Unraveling the Fundamentals of Brain Tumor Cell Migration

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This is to acknowledge that Dr. Bachoo has disclosed that he does have financial interests or other relationships with commercial concerns related indirectly to this program (PI of two Sponsored Research Agreements with Peloton Pharmaceuticals). Dr. Bachoo will not be discussing off label uses of molecular targeted therapies in his presentation.

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Interests:
Malignant Glioma
Basic mechanisms in gliomagenesis as related to gliogenesis
Mouse modeling of glioma
Brain tumor cell migration
Cancer metabolism

Purpose and Overview:

The goal of this presentation is to review the state of the field of malignant glioma including diagnosis and clinical management. In addition, a major focus will be on the failure of molecular targeted therapies to date and the important role that tumor cell migration plays in key aspects of tumor progression.

Objectives:
1. To understand the role of surgery, radiation therapy, and chemotherapy in the management of patients with all grades of glioma.
2. To identify the unique properties of gliomas that make them incurable with current therapies.
3. To identify the genetic mutations that have lead to a new classification of gliomas.
3. To understand the approach being taken on the basic and translational fronts to try and understand tumor cell migration and develop directed therapies.

Overview

Malignant gliomas (WHO grade II, III and IV) most often present with a single seizure or a few days of progressive headaches, and evaluation with MR imaging reveals a large mass that was likely present for months to years. Its stealth-like behavior belies the hallmark feature of this deadly cancer; that it infiltrates normal brain diffusely, early in the disease course and over long distances, making it impossible to detect early or to cure surgically. Lower grade II /III gliomas, classified as oligodendrogial, astrocytic or mixed, vary widely on clinical behavior (survival ranging from 2-15 years). Frequent hotspot somatic mutations in isocitrate dehydrogenase 1 or 2 (IDH1/2), are proving to be diagnostic and prognostic, and may be potential therapeutic targets. In contrast, median survival of Glioblastoma (GBM) (WHO grade IV) is 16 months with approximately 25% of patients alive at 2 years. The mainstay of treatment is external beam radiation in combination with the oral alkylating agent, temozolomide. The glioblastoma genome has been extensively characterized, yet no molecular targeted therapies have yet proven effective in the clinic. Improvements in outcome for this devastating disease will require a better understanding of the pathophysiology and the development of robust preclinical models for testing new therapies.

The following text includes Dr. Bachoo's recently published chapter on Glioblastoma, published in Rosenberg's Molecular and Genetic Basis of Neurological and Psychiatric Disease (Fifth Edition, 2014). Additional discussion of low-grade gliomas, genetics and tumor cell migration is included and will be covered in the Grand Rounds presentation.

Disease Characteristics

Glioblastoma (GBM) is a disease with a median survival of 15 months that strikes without warning, often manifested by a single seizure, new onset of a focal neurological deficit, or a few days of progressive severe headache. Initial evaluation with MR imaging usually reveals a heterogeneously gadolinium-enhancing mass on T1-weighted images with surrounding T2-weighted FLAIR signal[2]. It is glial in origin and classified by World Heath Organization (WHO) as grade IV/IV glioma. Diagnosis is made surgically, with an open craniotomy and tumor sampling or a stereotactic biopsy if surgical resection is deemed unsafe because of location of the tumor or the patient's clinical status. Since extent of resection may independently impact overall survival and recurrence rates[3,4], an attempt is usually made at the time of surgery to remove as much tumor as can be taken without causing a neurological deficit. The potential for a gross total resection depends on the location of the tumor and often a subtotal resection is achieved because of the safety concerns.

Diagnosis/testing

Histological analysis of the tumor reveals hypercellularity, paucity of neurons and glial cells, microvascular proliferation and usually the hallmark characteristic of GBM, pseudopalisading necrosis in which necrotic foci are typically surrounded by "pseudopalisading" cells[2]. Testing for methylation status of the DNA repair enzyme, O6-methylguanine methyl transferase (MGMT) is done routinely as a prognostic marker in academic centers [5] because methylation has been shown to be associated with better overall survival[6]. In addition, immunohistochemical analysis for mutated isocitrate dehydrogenase 1 (IDH1) is performed and, if negative, sequencing of commonly mutated regions of IDH1 and 2 are performed in some centers since the presence of the mutation identifies a good prognostic subgroup of patients [7].

Management

The current standard therapy for GBM, regardless of clinical or molecular subgroup, is external beam radiation combined with daily oral temozolomide for 6 weeks followed by 12 months of temozolomide on a 5 out of 28 day schedule[8]. Time to tumor recurrence is highly variable and patients are generally followed with serial MRI scans every 2-3 months until recurrence. Treatment for recurrent GBM generally includes use of the VEGF inhibitor, bevacizumab (Genentech), alone or in combination with a cytotoxic agent or molecular targeted therapy[9]. Duration of response is generally short and thus treatment on a clinical trial of a new agent or combination is encouraged.

Current Research

Extensive genomic, transcriptional, and epigenomic data has identified the landscape of molecular characteristics of GBM yet, to date, treatments based on targeting specific genes and pathways have not lead to improvements in overall survival. Research is focused on expanding the range of therapeutic targets, including vaccine therapy[10]and metabolic reprogramming[11].

Clinical Features

Historical overview

The etiology of GBM remains elusive. Risk related to chemical exposure, in particular Sarin nerve gas in Gulf War Veterans has been documented [12] although in the vast majority of cases, no etiologic factors can be determined. Signs and symptoms associated with brain tumors occur as a result of either increased intracranial pressure and/or focal cerebral dysfunction. The early signs of increased pressure include nausea, vomiting, headache and change in mental status and are sometimes accompanied by clinical findings of papilledema and loss of retinal venous pulsations. The late symptoms of uncal or cerebellar-foramen magnum herniation, associated with clinical findings of hemiplegia, hemianopia, pupillary dilatation and posturing constitute a surgical emergency for tumor debulking and relief of increased pressure. For cerebral hemispheric tumors, the symptoms reflect the neurological functions of the area affected by tumor. For example, temporal lobe tumors in the dominant hemisphere are heralded by progressive development of contralateral hemiparesis and expressive aphasia while tumors in the parietal lobe frequently manifest contralateral neglect, sensory inattention, dysesthesia, dyslexia, and dysgraphia. Frequently, temporal lobe tumors that have no overt focal neurological symptoms produce clinically subtle brief focal or partial seizures that may include olfactory or gustatory hallucinations, transient sensations of déjà vu or fear that prior to diagnosis may be misdiagnosed as panic attacks.

A major impact of the development and widespread use of MR imaging has been the earlier detection of GBM, such that late presentation with uncal herniation or complete hemiplegia from progressive symptoms has become much less common. Thus, earlier detection preserves neurological function and quality of life, making aggressive treatment more feasible although does not alter survival, since the major barrier to effective therapy is diffuse infiltration of highly resistant tumor cells. Historically, the treatment for GBM was debulking surgery followed by a 6 week course of external beam radiation. In 2005, the addition of the alkylating agent, temozolomide, to radiation therapy increased median survival from 9 months to 14.6 months[13]. With the results of 2 large phase 3 studies published in 2014 [14,15], no further increase in overall survival has been achieved with any single agent or combination therapy.

Mode of Inheritance and Prevalence

Neurofibromatosis and Li-Fraumeni syndromes predispose to gliomas[16], although most of the tumors are WHO grade II, not GBMs. Greater than 98% of all GBMs are sporadic. Rare families with multiple GBMs have been described, although with no clearly identified common genetic mutations [17].

The most comprehensive reporting of the prevalence of GBM is from the Central Brain Tumor Registry in the United States (CBTRUS), which combines data from the Center for Disease Control's (CDC) National Program of Cancer Registry (NPCR) and the National Cancer Institute's (NCI) Surveillance Epidemiology and End Results (SEER) Program. Most recently reported are data from 2006-2010, during which 50,872 GBMs were identified, accounting for 15.6% of all brain tumors and 45.2% of all malignant brain tumors [18]. The age-adjusted incidence of GBM during that time period was 3.19 cases per 100,000 people, with a 1.57:1 increased incidence in males vs females and 2.07:1 white: black.

Natural History

Age of onset

The median age at diagnosis for GBM is 64 years with the age-adjusted incidence increasing from 0.14 per 100,000 in pediatric patients (under 20 years), 0.4 in 20-44 year olds to 1.2 in 45-54 year old patients[18]. This is followed by a large increase to 8.08 per 100,000 in the 55-64 year old patients and then 13.09 in the 65-74 year olds and then remains relatively stable in the 75-84 age group (14.93 per 100,000)[18]. GBMs are far less frequent in the pediatric population. Among pediatric patients, gliomas represent 53% of all brain tumors in patients under 15 and 36% of brain tumors in the 15-19 year olds. Of these, GBMs occur far less commonly than in adults, accounting for only 2.6% of all gliomas in patients under age 15 and 2.9% in the 15-19 year olds[18]. A recent study comparing the molecular features of pediatric vs adult GBMs supports the notion that these tumors are derived along distinct genetic/epigenetic pathways leading to a common histopathologic endpoint [19].

Disease evolution and end of life mechanisms

GBM recurs after treatment with a variable time to progression ranging from a few months to a few years in a small number of patients (median ~6 months). Approximately 80% recur within 2 cm of the tumor resection margin and 20% recur at a site remote from the initial tumor location [20]. Except in very rare cases, GBMs do not metastasize outside the CNS. Aggressive treatment [21] of recurrent disease can result in stable disease for a period of time but inevitably the tumor grows leading to progressive neurological impairment with symptoms

related to the tumor location as well as progressive cognitive impairment leading to coma and death. The co-morbidities seen with progressive disease include a high incidence of deep vein thrombosis and pulmonary emboli, steroid-induced myopathies and diabetes, and seizures.

Disease variants

GBMs develop along 2 distinct clinical and pathological pathways. Ninety percent are primary or 'de novo' GBMs, which develop with a few month prodrome and no evidence of an antecedent precursor tumor. Ten percent of GBMs develop from a WHO Grade II (low grade) astrocytoma or oligoastrocytoma that is generally very slow growing for several years before transforming into a 'secondary' GBM. Low grade gliomas occur most commonly in young patients, with a median age of 30, and have a greater than 80% risk of transformation to GBM [22]. Secondary GBMs are histologically and clinically indistinguishable from primary GBM although have distinct genetic profiles [7,23]. Patients with low grade gliomas that do not progress to GBM die of widely infiltrating tumor.

Molecular Genetics

Insights from comprehensive molecular analysis of GBM

Recent large scale genomic and proteomic studies have provided new insights into the molecular complexity of GBM and identified recurrent gain of function mutations, which may serve as driver mutations [24,25]. The Cancer Genome Atlas project performed molecular analysis of 206 GBMs and identified common mutations in the major cancer-associated genetic pathways that impinge on critical activities of cell growth and survival. P53 signaling was altered in 87% of tumors (due to amplification of MDM2 or MDM4, homozygous deletion of CDKN2A, or mutation/deletion in TP53), leading to cellular proliferation, survival and translation RB signaling was altered in 78% (due deletions in CDKN2A, 2B, and 2C, amplification of CDK23, CCND2, and CDK6 or deletion of RB1) leading to G1/S progression. The receptor tyrosine kinase (RTK), RAS, PI3Kinase pathway was altered in 88% of tumors (due to most commonly to amplification of EGFR and/or loss of PTEN but other members of the pathway were also altered including amplification of PDGFRα, MET, AKT, mutation in ERBB2, or PI3K [24]. Analysis of an additional 250 GBMs has further delineated these pathways and identified novel mutated genes in these and other signaling pathways [25]. The complexity in GBM is further amplified by intratumoral heterogeneity by which different cells will harbor different combinations of mutations. For example, it has been shown that individual GBMs can harbor 2 more activating mutations or amplification of RTKs in the same cell or even in different populations of cells within the same tumor [26]. This has been raised as a possible explanation for a number of

ineffective clinical trials that target a single specific RTK suggesting that either the inhibitor can kill only the cells harboring the one specific RTK or that these are not the oncogenic drivers in the tumors.

Identification of IDH mutations in the secondary GBM pathway

For many years, low-grade gliomas have been characterized by loss of p53 in approximately 70% of cases with amplification/overexpression of PDGFRα in fewer than 20% of cases[27]. No other RTKs had been reported to be amplified or mutated in low grade gliomas or secondary GBMs, suggesting that these variants developed along distinct genetic pathways[23,28]. Transformation to GBM is commonly associated with loss of PTEN. In 2008, mutations were been identified in two isoforms of isocitrate dehydrogenase (IDH1 in the cytosol and IDH2 in the mitochondria) in >70% of grade II (low-grade) and III (intermediate-grade) gliomas and secondary GBMs[7,29,30]. However, fewer than 5% of primary GBMs harbor an IDH1/2 mutation. In gliomas, approximately 90% of the IDH mutations are found in IDH1 (R132H). Other tumors harboring these mutations in IDH1/2 include acute myelogenous leukemia [31], chondrosarcoma [32], and intrahepatic cholangiocarcinoma [33]. For gliomas, the presence of an IDH mutation has been shown to be associated with a better overall survival than IDH-WT tumors when compared grade for grade, and also is associated with a longer time to transformation in low grade gliomas when compared with tumors expressing only the wild type enzymes[7,30,34]. Acquired somatically, IDH1 and 2 mutations are activating mutations in the enzyme binding site which leads to production of high levels of 2 hydroxyglutarate (2HG) from α-ketoglutaric acid (αKG)[35]. The functional oncogenic and metabolic consequences of 2HG production include alteration of the epigenetic landscape and a block in cell differentiation [36].

Research efforts over the past 20 years to define the molecular underpinnings of lower-grade gliomas (LGG), defined as WHO grades II, low-grade, and grade III, intermediate grade gliomas, culminated in two recent landmark reports in *The New-England Journal of Medicine*[1,37]. These reports provide, for the first time, a robust molecular classification of LGG which is diagnostic and prognostic and which has not possible with routine histology, the benchmark for the past 100 years. These new findings are have prompted a revision of the WHO classification of brain tumors to include a molecular basis of classification.

The Cancer Genome Atlas Study (TCGA) Research Network report integrated, unsupervised (i) RNA-sequence, (ii) DNA copy number changes (iii) DNA methylation (iv) microRNA and (v) protein expression data from 293 LGG (WHO grade II and III) patients[1]. The accompanying report from the Mayo Clinic[37] selected three previously identified markers

(IDH1/2 mutation, 1p/19q co-deletion and mutation in the telomerase reverse transcriptase gene (TERT) promoter. Both reports, using either the unbiased approach (TCGA) or previously identified mutations, identified 3 non-overlapping prognostically significant clinical sub-groups. These were patients with only (i) IDH1 mutation, (ii) those with IDH1mutation and co-deletion of 1p/19q, and (iii) a small subset with neither IDH1 or 1p/19q co-deletion, which invariably had a TERT promoter mutation. The data is reprinted from the NEJM article (Figure 1). Grade II and III patients with IDH1 and 1p/19q codeletion had the most favorable outcome with median survival not reached at 10 years. Patients with only IDH1 mutation were the youngest at diagnosis (37 years of age) and had a median survival of almost 10 years. These two subgoups represented almost 90% of grade II and III glioma patients. Patients with neither an IDH1 mutation nor 1p/19q co-deletion had the worst outcome, with a median survival of 1.7 years. Notably, this subgroup could not be identified on the basis of routine histology or clinical profile, but often had mutations seen in Glioblastoma, including EGFR amplification, PTEN loss, Met amplification and CDKN2a loss. This subgroup may represent a glioblastoma in development, identified just prior to the development of all the characteristic features of glioblastoma. Overall, these molecular classifications are helping refine diagnosis and treatment planning for gliomas.

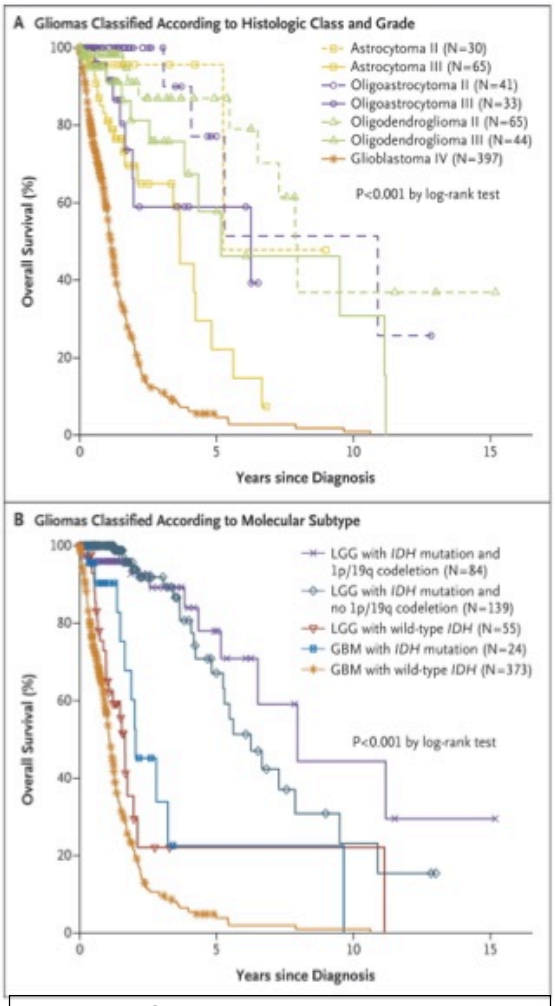

Figure 1. Clinical outcomes. From: Cancer Genome Atlas Research Network[1]

The field has progressed rapidly. Drugs targeting IDH1 and 2 (Agios, Cambridge, MA) that induce differentiation in mouse models of leukemia[38] and glioma[39] are in phase 1 clinical trials.

Disease mechanisms

GBM Cell-of-Origin and Genetically Engineered Mouse Models

Identification of the cell-of-origin of GBM remains an unanswered question but is

essential for the ultimate elucidation of the pathway(s) that are affected by oncogenic mutations early in tumor development. Such mutations may also be the most relevant therapeutic targets. In addition, defining the biochemical and biological changes that accompany the transformation of these target cells may provide clues to improvements in the treatment of GBM, to eradicate or halt tumor progression [40].

GBM may arise from malignant transformation of terminally differentiated astrocytes, glial restricted progenitors or multipotent neural stem cells. A number of reports have supported the role of a restricted population of neural stem cells found within the subventricular region as the cell-of-origin for gliomas [41] [42,43] [44,45] [46]. However, the studies on which these reports are based are limited by reliance on a constitutively active promoter (hGFAP, 2.2kb) that drives Cre expression during brain development resulting in activation of mutations in multiple lineages[47,48]. Although this strategy is efficient in generating high-grade glioma models, it is a poor representation of human adult GBMs, which have a median age of onset in the 6th decade. Thus, while these studies have identified progenitor cells as potential sources for glioma formation, they neither address nor preclude alternative mechanisms that could lead to glioma formation from a non-neurogenic niche precursor or more differentiated cell types. To circumvent the limitations of constitutive Cre-driver mice next generation inducible forms of Cre-Lox-P system have been developed. These rely on Cre recombinase fused to a mutated estrogen receptor, which is entrapped in the cytoplasm by binding to HSP90. Treating the mouse with tamoxifen at low doses allows the Cre-ERT^2 fusion to transiently enter the nucleus and excise lox-P flanked DNA regions. Such genetic models will be essential for addressing GBM cell of origin question. Using neural stem cell (nestin) and mature astrocyte-specific (PLA2G7) Cre-ERT^2 driver mice crossed to conditional oncogene and tumor suppressor mice has shown that both cell types are equally permissive for generating high grade gliomas (Bachoo, unpublished observations). This latest generation of genetically engineered mouse models, together with use of Cre-inducible lentiviral vectors have shown that both stem/progenitor cells as well as terminally differenated cells in the adult brain are susceptible to transformation and can give rise to gliomas [49,50].

Tumor Cell Migration

Diffuse single cell infiltration into normal surrounding brain is a pathological hallmark of all grades (grades II-IV) of malignant glioma and is the ultimate cause of death. Following periods of dormancy of months to years, mechanisms of recurrence are completely unknown and the clinical follow up of patients on treatment or during the period after treatment is with MRI

scans that document the changes in tumor size and invasion but without therapeutic options to halt the process. Recurrent tumors often occur in close proximity to the surgical resection, but can occur far removed (several centimeters) from the original tumor or even in the contralateral hemisphere. Whether infiltrating GBM cells are inherently more resistant to chemotherapy and radiation as a result of a unique mutational profile, or are partially protected from the cytotoxic effects of treatment-induced DNA damage by low rates of cell proliferation during treatment is unknown. Regardless of the precise mechanism(s) of therapeutic resistance, infiltrating GBM cells pose the single greatest challenge to improving the prognosis for primary brain tumor patients. Therapies, which could effectively block cell migration, could transform this fatal tumor into a local disease, one that could be effectively treated by surgery and/or high dose focal radiation.

For the past three decades, mechanisms of GBM migration and invasion have been studied under standard in vitro cell culture conditions and in vivo by transplanting established glioma cell lines into the brain of an immunocompromised mouse. Mechanistic insights from such studies are limited, however, by significant methodological problems: (1) standard GBM cell lines when transplanted into the SCID mouse, invariably grow as a circumscribed mass and show *no evidence of single cell infiltration, a pathognomonic* feature of GBM. Almost all in vitro studies have utilized two-dimensional (2D) surfaces such as cell culture dishes, glass coverslips or the transwell membranes that rely on chemo-attractant gradients to induce cell migration. These experimental models are poor representation of the normal brain interstitium since chemoattractant gradients or dense ECM matrix are not present in normal brain.

GBMs are capable of single cell infiltration through both cortical and subcortical grey matter, composed of tightly packed neuronal and glial processes, along white matter tracts and perivascular space lined by extracellular matrix (ECM). Migration through such diverse microenvironments raises the possibility that GBM cells may be capable of adhering to and gaining traction on a variety of different extracellular matrix molecules, which implies that infiltrating GBM cells may possess multiple mechanisms for invasion. Perhaps the most remarkable features of infiltrating GBM cells is their ability to infiltrate as single cells through an interstitial space that is estimated to be in the submicrometer range, without evidence of creating a path of proteolytic degradation or cellular destruction. At present there is little information on how GBM cells gain traction and generate sufficient contractile forces to overcome the mechanical challenge of migrating through the brains confined interstitial space. Understanding these steps will be critical to potential therapeutic targets to block tumor cell infiltration. Research efforts to understand the mechanics and biochemical mechanisms that

drive glioma cell migration are underway using primary human GBM cells that have been validated to be capable of single cell migration. Progress in designing assay systems that mimic the brain microenvironment are yielding interesting basic information about these processes. Data from the Bachoo lab will be presented during Grand Rounds.

Cancer Stem Cells

There is growing evidence that therapeutic failure in cancer is due to the relative refractoriness of rare populations of cells to all modes of treatment. Functionally identified as cancer stem cells or tumor-initiating cells the identification of this cell type has become a major focus in cancer, and specifically for GBM[51]. The implication is that to achieve durable therapeutic responses successful treatment regimens must target and successfully eliminate the cancer stem cell population within a growing tumor. To date, a major obstacle to eliminating GBM stem cells is the lack of methods for detecting and quantifying them for further studies.

Stem cells are uncommon cells defined by two exceptional features: the capacity for multi-lineage differentiation and self-renewal. These properties allow stem cells to generate an unlimited supply of progeny and are best exemplified by hematopoietic stem cells (HSCs) and embryonic stem (ES) cells. Observations from embryonic development and cancer biology have given rise to two popular concepts that link these fields: 1) tumors arise from stem cells; and 2) a small population within a tumor – so-called "cancer stems cells" – constitutes an inexhaustible source for tumor cells in the mass lesion. The identification of the cell type(s) from which GBM originates and through which its growth is sustained will be critical for a comprehensive understanding of tumor biology. Specifically, tumors arise and thrive within particular *permissive* cellular contexts that enable them to develop. Characterizing the key elements that define these contexts – namely cellular differentiation states, active signaling pathways, and/or developmental stages – will facilitate the understanding of the mechanism by which transforming mutations cause GBM and allow us to conceive of novel therapies and early detection strategies.

Patient derived tumor models

Although the latest generation of genetically engineered mouse models are very useful for testing specific hypotheses related to the cell of origin (astrocyte vs neural stem cell) and assessing the relative importance of particular driver oncogenes (EGFR vs c-MET, for example) and tumor suppressor genes (PTEN vs p53, for example), these models lack the complexity and the natural history of clonal selection associated with clinical tumors. Furthermore, they are

unable to address the existence of a cancer stem cell pool. The recent development of GBM patient-derived xenograft models in mice provide a better approximation of human tumors[52,53]. Despite the drawback due to reliance on an immune compromised host environment, these models have been shown to preserve the genomic, transcriptomic, and proteomic profiles of the patient's original tumor[53]. In addition, the xenografts preserve critical physiological pathways of the human tumor, such as the tumor's metabolic phenotype[52]. By maintaining GBM cells in a murine host microenvironment, without ever adapting the cells to culture conditions, these models represent the wide variety of genetic, epigenetic and transcriptional heterogeneity of the disease and, as such, can be very useful in basic investigation and preclinical evaluation of new therapeutics.

Testing

Molecular diagnosis

Molecular diagnosis currently focuses on the key genetic changes that have prognostic importance. IDH1/2 sequencing and assessment of methylation status of MGMT are becoming more routine in clinical practice [5,54]. Assessment of EGFR amplification and the presence of the EGFR VIII mutant are done when the specific EGFR targeted therapies are being considered. This is not standard in clinical practice because the anti-EGFR therapies have not proven to be effective.

Noninvasive imaging of 2-hydroxyglutarate as a clinical biomarker

The demonstration of high levels of 2HG in glioma cells with mutations in IDH1 or 2 has provided an unexpected and highly valuable biomarker for non-invasive brain tumor imaging. Methods using MR spectroscopy (MRS) at 3Tesla (T) have been developed a method to detect and quantitate 2HG [55,56]. Since the presence of an IDH mutation is associated with a better prognosis in GBM, imaging with 2HG can be diagnostic and prognostic as well as potentially important as a dynamic biomarker in the ongoing follow up of patients with IDH-mutated gliomas. We have demonstrated that the concentration of 2HG is reproducible between MR scans in the same patient, is stable when the tumor is stable, increases when the tumor is growing and decreases when the tumor is responding to treatment (Maher, unpublished observations). In addition to its clinical value, 2HG represents the first direct link between a genetic mutation and non-invasive imaging biomarker, which is a significant breakthrough in cancer diagnostics and imaging.

Management

Approach to management of GBMs relies first on maximal safe surgical resection, although due to the infiltrative nature of gliomas, none are curable by surgery alone. Post-operative radiation therapy has been shown to significantly improve survival for high-grade astrocytomas[57,58]. It has been established that external beam radiation to the tumor and adjacent brain is as effective as whole brain radiation and is less morbid[59]. Based on these data, the standard of care for GBM until 2005 (see below) was post-operative radiation (54-60 Gy over 6 weeks) to the tumor and a 2 cm margin.

Addition of chemotherapy either in combination with or following radiation did not alter overall survival until the development of temozolomide (TMZ, Merck), a second generation alkylating agent with excellent bioavailability and blood brain penetration. TMZ was approved for the treatment of recurrent high-grade astrocytomas in 1999 and further development of this drug included investigation in GBM. A landmark phase III study conducted by the European Organization for Research and Treatment of Cancer (EORTC) was published in 2005 that showed that addition of TMZ to radiation in newly diagnosed GBM patients improved median overall survival by 2.5 months [8]. In addition, the 1-year survival increased from 9% to 27%, and 2-year survival from 2% to 11% compared with radiotherapy alone[8]. Based on this study, the FDA approved TMZ for the treatment of newly diagnosed GBM in 2005 and the new standard of care regimen was established as post-operative treatment with concurrent radiation plus daily TMZ (75 mg/m2/day) for 6 weeks followed by adjuvant TMZ (150-200 mg/m2/day x 5 out 28 days) for 6-12 months. In a subset of patients from this study tumor was available for correlative studies. Methylation of the DNA repair gene O6-methylguanin-DNA methyltransferase (MGMT) was found to be correlated with better overall survival[6], thought to be a result of the tumor cell's inability to repair DNA after treatment with the alkylating agent, TMZ, leading to apoptosis. Based on these data, it was hypothesized that depleting MGMT in tumors that were not methylated and therefore had active MGMT would achieve the same goal of "inactivating" this mechanism. Several regimens of TMZ treatment were investigated and the regimen of 21 days treatment followed by 7 days off was shown to effectively deplete intracellular levels. A phase III randomized study for newly diagnosed GBM was conducted by the Radiation Therapy Oncology Group (RTOG) in which the 'dose intensive regimen' (21 days on/7 days off) was compared with standard dose (5 days on/23 days off) in the adjuvant phase after concurrent radiation and TMZ was completed. It also prospectively evaluated the status of MGMT in predicting response and/or survival benefit. A total of 833 were randomized between the 2 arms. No statistically significant difference was observed for median overall

survival (16.6 vs 14.9 months, respectively) or progression free survival (5.5 vs 6.7 months)[60]. MGMT methylation was associated with a marked increase in overall survival (21.2 vs 14 months) as well as response to treatment [60]and therefore has been identified as an important prognostic factor in GBM.

In 2009, the FDA approved Bevacizumab (Genentech), a vascular endothelial growth factor (VEGF) inhibitor for the treatment of recurrent GBM, based on 2 phase II clinical trials that showed responses in ~20% of patients with effects lasting, on average, approximately 4 months[61]. There has been considerable controversy as to whether bevacizumab is effective in combination with other agents due to variability in results of small phase II clinical trials. A major interest was in determining whether the addition of bevacizumab to radiation/TMZ and adjuvant TMZ would improve overall survival. Two large phase III studies have been completed and were published in 2014[14,15]. Each study randomized ~1000 patients to either the standard regimen with placebo or the standard regimen with bevacizumab given intravenously every 2 weeks throughout the full course of radiation/TMZ and adjuvant TMZ. Neither study was able to show an improvement in median survival with the addition of bevacizumab[14,15]. Moreover, there is some indication that the treatment was associated with increased neurocognitive toxicity[14]. While bevacizumab is an active drug in GBM but not effective in the upfront treatment setting, more work needs to be done to determine how best to use it[62].

The future of GBM therapeutics

The ability to do large, multi-center, international studies in GBM with correlative tissue collection and biomarker analysis has been established. This is undoubtedly a major advantage for the development of future therapeutics. However, the lack of efficacy of a wide range of treatments including the molecularly targeted therapies, alone or in combination with bevacizumab and TMZ, has raised the question as to where the next improvement in median survival will come from. There is a large effort directed at developing vaccines and harnessing the immune system for therapeutic benefit in GBM[63]. To date, vaccines have been shown to be safe with no demonstrable autoimmune activity in the brain. However, efficacy is currently lacking and future studies will be directed at optimizing antigen choices and minimizing immunosuppression due to the tumor itself and treatment with dexamethasone.

It is becoming clear that improved treatments are highly dependent on developing better mouse models of GBM for preclinical testing, as discussed above. Currently, almost no preclinical testing is done and drugs are taken straight from development to clinical trials. This is a very expensive, time consuming, and overwhelmingly disappointing and inefficient process. The patient-derived xenograft models that recapitulate the key features of the human disease

are critical for future drug development. Using the models to assess treatment response, develop biomarkers and understand resistance will mark a new era in GBM therapeutics and are the best chance for finally improving overall survival and heading for cure in this devastating disease.

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