

## Chapter 38

# Evolution of Management Strategies for Cerebral Gliomas: The Effects of Science and Technology

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The evolution in the management of gliomas will require a new understanding of the factors that predispose patients to develop gliomas. Much of the past literature has involved a questionnaire-type strategy to accumulate data regarding previous exposures to various environmental factors. However, the field of epidemiology has evolved into a molecular based approach that assesses the exposure of a given agent with regard to its dose that would cause a biological effect, as well as the subsequent detection of that biological effect including molecular genetic aberrations that lead to altered gene structure and function. Ultimately, this produces the clinical correlate of a brain tumor and, subsequently, is what is known about progression markers based upon not only our understanding of genomics, but also proteomics and transcriptomics. One of the clues that we are taking from molecular epidemiology comes in the form of assessing genomic polymorphism of certain DNA-repair genes. Data from the San Francisco Bay area adult glioma study demonstrated that certain DNA-repair genes such as ERCC1 and ERCC2 have particular variant alleles that indicate a susceptibility to the development of gliomas (Dr. Margaret Wrensch, personal communication). In that study, patients were threefold more likely to develop a glioma than the control population when these variant alleles were identified in these two particular DNA-repair genes (Fig. 38.1). Thus, this is a clue that susceptibility of gliomagenesis can be determined based on the assessment of constitutive or germline single nucleotide polymorphism.

Our ability to determine a diagnostic classification of gliomas has recently changed with the discovery of DNA expression arrays. The information gained with this methodology has allowed us to group various genes together based upon the level of expression or underexpression (8). Using various bioinformatics approaches, expression patterns can be seen in various grades gliomas that support the histological classification based upon standard hematoxylin and eosin morphology. For example, overexpression of epidermal growth factor receptor (EGFR) and MDM2 is typically seen in the high-grade glioma expression array groups, as are other markers of cell cycle dysregulation and invasion. Recently, a study performed by the University of California Los Angeles group demonstrated that glioblastomas could be sub-typed based on gene clusters (2). These gene clusters were based on the finding of the expression profiles associated with neurogenesis as a marker of a slow growth potential and a better overall prognosis. This gene cluster was differentiated from genes overexpressed from mitosis or extracellular matrix remodeling, such that both of these clusters were seen to be overexpressed in patients who did very poorly. When compared with histology, the comparison was maintained based on the overall survival of these patient populations. Therefore, clues will be determined about outcome within subtypes of gliomas based on expression profiles of genes known to perform certain functions that can result in a more aggressive phenotype. However, in the past, many genes that were potentially inactivated as tumor suppression genes and thus part of the glioma formation profile were unknown because of the genomic-based approach. However, looking at epigenomic approaches, such as in the methylation pattern of promoter regions of genes, it has been determined that there are significant number of tumor suppressor genes that are inactivated and thus not transcribed because of the methylation status of the CpG islands within these promoter regions. In work from Joe Costello and his laboratory of the Brain Tumor Research Center at the University of California San Francisco (UCSF) (Dr. Joseph Costello, personal communication), it is quite apparent that methylation-based gene silencing using the techniques of restriction landmark genomic scanning

(RLGS) and reverse transcription-polymerase chain reaction (RT-PCR) are much more prominent than the standard genetic alterations seen and linked to tumor suppression gene inactivation (Fig. 38.2). Thus, both genetic and epigenetic approaches will be required to develop an integrative view of glioma genome in the future.

Much has been learned about the molecular pathways associated with glioma progression in the past several years. A great deal of this information is based on our understanding of extracellular ligands that bind the receptors on the cytoplasmic membrane and subsequently activate signaling pathways within the cytosol. Ligand-binding, receptor tyrosine kinase activation occurs, and various pathways have been targeted that result in angiogenesis, protein synthesis, cell cycle regulation, cell survival, transcription, and proliferation (Fig. 38.3). Although this has become quite complex, there have been a number of components of this pathway, which have been targeted with small molecule inhibitors resulting in alteration in these growth-promoting pathways (Fig. 38.3). A classic example of this is with the EGFR and its ligand EGF, which can be blocked in terms of its signaling pathway with compounds such as ZD1839 and OSI774. Other components of the pathway that are being targeted, including Ras activation using farnesyl transferase inhibitors along with blocking the PI3 kinase pathway downstream at mTOR using CCI779 and RAD001. Many more signal transduction inhibitors will be developed in partnerships with industry and will subsequently be tested in the clinical setting in the future. At UCSF in the Brain Tumor SPORE program, one of the projects has concentrated on the rationale for glioma-targeted therapy looking at EGFR blockade. It is commonly known that EGFR is overexpressed at approximately 40 to 90% of glioblastomas, and EGFR gene amplification is seen in nearly half of these cases. Frequent overexpression of EGFRvIII deletion mutants is known to exist in glioblastomas, along with the PI3-kinase pathway, which is a key signaling component in glioma pathogenesis. The EGFR tyrosine kinase inhibitor erlotinib (Tarceva or OSI-774) has shown promising response rates in malignant gliomas. This is based on the fact that in some clinical trials, inhibitors of other markers endemic to different tumors, such as breast cancer and CML using inhibitors of HER2/neu and c-ABL, i.e., Herceptin and Gleevec, have shown that targeted therapies can be successful to yield objective response rates and enhance patient survival.

We have assessed the EGFR status by fluorescence in situ hybridization (FISH) and immunohistochemistry in our glioma patients receiving OSI-774. We determined that there was a significant association with EGFR amplification, as measured by fish in response to erlotinib. Among patients whose tumor displayed EGFR amplification, 40% responded to erlotinib and about 60% did not. Among patients whose tumor did not display EGFR amplification, 14% responded to erlotinib, whereas 86% did not respond. We also determined that there was as strong and significant association between phosphorylated AKT (phospho-PKB/Akt) expression in response to erlotinib. In addition, phospho-PKB/Akt was a significant predictor of time-to-tumor progression. Among patients whose tumors were positive for phospho-PKB/Akt, none responded to erlotinib. Among patients whose tumors were negative for phospho-PKB/Akt, 44% responded to erlotinib and 56% did not respond. Thus, for those patients who did not respond to EGFR blockade with erlotinib despite having an EGFR amplification, virtually all of those patients had phosphorylated or constitutively active AKT despite the fact that only two of these patients had a mutation in the tumor suppression gene PTEN. It was also seen that the four patients, despite EGFR amplification that responded to erlotinib, did not have overexpression or activation of AKT. Thus, we have gained insight into the role of EGFR blockade based not only on EGFR expression patterns in gliomas, but also on downstream activated targets of the pathway linked to EGFR expression. Clues such as these are very important in being able to predict responses to therapy in the future.

Imaging continues to be critically important in our understanding of not only the anatomy of these glial tumors, but

also the physiologic and metabolic activity of these lesions. Magnetic resonance spectroscopy (MRS) has been critically important in allowing us to determine areas of active tumor versus necrosis or treatment related effects (7). The classic example is that of the choline to NAA index (CNI) being graded as 2, which is highly indicative of a neoplastic process. Our ability to take this information and to intrapolate this CNI image and superimpose it into a navigational workstations allows us to determine areas of relative risk with regard to tumor activity that are not typically seen with pure magnetic resonance anatomical images (6). Thus, MRS must be included in all aspects of tumor imaging both at the time of the initial diagnosis as well as in follow-up. However, one question that remains is whether MRS can be used to develop a profile of surrogate markers associated with various aspects of glioma activity. For example, if we had a way to measure EGFR amplification without obtaining a tissue sample, this would be a very useful noninvasive modality, although this currently does not exist. We have been able to correlate the CNI index with various aspects of the cell cycle, relating the MIB labeling index and cell density with the CNI, showing that tumors with a high CNI have a greater likelihood of having a mitotically active tumor. The use of dynamic bolus contrast imaging or perfusion weighted magnetic resonance imaging has also been exceedingly valuable in terms of evaluating cerebral blood volume and vessel permeability (1). This is based on the signal versus time intensity curve after a bolus administration of gadolinium during a 60-second period. Using an extrapolation paradigm, one can determine a relative cerebral blood volume map and potentially use the data obtained in this imaging technique to correlate the degree of angiogenesis based on VEGFR immunohistochemistry with the degree of vessel leakiness. Other imaging based modalities that may serve as surrogate markers include lactate based MRS as a marker of hypoxia and T2 quantitation as a marker of cellular infiltration. However, newer imaging modalities in which reported genes can be identified using nuclear medicine strategies will be highly useful in identifying critical molecular targets. These new techniques include enzyme-based bioluminescence imaging, enzyme- and receptor-based PET imaging and receptor-based magnetic resonance imaging (MRI). A thorough review of this emerging field can be found in an article by Massoud and Gambhir (5). However, these techniques are clearly limited to animal imaging at the present time, although in the future we should be able to use this in our patient population.

One of the major obstacles in the successful treatment of brain tumors is the inability to deliver not only conventional chemotherapy but also small molecule inhibitors and new agents to the tumor itself. A recent strategy that has evolved with some success in vitro and in animal systems is the use of small liposomal nano particles, i.e., 30 to 40 nm to package drugs, to enhance delivery to the tumor and the brain adjacent to the tumor. The current liposomes available seem to be quite safe and biodegradable. In particular, the stealth version is not immunogenic and has a long circulating half-time. These have been clinically validated in the form of liposomal anthracyclines, such as Doxil, and thus may serve as an effective agent for brain tumors and breast cancer. However, limitations to these liposomes exist, and the next generation of these nano-particles will have to have increase versatility of delivery as well as molecular targeting such as the use of monoclonal antibodies to create immunoliposomes. Thus far, we have been able to produce an EGFR-targeted immunoliposome using monoclonal antibody fragment against the EFGRvIII target (3). This has been done primarily with the commercially available C225-mono-clonal antibody fragment, which binds both EFGR and EFGRvIII. However, a number of single-chain human fage library antibodies are currently becoming available for more selective targeting against gliomas. Once again, the monoclonal antibody fragment conjugation is compatible with the stabilized liposome and can be subsequently loaded with drugs or imaging probes. Thus far, we have found excellent EGFR targeting with the immunoliposome in vitro and increased uptake in EGFR-overexpressing U87 cells, as compared with nontargeted liposomes, demonstrating excellent intracellular localization. Compared with the use of free Doxil or the Doxil liposome, both EGFR- and VEGFR-targeted immunoliposomes with Doxil seem to be quite effective in the flank U87 model (Fig. 38.4). However, one of the potential limitations is

systemic degradation or limitation of a large enough concentration within the tumor when given systemically, unless other ways to deliver this compound are being explored. For example, convection enhanced delivery (CED) is an excellent strategy to increase local distribution of drugs or small molecules. This is based on the principle of interstitial microinfusion using a pressure gradient and constant infusion, as opposed to diffusion down a concentration gradient such as with the use of polymers. Interstitial microinfusion results in a higher peak concentration over a greater volume of tissue compared with diffusion and thus would be an excellent strategy with the use of liposomes to enhance penetration and improve biodistribution within gliomas. The liposomal nano-particles can also be loaded with gadolinium-DTPA, in addition to other drugs, such that these liposomes can be imaged to determine the extent of distribution within the tumor based on MRI. Early experience with this technique shows that the gadolinium liposomes are not only safe, but they also provide reliable data, compared with fluorescence imaging, to demonstrate excellent penetration throughout the tumor in the brain adjacent to the tumor in virtually all settings tested (4, 9). A volume of 20  $\mu$ L can cover an entire U87 intracranial implant when distributed over a short period of time using the CED strategy. Other strategies are being tested with the liposomal approach, such as the use of TRAIL, which is a proapoptotic factor in combination with chemotherapy using temozolomide. Temozolomide given a 350mg/m<sup>2</sup> per day intraperitoneally  $\times$  5 days along with a 2  $\mu$ g per 20  $\mu$ L infusion of TRAIL results in nearly 70% survival of animals with U87 intracranial implants beyond 50 days (10). TUNEL staining demonstrates the presence of increased apoptosis throughout the tumor without any toxicity to normal brain. Another agent that has recently been tested in a form of a liposome is topotecan, which has been administered in a single dose via CED in rats with U87 intracranial tumor model. This has superb effects given in a single dose of 5 mg/mL in 20  $\mu$ L over a continuous infusion, which resulted in nearly 85% survival in the animal treatment groups after 80 days (Fig. 38.5).

Convection enhanced delivery has been successfully used in patients with gliomas. The compounds tested primarily involved immunotoxins conjugated to ligands that bind to frequently expressed receptors in gliomas. To date, based on the studies reported, transient effects have been noted, but very few permanent irreversible deficits have resulted. All the indications point to this being a safe therapeutic strategy. Kunwar et al. from UCSF have demonstrated that the survival time for patients who receive the IL-13PE38 construct with CED was significantly improved if more than two catheters were placed within the tumor and the adjacent brain (Dr. Sandeep Kunwar, personal communication (Fig. 38.6). Thus, the biodistribution is greater and the results are significantly better than using less than two catheters.

Finally, one of the most important issues in neurooncology has to do with our understanding of the origin of human gliomas. In an article published in the February issue of Nature of this year, Sanai et al. (11) from UCSF describe, for the first time, the components of the human subventricular zone such that GFAP positive bundles adjacent to the ependyma were identified along with a unique ribbon of subventricular zone astrocytes. These astrocytes are mitotically active and can be seen to be adjacent to the entire ependymal region around all of the ventricular systems in the adult brain. This has a great deal of importance because these cells could be the cells of origin for gliomas, thus helping us to further elucidate the developmental neurobiology of glioma genesis. Similar to medulloblastomas, gliomas have reactivated central nervous system developmental pathways involving the Hedgehog-signaling cascade. New compounds are available such as cyclopamine that could target the Hedgehog-signaling pathway such as to block downstream activation of target genes, which result in uncontrolled growth and proliferation. Thus, it may one day be possible to eradicate the cells from which gliomas form that would be the ultimate therapy for this difficult-to-treat disease.

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- FIG. 44.1 DNA nucleotide excision repair genes ERCC1 and ERCC2 lie near each other on chromosome 19q13.3 near a putative glioma suppressor region. Glioma patients were three times more likely than controls to be homozygous for variants in two SNPs within ERCC1 and ERCC2, suggesting the possibility that these genes or others to which they are linked might influence susceptibility to glioma.
- FIG. 44.2 Methylation-based gene silencing using the techniques of restriction landmark genomic scanning and reverse transcription-polymerase chain reaction are more prominent than the standard genetic alterations linked to tumor suppression gene inactivation.
- FIG. 44.3 Components of growth-promoting pathways that have been targeted with small molecule inhibitors.
- FIG. 44.4 Compared with the use of free Doxil or the Doxil liposome, both EGFR- and VEGFR-targeted immunoliposomes with Doxil seem to be quite effective in the flank U87 glioma model.
- FIG. 44.5 Efficacy of nanoliposomal topotecan after single dose via CED in rats with U87 intracranial tumor.
- FIG. 44.6 Survival time for patients who receive the IL-13PE38 construct with CED is significantly improved if more than two catheters are placed within the tumor and the adjacent brain