptosis. The p53 gene mediates G1 phase arrest partly by inducing the expression of p21\(^{CIP1}\) and p27\(^{kip1}\), which control entry into S phase by strongly inhibiting CDKs. The RB pathway was discovered in the context of familial RB. G1–phase cyclin D–dependent kinases phosphorylate RB, which in turn controls expression of gene products important for S-phase entry. In its phosphorylated form, RB binds to a subset of E2F complexes converting them to repressors; furthermore, knocking out E2F-1 in animals leads to tumor formation. Inhibitors of cyclin D–dependent kinase activity (p16, p15, p18, and p19; and the so-called INK-4 proteins) cause G1-phase arrest.30

Transfer of the p16, p53, RB, and E2F-1 genes into gliomas is discussed by Fueyo et al\(^{29}\) in this issue of the ARCHIVES. The MYC family of proto-oncogenes also function in regulating cellular proliferation and differentiation; aberrant expression of these genes is believed to enable unrestrained progression through the late G1-phase checkpoint under growth-promoting conditions. Inhibiting MYC by adenoviral-mediated overexpression of MAD, an MYC repressor, induces growth arrest and inability of astrocytomas to form tumors in vivo.30

Another strategy targets glial cell surface molecules. Unlike glial cells, which express high amounts of transferrin receptors, normal brain expresses transferrin receptors only at the luminal surface of brain capillaries. Patients with malignant brain tumors refractory to conventional therapy were treated by transferring CRM107, a conjugate of human transferrin and a genetic mutant of diphtheria toxin (CRM107) that lacks native toxin binding. Tumor response occurred in 9 of 15 patients who could be evaluated, as evidenced by a 50% reduction in tumor volume visible on magnetic resonance images.31 A similar approach exploits glioma-specific chloride channels that selectively bind chlorotoxin.32

Infinite cellular divisions are the hallmark of cancer. Normal cells become senescent after certain cycles of division, and their ability to proliferate is tightly restricted. Human telomeres consist of tandem hexameric repeats (TTAGGG)\(_n\) at the end of chromosomes. Normal cells usually lose 50 to 100 base pairs of the terminal telomeric DNA with each cell division. This shortening of telomeres is believed to constitute the mitotic clock that controls the onset of cellular senescence. Telomerase is a ribonucleoprotein enzyme complex that elongates telomeric DNA; its reactivation is believed to be obligatory for cellular immortalization and infinite tumor growth. Telomerase activity is detected in 61.7% of neuroepithelial brain tumors; it is present in 0% to 20% of grades I and II astrocytomas, 40% of anaplastic astrocytomas, and 72% to 100% of glioblastoma multiforme. High telomerase activity is present in all primitive neuroectodermal tumors, anaplastic oligoastrocytomas, neuroblastosomas, and oligodendrogliomas. Furthermore, targeting malignant human gliomas with antisense directed against telomerase RNA elicits (1) inhibition of telomerase activity, (2) suppression of tumor cell growth in vitro, (3) a 50% reduction of tumor mass in vivo, and (4) tumor cell apoptosis.33,34 Other novel and interesting strategies include transfer of apoptosis-inducing genes or suicide genes under inducible promoters.35

**CONCLUSION**

One could argue that the “magic bullet” for “curing” malignant brain tumors will not be a conventional approach of radiation and cytotoxic chemotherapy. Ongoing, rapidly expanding spectacular advances in molecular medicine are creating smart weapons that bear the promise of taming the beast.

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