Targeted Brain Tumor Treatment-Current Perspectives

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Abstract: Brain tumor is associated with poor prognosis. The treatment option is severely limited for a patient with brain tumor, despite great advances in understanding the etiology and molecular biology of brain tumors that have lead to breakthroughs in developing pharmaceutical strategies, and ongoing NCI/Pharma-sponsored clinical trials. We reviewed the literature on molecular targeted agents in preclinical and clinical studies in brain tumor for the past decade, and observed that the molecular targeting in brain tumors is complex. This is because no single gene or protein can be affected by single molecular agent, requiring the use of combination molecular therapy with cytotoxic agents. In this review, we briefly discuss the potential molecular targets, and the challenges of targeted brain tumor treatment. For example, glial tumors are associated with over-expression of calcium-dependent potassium (K_{Ca}) channels, and high grade glioma express specific K_{Ca} channel gene (gBK) splice variants, and mutant epidermal growth factor receptors (EGFRvIII). These specific genes are promising targets for molecular targeted treatment in brain tumors. In addition, drugs like Avastin and Gleevec target the molecular targets such as vascular endothelial cell growth factor receptor, platelet-derived growth factor receptors, and BRC-ABL/Akt. Recent discovery of non-coding RNA, specifically microRNAs could be used as potential targeted drugs. Finally, we discuss the role of anti-cancer drug delivery to brain tumors by breaching the blood-brain tumor barrier. This non-invasive strategy is particularly useful as novel molecules and humanized monoclonal antibodies that target receptor tyrosine kinase receptors are rapidly being developed.

Abbreviations: BBB: blood-brain barrier; BTB: blood-tumor barrier; K_{Ca}: calcium-dependent potassium channels; NS-1619/NS 004: 1,3-dihydro-1-5-(trifluoromethyl)-2H benzimidazol-2-one; HBMVEC: human brain microvascular endothelial cells; FACS: fluorescence activated cell sorting; PDGFR: platelet-derived growth factor receptor; RTKIs: receptor tyrosine kinase inhibitors; EGFR: epidermal growth factor receptor; EGFRvIII: variant III of the human EGFR; gBK channel: glioma specific spice variant of K_{Ca} channel gene; K_{ATP}: ATP sensitive potassium channels; Minoxidil sulfate (MS: K_{ATP} channel agonist); Trastuzumab (Herceptin, Her-2 inhibitor, Genentech Inc.).

Keywords: Brain tumor, BBB, drug delivery, therapeutic targets in brain tumors

Introduction

Brain tumor

Nearly 20,000 new primary brain tumors and about 200,000 metastatic brain tumor cases are reported each year in the U.S.A. (Levin, 2007). The overall survival of these patients is dismal and the majority of survivors suffer disabling toxicities from their treatments. Standard treatment for brain tumors includes combination of surgery, radiation therapy, and chemotherapy. Brain tumor poses unique challenges due to its distinct biology, genetics, treatment response, and survival. Despite extensive characterization of the brain tumor pathways, molecularly targeted approach is not available to brain tumor patients. Future research in brain tumors needs to focus on strategies for improving drug delivery, disruption of blood-brain-barrier (BBB), and molecular profiling of tumors. In addition, careful studies are needed to delineate pathways that aid and abate brain tumor progression. Identification of potential markers (genes and proteins) for targeted therapy will definitely help the clinicians to design the treatment accordingly. Usually, after surgical treatment, brain tumor recurs, severely shortening life expectancy (Friedman, Kerby and Calvert, 2000). Conventional treatments using radiation and intravenous chemotherapy are not sucessful

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because the cancer cells develop resistance to treatment. Anti-cancer drugs fail to penetrate the BBB in sufficient quantities (Pardridge, 2001), allowing cancer cells to develop resistance to these agents. Therefore, understanding the biochemical regulation of the BBB (Fig. 1) in normal and tumor-invaded brain is of great importance to develop therapeutics that breach or circumvent BBB and directly target brain tumor cells (Ningaraj, 2006). The focus is now on the targeted cancer therapies (Butowski and Chang, 2005) that complement conventional treatments and reduce the drug resistance in cancer cells and the toxicity in normal brain (Newton, 2003). Novel cancer therapies include anti-angiogenic agents, immunotherapy, bacterial agents, viral oncolysis, cyclin-dependent kinases and receptor tyrosine kinase inhibitors (RTKIs), anti-sense agents, gene therapy, microRNA (miRNA), and combinations of various methods (Butowski and Chang, 2005).

Chemotherapy

Chemotherapy is a form of targeted therapy where cytotoxic drugs act on multiplying tumor cells. The drugs can also be used as sensitizers to augment the effects of radiation therapy. Chemotherapeutic drugs can be delivered directly to brain tumors through a polymer wafer implant such as a biodegradable wafer soaked with BCNU (Carmustine). Besides BCNU, several other chemotherapy drugs are used to treat brain tumors, which are administered by various routes. The chemotherapeutic drugs taken orally include Temozolomide (TMZ, Temodar), procarbazine (Matulane), and lomustine (CCNU). The intravenously administered drugs include vincristine (Oncovin or Vincasar PFS), cisplatin (Platinol), carmustine (BCNU, BiCNU), Carboplatin (Paraplatin), while Methotrexate (Rheumatrex or Trexall) may be taken orally, by injection, or intrathecally. Treating brain tumors with chemotherapy can be difficult because the brain is protected by BBB, which keeps out harmful substances such as bacteria and chemotherapeutic drugs. Among many cytotoxic agents in the clinician's arsenal, Temozolomide (TMZ) has shown some promise in treatment of low grade gliomas (Friedman, Kerby and Calvert, 2000), however, the effect on patient survival was modest (Balana et al. 2004). The problem is that glioblastoma multiforme (GBM) exhibits varying responses to TMZ (Hirose, Berger and Pieper, 2001), and in some cases gliomas have increased O^6-methyl guanine methyl transferase (MGMT) activity, which results in complete resistance to TMZ (Bocangel et al. 2002). The clinical utility of TMZ against all types of brain tumors remains limited due to its BTB penetration (some authors claim TMZ metabolite (MTIC) concentration in CSF to be as high as 30%), which demand repeated high doses to achieve *in vivo* therapeutically effective concentrations in brain tumors (Yung et al. 1999), and different phenotypes and genotypes that render some form of resistance against TMZ (Kanzawa et al. 2003). Most importantly, an extensive literature search and preliminary work on BBB/BTB penetration of TMZ did not convince us that sufficient amount of drug penetrates the BBB or BTB to elicit anti-tumor effect. To circumvent the penetration problem, chemotherapy drugs can be delivered by

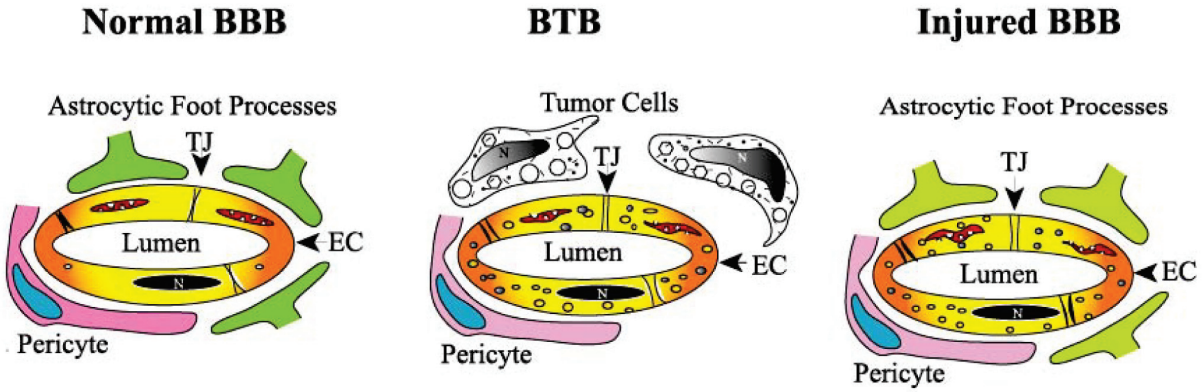

Figure 1. A schematic representation of normal and abnormal blood-brain barriers. We reason that genes in brain cancers/vasculature are distinct from normal brain/vasculature. They are attractive targets for the design of therapies that can penetrate the BTB and selectively kill brain cancer cells. We are studying the genes that direct the formation of the normal and abnormal (cancer) human brain vasculature, and with this knowledge develop new treatment strategies for brain tumor patients. EC: endothelial cells; TJ: tight junction proteins; N: nucleus.

intratumoral route or by drug impregnated wafers to attain higher concentration of drugs in the tumor cells, but the procedures are highly invasive.

Targeted brain tumor treatment

The human genome project has raised the expectation of the development of novel therapies for brain tumor because the conventional treatment strategies have not yielded any significant clinical outcome. Brain tumor treatment differs according to the grade and location of the tumor. Hence, combination of surgery, chemotherapy, and radiotherapy can be used in treating brain tumor patients (Stupp et al. 2005). Most promising anti-cancer drugs for pediatric and adult patients that are effective against cancers outside the brain have failed against brain tumors in clinical trails, in part, due to poor penetration across the BBB. For instance, aberrant expression of src family kinase (LCK) (Fabian et al. 2005) or mutation of c-KIT are involved in the pathogenesis of many cancers. Studies using imatinib mesylate (STI 571, Gleevec, Novartis, U.S.A.), an inhibitor of the tyrosine kinases BRC-ABL, c-KIT, and PDGFR, have shown significant response in patients with chronic myelogenous leukemia (CML) and gastrointestinal stromal tumor (GIST). Clinical trials were recently conducted to test the efficacy of Gleevec in brain tumors (Reardon et al. 2005; Wen et al. 2006; Pollack et al. 2007). Gleevec is an effective agent that targets specific gene/protein in cancer cells without harming normal cells and tissues. Drugs like Gleevec and Temozolomide attack abnormal chemical signals or molecules inside the cells or on the surface of the cells that have enabled brain tumor cells to escape the normal growth controls. Therefore, combating many forms of cancer will probably require a variety of targeted drugs used in combination, as cancer involves different types of dysfunctional genes and no single or two drugs will be sufficient. Some cancers, particularly primary and metastatic brain tumors of the breast and lung are difficult to treat because they are caused by multiple signaling pathways that are running amok, rather than just one, as observed in CML and GIST (Butowski and Chang, 2005). Gleevec may potentially target the above mentioned oncogenes in brain tumor (Holdhoff et al. 2005) provided it penetrates the BBB (Leis et al. 2004). Careful molecular studies would identify the stem cell factor/c-kit pathways in pediatric brain tumors, which might be the target of Gleevec. Characterizing the genetic and proteomic events that play a role in the biology of these tumors may allow molecular sub-typing which could lead to the development of novel therapeutic strategies, including treatment with Gleevec or with potassium channel modulators targeting tumor and tumor blood vessel endothelial cells (Ningaraj, 2006).

Targeting brain tumors

Targeting tumor and tumor blood vessel-specific marker(s) is a good strategy to control tumor growth (Robinson et al. 2003). It is, however, critical to study whether tumor-specific drug delivery has the potential to minimize toxicity to normal tissues, and to improve the bioavailability of cytotoxic agents to neoplasms. Existing site-specific drug delivery systems include delivery to endothelial receptor $\alpha_v\beta_3$, and tumor specific antigens. Antibody conjugation to cytotoxic agents has shown promise in achieving the goal of tumor-targeted cytotoxicity. This approach may be limited by the small subsets of tumors that can be targeted by these antibodies and by poor biodistribution of these antibodies into solid tumors. Alternative approaches to target all neoplasms exploit differences in human tumor blood vessel characteristics when compared to normal brain blood vessels (Black and Ningaraj, 2004; Ningaraj et al. 2002; Ningaraj, Rao and Black, 2003a).

Epidermal growth factor receptor (EGFR) is often amplified and mutated in human gliomas, but the expression is low or undetectable in normal brain. Recently, EGFR's mutant isoform, variant III of the human EGFR (EGFRvIII), is under intensive investigation as potential molecular target for the specific delivery of the diagnostic and the therapeutic agents to brain tumors (Yang et al. 2005). The therapeutic monoclonal antibodies (MAb) targeting growth factor pathways are being developed. The purpose of antibody treatment of cancer is to induce the direct or indirect destruction of cancer cells, either by specifically targeting the tumor or the tumor vasculature (Butowski and Chang, 2005). Examples of therapeutic antibodies which are effective in treating cancer includes the humanized IgG antibody Herceptin for the treatment of breast cancer, Cetuximab, ABX-EGF, EMD 720000 and h-R3 directed at extracellular receptor domain that inhibits the ligand-receptor interactions. Other antibodies

like Y10 and MAb806, which are directed towards the extracellular portion of EGFRvIII in gliomas have also shown some activity in clinical trials (Rich and Bigner, 2004). Suramin (polysulfonated napthylurea), which acts by interfering with the binding of several growth factors-including EGF, platelet derived growth factor (PDGF), and insulin growth factor (IGF1) with their putative receptors, is being tested in clinical trails. These MAbs, however, have poor penetration into brain tumors, which results in recurrence in brain tumor patients.

Kinase inhibitors

Kinase inhibitors show great promise as a new class of therapeutics to control gliomas. The specificity of RTKIs, including those that are in clinical use or in development widely varies, and is not strongly correlated with chemical structure of the identity of the intended target. Many novel interactions were recently identified (Fabian et al. 2005). EGFR and PDGFR are abnormal genes identified in gliomas (Rich and Bigner, 2004), whose expression is linked to an increased rate of tumor cell proliferation, resistance to chemotherapy, invasion, and apoptosis, and hence decreased survival in patients with malignant gliomas. PDGF ligands bind to PDGFRs to induce phosphorylation and activation of downstream signaling pathways such as RAS, MAPK, and Akt. Therefore, therapies using Gleevec, Suramin, and MAbs are directed at PDGFR to control glioma growth. PDGFR inhibitors may also provide additional benefit by blocking pericytes-assisted angiogenesis (Bergers et al. 2003). Clinical trials with EGFR and PDGFR inhibitors have shown promise for glioma therapy, although their ability to penetrate BBB in sufficient amounts is largely unknown. We transiently opened the BTB with K_{Ca} and ATP-sensitive potassium (K_{ATP}) channel agonists (Black and Ningaraj, 2004; Ningaraj et al. 2002; Ningaraj, Rao and Black, 2003a,b; Rao and Ningaraj, 2001) to increase the delivery of Gleevec and Herceptin to human glioma xenografts grown in murine brains.

K_{Ca} channels in gliomas

Membrane ion channels are essential for cell proliferation and appears to play a role in the development of cancer (Ningaraj, 2006). The K_{Ca} channels are highly expressed in gliomas (Weaver, Liu and Sontheimer, 2004) supporting the hypothesis that these channels play an important role in brain tumor growth and possibly the progression of low grade anaplastic astrocytomas (grade II) to a deadly high grade GBM (WHO grade IV). In addition, studies have shown that modulation of the biological function of K_{Ca} channels with specific inhibitors attenuate glioma growth (Rao and Ningaraj, 2001). Another study showed that the activation of intermediate K_{Ca} channels with its opener caused down-regulation of these channels and attenuated the non-excitable cell growth and its proliferation (Kraft et al. 2003). We showed that chronic activation of K_{Ca} channels with its specific openers NS-1619 and NS-004 elicited apoptosis in vitro and in vivo (Rao and Ningaraj, 2001). However, the role of K_{Ca} channels in progression from a treatable low grade to an untreatable high grade glioma in pediatric as well as in adult patients is not fully understood. Recently, glioma K_{Ca}/BK channels (gBK) splice variant of the KCNMA1 gene was characterized by enhanced sensitivity to intracellular calcium levels (Weaver, Bomben and Sontheimer, 2006). The study also showed that the expression of functional gBK channels appears to be regulated in a growth-factor-dependent manner. It is well established that EGF activates EGFR. Several molecular agents targeting EGFR are undergoing clinical trails as potential therapies in neurooncology (Rich and Bigner, 2004). For example, ZD1839 (Iressa) an orally active, selective EGFR-tyrosine kinase inhibitor has anti-tumor activity against malignant human cancer cell lines (31). Glioma cells also show up-regulation and constitutive activation of Her-2 neu, and its expression which correlates positively with aggressive malignancy (Mellinghoff et al. 2005). A correlation has been demonstrated for the expression of gBK/K_{Ca} channels and Her-2 neu, which implies gBK/K_{Ca} channels as a downstream target for Her-2 neu signaling (Olsen et al. 2004). How K_{Ca} channel modulates EGFR tyrosine kinase or vice versa is poorly understood. It appears to occur via changes in intracellular calcium levels without change in channel expression or phosphorylation (Weaver, Bomben and Sontheimer, 2006). In a transgenic glioma mouse model, a loss of EGFR overexpression was observed by EGFRvIII introduction

(Gullick, 2001). This model of high grade glioma is useful in evaluating targeted molecular therapies in brain tumor.

Targeting angiogenesis in brain tumors

Angiogenesis plays a crucial role in malignant primary brain tumor growth. Several preclinical and clinical studies have confirmed that the vascular endothelial cell growth factor (VEGF) and the bFGF bind to their receptors to promote glioma growth. VEGFR is expressed in human high grade glioma but not found in normal brain. Increased concentration of angiogenic factors and their receptors is correlated with tumor vasculature and malignant human gliomas. Furthermore, it is shown that the endogenous inhibitor of angiogenesis, thrombospondin-1 (TSP-1) is produced by normal brain and low grade gliomas, but is completely absent in high grade gliomas. The GBM is among the most "endothelial rich" brain tumors studied. In children with brain tumors, microvascular density correlates with tumor recurrence, and patient mortality. As tumor vascularity is highly correlated with disease outcome in neuroblastoma, novel therapeutic that targets the vascular endothelium is a suitable clinical trial target candidate. The molecules like VEGF, bFGF, PDGF as well as endothelial integrins are linked to advanced malignancy, which provided the rationale for developing anti-angiogenic therapies in brain tumors. The potential of anti-angiogenic therapy in human brain tumors is demonstrated in experimental brain tumor models. A wide range of anti-angiogenic agents such as endogenous angiogenesis inhibitors, synthetic angiogenic inhibitors, antibodies, and anti-angiogenic gene therapy are investigated with radiation therapy. Anti-angiogenic drugs have low potential for toxicity and resistance because they specifically target endothelial cells. The potential of anti-angiogenic agents to augment the anti-tumor activity of standard cytotoxic chemotherapeutic agents is being investigated (Bernsen and van der Kogel, 1999; Reijneveld, Voest and Taphoorn, 2000; Takano et al. 2004).

The evidence for glioma anti-angiogenesis therapy, with or without chemotherapy has been described in several preclinical animal models. The anti-angiogenic function of TSP-1 is known for a long time. The TSP-1 transfected glioma cells lacked VEGF expression ability, which supports the rationale for using VEGF and bFGF antibodies in clinical trails. Anti-angiogenic drug, Thalidomide exhibits synergistic anti-glioma activity when combined with DNA alkylating agent Temozolomide, and increased median survival from 63 weeks to 103 weeks compared to Thalidomide only group (Baumann et al. 2004). While evaluating anti-angiogenic drugs for clinical development, it is important to analyze if such drugs penetrate the BBB, and survive p-glycoprotein-mediated drug efflux system. At present, there is a great deal of interest in combination therapy using conventional cytotoxic therapy with chemotherapeutics and radiotherapy. Anti-angiogenic agents like TNP-470, angiostatin, DC 101, SU5416, anti-VEGF and VEGF-R antibodies and VEGF monoclonal antibody A4.6.1, tyrosine kinase inhibitors, COX-2 inhibitors, and anti-EGFR inhibitors are used in combination with radiation. The synthetic fumagillin analogue, TNP-470 was shown to interfere with angiogenesis through inhibition of endothelial cell proliferation and migration in murine and human neuroblastoma xenograft model. Now it is being evaluated in phase I/II clinical trials. In brain tumor models, TNP-470 and Minocycline together increased 9L glioma sensitivity to BCNU and andriamycin (Shusterman et al. 2001), while Lund, Bastholm and Kristjansen, (2000) found that TNP-470 increased radiation sensitivity of human U87 glioblastoma xenografts. A phase II study with anti-angiogenic monoclonal antibody bevacizumab (Avastin) and anti-cytokine Irinotecan in brain tumor patients is also being conducted (NCT00381797). Endogenous inhibitors of angiogenesis such as angiostatin, endostatin, PEX, pigment epithelial-derived factor, and thrombospondin (TSP-1&2) are shown to be efficacious. They exert their effects through multiple mechanisms, including induction of apoptosis of micro vascular endothelial cells, inhibition of proliferation of endothelial cells, inhibition of function, and regulation of proangiogenic molecules. These endogenous inhibitors offer a novel treatment option because they are unlikely to trigger a host immune response. Angiostatin, a proteolytic fragment of plasminogen inhibits angiogenesis and attenuate the growth of primary and metastatic tumors. Angiostatin was effectively used in combination with

fractional radiation therapy in human glioma models (Mauceri et al. 1998; Rege, Fears and Gladson, 2005). Recently, gene therapy has hit a snag, but offers a promising alternate treatment strategy.

Brain tumors are attractive for gene therapy because the brain is an immunologically privileged organ, and the BBB provides a natural immunological barrier. Mice when treated with a retrovirus encoding a dominant negative mutant of the VEGF receptor Flk-1 resulted in reduced tumor growth and decreased blood vessel density. Recombinant adeno-associated virus (AAV) vector with the angiostatin gene was used to reduce tumor growth and angiogenesis in a C6 glioma model. Anti-angiogenic therapy using Semliki Forest Virus (SFV) carrying endostatin gene significantly reduced the tumor growth in animals. Therefore, gene therapy with endostatin delivered via SFV may be a viable treatment strategy for brain tumors (Ma et al. 2002; Yamanaka et al. 2003). Although, the gene therapy in general is in its infancy, it provides an alternate strategy to treat hard-to treat brain tumors.

Epigenetic genes as brain tumor targets

Epigenetic events are genetic modifications (DNA methylation and covalent histone modifications) that are heritable through cell division, which affect gene expression without causing changes to the DNA coding sequence. Cancer cells exhibit global hypomethylation of the genome accompanied by region-specific hypermethylation events. The hypomethylation mainly occurs in the repetitive sequences leading to genomic instability and tumor formation. Aberrant hypermethylation occurs at CpG islands found in the promoter region of genes, which is usually associated with the transcriptional silencing of that gene (Baylin et al. 2001). DNA methylation changes (Palanichamy, Erkkinen and Chakravarti, 2006), particularly CpG island hypermethylation is frequent, early, and common event (as common as mutations) in many types of cancers leading to the inactivation of tumor suppressor genes.

Several genetic changes have been identified in AAs and GBMs involving heterozygous deletion of 19q13, inactivation/deletion of tumor suppressor genes namely p16INK4A (Hegi et al. 1997), p14ARF (Ichimura et al. 2000), RB1 (Ichimura et al. 1996), PTEN and p53 gene (Mashiyama et al. 1991) and amplification of EGFR gene (Libermann et al. 1985). Epigenetic research in glioma pathogenesis revealed several epigenetic genes silenced by promoter CpG island hypermethylation, such as, cell cycle regulatory proteins RB1 (Nakamura et al. 1996), p16INK4A (Costello et al. 1996; Fueyo et al. 1996), myelin related gene EMP3 (Alaminos et al. 2005), and matrix metalloproteinases inhibitor TIMP3 (Bachman et al. 1999). Comprehensive whole-genome microarray studies using inhibitors of epigenetic modification have identified several genes including CST6 (putative metastatic suppressor), BIK (apoptosis inducer), TSPYL5 (unknown function), BEX1, and BEX2 (uncharacterized function) as putative tumor suppressors that are frequently methylated in primary gliomas (Kim et al. 2006; Foltz et al. 2006). Another genome-wide study using restriction landmark genomic scanning has identified as many as 1500 CpG islands to be aberrantly methylated in low grade gliomas (Costello et al. 2000). These studies have highlighted a role for DNA methylation in gliomagenesis. To date very few genetic assays are available to accurately provide information regarding patient prognosis or response to therapy. It has been hypothesized that aberrant DNA methylation plays a key role in tumor initiation. Therefore identifying such modifications helps in early detection of cancer, and might also provide information regarding the mechanisms that control glioma progression (Costello, 2003). In addition to being a diagnostic marker, DNA methylation can also serve as an useful prognostic marker as shown by the methylation of the DNA repair gene, MGMT, in gliomas. Epigenetic silencing of the gene (involved in the repair of DNA damaged by alkylating agents) is associated with the increased survival in patients treated with alkylating drug Temozolomide (Esteller et al. 2000; Komine et al. 2003). Current laboratory studies are aimed at discovering novel methylation markers in tumor tissue as well as in the patient's body fluids (Belinsky et al. 2006; Cairns et al. 2001). Since the primary DNA sequence of epigenetically modified genes remains intact, it is possible to reactivate genes using inhibitors of DNA methylation or histone modifications (Daskalakis et al. 2002; Plumb et al. 2000). Clinical trials using DNA methylation and histone deacetylase inhibitors, which reactivate silenced genes in cancers, are in various development stages. The DNA methyltransferase inhibitors,

5-azacytidine (Vidaza) and 5-aza-2′-deoxycytidine (Decitabine), are used with reasonable success in the treatment of hematologic malignancies (Lubbert, 2000), but have limited success in solid tumors. Combination of HDAC inhibitors with DNA methyltransferase inhibitors appear to synergistically induce the expression of silenced genes (Cameron et al. 1999). However, these drugs have drawbacks such as extreme instability, serious side effects, and sometimes these drugs at high doses may promote malignant transformation. Alternative approaches include the use of siRNA targeted against the DNA methyltransferase enzyme (Goffin and Eisenhauer, 2002) and developing stable small molecule inhibitors that can overcome the BBB.

Small interfering RNA (siRNA) to target brain tumor gene(s)

The *siRNA* directs the targeted destruction of mRNA encoding a specific protein, in a process known as RNA interference (RNAi). This process stops translation of the targeted mRNA into protein, effectively silencing the gene. RNAi is a recent discovery, identified in mammalian cells in 2001, but it has rapidly advanced into practical technique, and is being used increasingly to investigate mammalian gene function. Tools are available to induce RNAi in cell lines, intact tissue preparations, and even in *in vivo*. Depending on the method used, loss of gene expression may be transient or sustained, enabling a wide range of functions to be investigated. The RNAi is a powerful technique that can be used to produce targeted knockout of genes in mammalian cells (Gurney and Hunter, 2005). Its applications potentially include identification of protein function in health and disease, identification of novel genes, and drug target validation. Effective RNAi requires an appropriate siRNA sequence to be designed and an efficient method for delivering the siRNA to the cells of interest. Since not all potential siRNA sequences are effective, it is important to verify the loss of gene expression by measuring the level of protein remaining. Limitations for delivering siRNA are one of the main obstacles to produce efficient RNAi, especially in intact tissue preparations. A successful *in vitro* method for targeted RNAi against the TASK-1 potassium channel gene (Gurney and Hunter, 2005) was described. Increasing evidence show that microRNA (miRNA) represent a new class of genes involved in oncogenesis (Ciafre et al. 2005).

miRNAs as druggable targets

The miRNAs are non-coding, double stranded RNA molecules with an average size of 22 bp, and serve as posttranscriptional regulators of gene expression in higher eukaryotes. The miRNAs play an important role in development and other cellular processes by hybridizing to complementary target mRNA transcripts and destabilizing the latter by preventing their translation (Ambros, 2003; Bartel and Bartel, 2003; Bartel, 2004). Although a few hundred miRNAs have been discovered in a variety of organisms, little is known about their cellular functions. They have been implicated, among others, in regulation of developmental timing and pattern formation, restriction of differentiation potential, regulation of insulin secretion, resistance to viral infection, and in genomic rearrangements associated with carcinogenesis or other genetic disorders, such as the fragile X syndrome. Recent evidence suggests that the number of unique miRNA genes in human ranges from 1000 to 20,000. It is estimated that 20%–30% of all human mRNA genes are miRNA targets, and hence special attention has been given to miRNAs as candidate drug targets in brain tumor.

Several recent reviews and research articles have illustrated the involvement of miRNAs in cancer (Calin and Croce, 2006a, 2006b; Jannot and Simard, 2006; Kent and Mendell, 2006; Jovanovic and Hengartner, 2006; Dalmay and Edwards, 2006; Hutvagner, 2006; Osada and Takahashi, 2006; Zhang and Coukos, 2006). Therefore, we will restrict this section to the general concepts. In a recent study the miRNA expression levels in GBM was investigated (Ciafre et al. 2005; Chan, Krichevsky and Kosik, 2005). The analysis of both glioblastoma tissues and glioblastoma cell lines showed a significantly altered miRNA expression. The most interesting miRNA is miR-21, which is significantly upregulated in glioblastoma. In another study, knockdown of miRNA-21 in cultured glioblastoma cells triggers activation of caspases that leads to increased apoptotic cell death (Chan, Krichevsky and Kosik, 2005). These data suggest that aberrantly expressed miR-21 may contribute to the malignant phenotype by blocking expression

of critical apoptosis-related genes. A set of brain-enriched miRNAs, miR-128, miR-181a, miR-181b, and miR-181c, are down-regulated in glioblastoma is also discovered (Ciafre et al. 2005; O'Driscoll, 2006). One of the early works that demonstrates miRNAs as potential candidate drug target was performed by obstructing the adipocyte differentiation process in human primary adipocytes (Esau et al. 2004). Major hurdles are expected before a miRNA-based drug is successfully developed against cancer. In fairness this is only the beginning of the impact of the discovery of miRNA on understanding the brain tumor etiology, and developing cancer treatment strategies.

Anti-cancer drug delivery to brain tumor

Drug delivery in the treatment of brain tumors is a crucial consideration in the development of anti-cancer agent because the delivery of all substances into the brain is tightly regulated by BBB. Brain tumor cells diffuse into the normal brain and are protected by intact BBB (Rich and Bigner, 2004), where anti-cancer drug delivery is very critical. We showed that improved drug delivery in human glioma xenograft models (Ningaraj, 2006) has the potential to be extrapolated to patients with brain tumors for better control of the disease. In this direction, our laboratory is developing methods for high-throughput screening of RTKIs for selective delivery to brain tumors, simultaneously monitor dosing, delivery, and pharmacological efficacy of RTK inhibitors in animal brain tumor models. The challenges and opportunities of the biochemical modulation of BBB for selective drug delivery to brain tumor was reviewed recently (Ningaraj, 2006). We showed that intravenously administered, potassium channel agonists increase TMZ (Fig. 2) and Her-2 MAb (Herceptin) (Ningaraj, Rao and Black, 2003b) delivery across the BTB to elicit anti-tumor activity and increase survival in nude mice with intracranially implanted human glial tumor. Our study suggested that the BTB allows a small amount of TMZ into brain tumors. Potassium channel agonist-mediated biochemical modulation significantly increased BTB permeability allowing greater amounts of

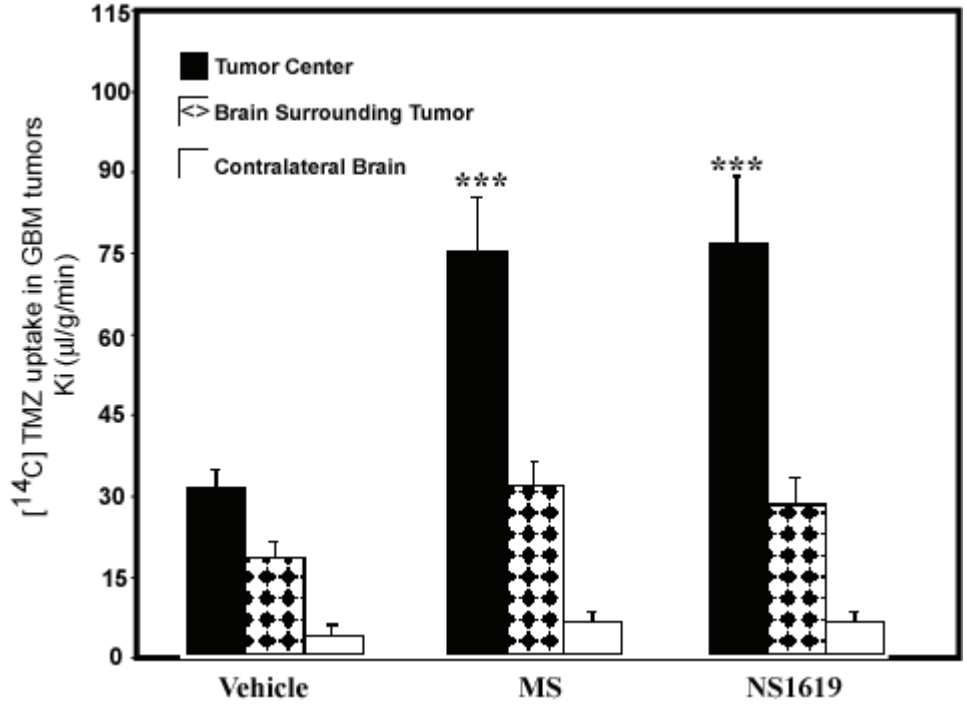

Figure 2. Quantitative increases in BTB permeability. A significant increase in the mean K_i for [^{14}C]-Temozolomide (TMZ) after i.v. infusion of 100 µg/kg/min for 15 min of NS-1619 and MS compared to a vehicle-treated group was observed. The increase in [^{14}C]-TMZ uptake in tumor center was significant although a slight increase in uptake of the radiotracer was observed in the brain tissue-surrounding tumor. No [^{14}C]-TMZ uptake in contralateral normal brain, which served as internal control, was observed in all the groups. Data are presented as mean ± S.D (N = 6), ***P < 0.001 versus vehicle-treated group. Precaution was taken to avoid necrotic area during the K_i measurement by comparing the QAR brain section with a corresponding H&E stained serial brain tumor section.

TMZ, selectively to reach brain tumor and brain tissue surrounding tumor, which represents proliferating edges of tumor where the BBB may be intact (Pardridge et al. 1992). Furthermore, we showed that Trastuzumab combined with TMZ co-administered with potassium channel agonists significantly increased survival rates in mice with intracranial GBM xenograft (unpublished data). These results are consistent with our earlier study, where we showed that potassium channel activator (Minoxidil sulfate: MS) infusion selectively enhanced carboplatin delivery to tumor tissue without increasing delivery to normal brain (Ningaraj, Rao and Black, 2003b). MS co-infusion with carboplatin in rats resulted in tumor regression, significantly increasing survival (Black and Ningaraj, 2004). The ability to deliver Her-2 neu targeting drug Herceptin (Trastuzumab) by potassium channel-mediated BTB modulation in human xenografts may be clinically useful because GBMs frequently have altered receptor tyrosine kinase genes (Fuller and Bigner, 1992), including Her-2 neu that is over expressed in about 17%–20% of GBM patients (Forseen et al. 2002) resulting in poor prognosis and patient survival. A molecular target-based therapy using Trastuzumab and Pertuzumab (Omnitarg, 2C4) is developed by Genentech Inc., for brain tumor, but their delivery across the BTB remains a major concern.

Molecular medicine

To conclude, the future of molecular targeted therapy is to achieve customized treatment strategy for brain tumor patients, where individual patient treatment will be based on the molecular profile of the disease. The information based on the changing levels of active genes/proteins inside tumor cells in response to an anti-cancer drug, could help physicians to determine early in the treatment whether a drug works effectively or not. Researchers have identified gene/protein markers that are useful in individualizing treatment in prostate, breast, and ovarian cancer patients. Although, brain tumor tissue is heterogeneous, the genetic profiling of tumor tissue gives valuable molecular information. As a case in point, high-throughput gene profiling of brain tumor biopsy samples by gene array technique can be compared with genomic data generated using tumor samples to predict whether patients would benefit from anti-cancer drug (like Gleevec) treatment or by potassium channel modulation in tumor and tumor vascular endothelial cells.

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