Molecular Neuro-oncology and the Challenge of the Blood-Brain Barrier

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Despite epochal advances in brain imaging, radiation delivery, and neurosurgical sophistication, the survival of patients with primary malignant astrocytomas has not been met with commensurate progress. While temozolomide, an alkylating agent, has demonstrated a survival benefit, median survival in the past decade of patients with glioblastoma (GBM) remains an obdurate 15 months and add-on therapies have not significantly prolonged life. It is likely that further advances may need to await additional discoveries that are slowly being revealed by molecular exploration of the tumor genome. This review summarizes many recent developments in molecular neuro-oncology and examines formidable challenges imposed by the highly restrictive properties of the blood-brain barrier (BBB) that impact effective drug delivery.
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Abundance of knowledge does not teach men to be wise.
–Heraclitus
How small a thought it takes to fill a life.
–Wittgenstein

Despite revolutions in neurosurgery, brain imaging, and conformal radiation delivery, long-term control of most primary brain tumors remains elusive. In the modern age of chemotherapy, median survival of patients with glioblastoma (GBM), the most common of these most malignant primary brain tumors, remains resolute at about 15 months. When relapse occurs, treatment options are limited and response is generally shorter. The addition of bevacizumab to temozolomide in newly diagnosed GBM does not extend overall survival (OS), although it does improve progression free survival (PFS).[1,2] Failure to prolong OS has emerged as a recurring theme in several phase II and III brain tumor trials reported in the past 2 years. The third rail of treatment and arguably the most important, chemotherapy, may need to await molecular interrogation of the tumor genome to reveal new and exciting insights that may render them much more amenable to treatment.

The classification of astrocytic tumors is still based on graded alterations in cell and tissue architecture: degrees of anaplasia, mitoses, endothelial proliferation, and necrosis to provide prognosis. Some molecular markers add diagnostic and prognostic clarity in histologically similar tumors. This review will discuss a number of advances in brain tumor molecular pathology. Some may lead to treatments that are unique and specific, and that significantly extend life. Others may emerge as collateral targets that may guide further therapies. This review also will address some considerable challenges additionally posed by the highly selective blood-brain-barrier (BBB) and blood-brain-tumor-barrier to the delivery of any potentially effective treatment.

MGMT

The *MGMT* gene (O^6 methyl-guanine methyltransferase), located on chromosome 10q26, encodes an enzyme that removes alkyl groups from the O^6 position of guanine. This DNA repair mechanism reverses the effect of alkylating agents. Tumors with high MGMT activity become resistant. Methylation of the MGMT promoter, thought to be an early event in glioma pathogenesis, is associated with improved response to chemotherapy with temozolomide and other alkylating agents and longer patient survival and is an independent prognostic and predictive marker.[3,4] MGMT promoter methylation is present in about 40% of GBMs, 38%–75% of grade II astrocytomas, and 25%–70% of grade III astrocytomas. The

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effect of concomitant and adjuvant temozolomide versus radiation alone was evaluated in the European Organization for Research and Treatment of Cancer (EORTC)/National Cancer Institute of Canada (NCIC) 26981/22981 phase III clinical trial. The benefit of combined chemo-radiation was higher in the MGMT methylated patients (23.4 *v* 15.3 months; $P = .004$) than in unmethylated patients (12.6 *v* 11.8 months; $P = .035$).[5,6] As a result of these and other related findings, it has become standard practice to perform MGMT analysis on GBMs.

While MGMT methylation is both predictive and prognostic, it is limited by uncertainty regarding what is the optimal measurement technique. MGMT status by immunohistochemistry is not reliably associated with MGMT promoter methylation or outcome and has significant inter-observer variability. Determination of methylation status by methylation-specific polymerase chain reaction (PCR) or pyrosequencing of bisulfite-mediated DNA, is a reliable method.[7] In this study, methylation-specific PCR was used, but the method is qualitative and lacks automation. Semi-quantitative and predictive methods have been developed, but these techniques do not evaluate the same region of the promoter. Since promoter methylation can be heterogeneous, some patients can be classified as methylated or unmethylated depending on the technique used. Two recent studies suggest that pyrosequencing was the most reliable technique and had the best predictive effect.[7,8]

ISOCITRATE DEHYDROGENASE

One of the most important findings to emerge from non-hypothesis driven research in The Cancer Genome Atlas (TCGA) pertains to the importance of isocitrate dehydrogenase (IDH) mutations in gliomagenesis. IDH is an enzyme in the Krebs cycle that catalyzes the oxidative decarboxylation of isocitrate and produces α-ketoglutarate (α-KG) and CO_2. IDH1 is the isoform localized to the cytoplasm and perioxisome[9] and IDH2 and IDH3 to the mitochondria. The vast majority of IDH mutations are IDH1 (97%) and a small number are IDH2. Mutations in IDH1 and IDH2 decrease the affinity of the enzyme for isocitrate and increase the affinity to bind α-ketoglutarate as the alternative substrate. This leads to the neomorphic oncometabolite, 2-hydroxyglutarate (2-HG). The vast majority of mutations occurs at codon 132 and involved base substitution of a guanine for adenine, which results in an amino acid change from arginine to histidine (R132H). 2-HG is an antagonist of α-KG and several α-KG–dependent deoxygenases. These include histone demethylases and 5-methylcytosine hydroxylases, which demethylate DNA and are essential for gene expression. IDH-mutations are rare or absent in grade 1 gliomas and ependymomas. They are often identified in tumors of young adult patients, although they are rare in pediatric gliomas. IDH-mutated gliomas contain hypermethylated DNA and may have a more favorable prognosis than same-grade non–IDH-mutated tumors.[10,11] 2-HG levels are very low in normal brain. In an IDH-mutated setting, the increase in 2-HG levels is about 3 orders of magnitude greater and levels can be detected by 3T magnetic resonance imaging (MRI) spectroscopy (MRS). Using MRS, 2-HG on MRIs of patients with IDH-mutated gliomas also correlates with IDH mutations by gene sequencing.[12]

IDH mutations are an early-stage event in glioma pathogenesis because they are found in astrocytomas, oligodendrogliomas, and oligoastrocytomas. They may lead to similar early-stage molecular events that may direct differentiation along certain histological pathways. They are also much more common in secondary GBMs, which are tumors that arise from lower grade astrocytomas than primary (or de novo) GBMs.

Molecular gene profiles have defined four separate GBM subclasses, termed proneural, neural, classic, and mesenchymal subtypes, which correlate to specific mutation patterns (and response to existing chemotherapy and radiation). The proneural subtype is associated with *PDGFA*, *IDH1*, and *TP53* mutations and *EGFR* mutations/amplification. It has been divided further into non–G-CIMP (glioma CpG island methylator phenotype) and G-CIMP subgroups. The classic subtype is characterized by *EGFR* amplification/mutations and does not have *PDGFA* alterations or *IDH1* and *TP53* mutations. *NF1* mutations are typically expressed in the mesenchymal subtype.[13]

IDH-mutant tumors have been suggested to predict a more favorable response to front-line temozolomide chemotherapy in low-grade astrocytomas, but this requires validation. An analysis of the outcome of IDH mutations in a phase III Radiation Therapy Oncology Group (RTOG) trial of radiation (RT) + PCV (procarbazine, lomustine, and vincristine) in favorable low-grade glioma and a phase III EORTC/NCIC trial of upfront RT versus RT and temozolomide in progressive low-grade glioma is expected to define the predictive value of IDH mutations in low-grade glioma.[14]

CO-DELETIONS OF 1P AND 19Q

Co-deletion of 1p and 19q chromosomes is present in about 60%–80% of oligodendrogliomas.[15] This results in an unbalanced t(1;19)(q10;p10) translocation in which one copy of the short arm of chromosome 1 (1p) and one copy of the long arm of