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REVIEW ARTICLE

IMMUNOLOGICAL OBSERVATIONS ON GLIAL CELL NEOPLASM WITH CURRENT PROMISING MOLECULAR THERAPEUTIC OPTIONS

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ABSTRACT

Glioblastoma (GBM) is a highly malignant brain cancer characterized by uncontrolled cellular proliferation, diffuse infiltration, a tendency for necrosis, significant angiogenesis intense resistance to apoptosis, and widespread genomic aberrations. Glioblastoma multiforme results from a cascade of genetic alterations that begin in a target brain cell and, through unregulated cell division and a panoply of other molecular abnormalities, lead to an expanding mass lesion Among the therapeutic triad of surgery, radiation therapy, andchemotherapy only radiation therapy has been shown to improve survival. Despite aggressive treatments, malignant GBM hasremained difficult to treat, and its overall response to treatment hasremained poor, as has outcome in patients harbouring this lesion. Greater understanding of the tumour biology of GBM has been achieved in the past decade, leading to the prospect of novel targeted therapies and biomarker-based individualization of therapy. The aim of this review is to analyse the tumour biology and the pathologic features of GBM which includes guidelines for classification and diagnosis, the current status of prognostic and predictive biomarkers, and the role of the blood-brain barrier in delivering therapy for GBM.

INTRODUCTION

Glioblastoma multiforme is the most common and deadly malignant brain tumour. It has a very poor prognosis and is associated with low quality of life during the course of treatment.Glioblastoma Multiforme (GBM) is a grade IV neoplasm of the brain characterized by a heterogeneous group of cells that are unstable genetically, angiogenic, resistant to chemotherapy and highly infiltrative. In other words they are biologically aggressive tumours that present highly unique therapeutic challenges due to the following characteristics: 1) The tumour is localised in the cranial cavity; 2) The lesions show intrinsic resistance to conventional therapy; 3) The brain has very limited capacity to repair itself; 4) the spread of malignant cells into brain parenchyma; 5) the neurotoxicity of treatments directed at glioblastomas; 6) Tumour capillary leakage, with resultant peritumoral oedema and intracranial hypertension; 7) the limited response to therapy; and 8).Drug delivery to the tumour is very complicated due to the variably disrupted blood–brain barrier. GBM tumours present a series of mutations that provide cells with selective growth advantages that promote survival and proliferation in a hostile and hypoxic environment where normal cells cannot survive.Tumour suppressor genes, such as p53, p21, p16, and PTEN are commonly mutated in GBMs, pointing to the highly unstable nature of the cells (**Chen *et al*., 2012**). GBM tumours are pathologically characterized by the presence of necrotic areas and an aberrant vasculature comprised of glomeroid tufts, leaky hyper proliferative, and unorganized blood vessels At present the standard of care is surgical resection along with ionizing radiation (IR) followed by the administration of chemotherapeutic agent temozolomide (Temodar, Temodal, TMZ). However, this treatment only provides GBM patients with a 12–14 month survival period post- diagnosis.Almost all GBM patients undergo tumour recurrence despite aggressive surgical resection and chemotherapy. Recurrence at the primary site is observed in more than ninety percent of GBM tumours. The highly infiltrative nature of the tumour makes complete resection of the tumour with clean margins nearly impossible. In addition, GBM tumours can have extensive regions of hypoxia. The oxygen reduction limit the efficacy of IR as the generation of DNA-damaging free radicals is decreased. The capacity of GBM chemotherapeutic drugs to cross the blood brain barrier (BBB) and enter the tumour limits efficacy (**Kesari *et al*., 2011**). The tumour vasculature causes high hydrostatic pressure in the tumour hence drug delivery in the tumour is markedly supressed. These obstacles can by

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reduced by placing dissolvable chemotherapy wafers (Gliadel) in the tumour bed (**Panigrahi *et al.*, 2011**). However, even with IR, TMZ and Gliadel combined treatments, GBMs may exhibit a group of cells that survive the IR and TMZ treatments and may form a group of highly chemotherapy-resistant cells.

Tumour biology

Successful treatment of GBM requires a thorough understanding of tumour biology. These include difficulties in overcoming resistant cancer stem cells (CSCs) and multiple interactions with the tumour microenvironment, the characteristic tumour heterogeneity of GBM, and the circulating tumour cells.

GBM Stem Cells

The biology of glioblastoma has been well studied and interpreted in recent years. CSCs have been found in several cancers, including GBM.CSCs have a unique ability to self-renew thereby giving rise to fresh malignant stem cells. It also has the potency to proliferate into a variety of non-tumorigenic cells within the neoplasm. CSCs are believed to play a pivotal part in malignant glial cell tumour initiation, progression, as well as angiogenesis.

Tumour Microenvironment

The modifications of the tissue microenvironment can contribute to tumour growth and progression. Knowledge on the tumour microenvironment, the vasculature, inflammatory/immune cells, extracellular matrix, and growth factor signalling, may lead to the development of viable therapeutic targets for GBM. Among solid tumours, GBM is one of the most highly vascular among all neoplasms and, consequently, there has been great interest in the evaluation of anti-angiogenic therapy. A recent study reported by Soda and colleagues (**Soda *et al.*, 2011**) provided preclinical evidence that GBM cells trans-differentiate into endothelial cells. These tumour-derived endothelial cells (TDECs) were functional (formed vascular structures) and did not respond to anti-VEGF receptor inhibition. The authors suggested that the involvement of TDECs in tumour angiogenesis could be a factor in the resistance to anti-VEGF therapy that is often observed in patients with GBM. (**Soda *et al.*, 2011**) As GBM tumors increase in size, tumor cells must sustain balance between adaptation to hypoxia and cel death (**Tafani *et al.*, 2011**). The release of pro-inflammatory proteins is one such adaptation to the hypoxic conditions that occur within GBM tumours. A recent study indicated that a coordinated upregulation of proinflammatory proteins is activated in GBMs, and that this upregulation was more apparent in tumor cells than in peritumor and host tissue (**Tefani *et al.*, 2011**). The upregulation of pro-inflammatory proteins was also seen in hypoxic GBM CSCs, indicating that stem cells use such mechanisms to survive under hypoxic conditions. (**Tefani *et al.*, 2011**) Malignant gliomas express tumour-associated and tumour- specific antigens which the immune system normally recognises. The GBM patients exhibit profound immunosuppression.A recent study (**Wei *et al.*, 2010**) reported that showed that cancer initiating cells may play a role in many of the immunosuppressive features of GBM.. The results demonstrated that the mechanisms of this immunosuppression were by cell-to-cell contact and secretion of products, resulting in inhibited T-cell activity and proliferation, induction of regulatory T cells, and initiation of T-cell apoptosis (**Wei *et al.*, 2010**). Immune reactions in GBM are not well understood, but several novel immune-based therapies are currently under investigation (**Rolle *et al.*, 2010**). Bonavia and colleagues have suggested a hypothesis as to how glioma cells interact with other cells and the microenvironment to facilitate tumour growth. They propose that certain clones within a tumour acquire oncogenic mutations, resulting in a pro-oncogenic phenotype that favours other nearby clones. Furthermore, results reported epidermal growth factor receptor (EGFR) cells release interleukin-6 and leukaemia-inhibitory Factor, providing a microenvironment in which wild type EGFR cells are able to proliferate. This leads tosubsequent tumor growth in heterogeneous tumors compared to wild-type EGFR homogeneous tumors (**Bonavia *et al.*, 2011**). Extensive research has been conducted to elucidate the mechanisms of the major signalling pathways that are important in malignant gliomas In particular there has been considerable interest in the receptor tyrosine kinases EGFR, PDGFR (platelet-derived growth factor receptor), and VEGFR (vascular endothelial growth factor receptor). These signaling pathways have been reviewed previously (**Lo *et al.*, 2010**). In addition, a variety of small molecule targeted agents that inhibit signalling pathways are currently under investigation for GBM.There is increasing evidence that mesenchymal stromal cells may contribute to tumour pathogenesis and progression of malignant gliomas. Specifically, it has been suggested that stromal cells may contribute to tumor vasculature and/or premetastatic niche formation through the expression of endothelial markers such as VEGFR1 and VEGFR2 (**Kucerova *et al.*, 2010**).

Tumour Heterogeneity

Significant intratumoral heterogeneity is present in GBM; cells of an individual glioma may differ in their morphology, genetics, and biological behavior (**Bonavia *et al.*, 2011**). This heterogeneity occurs within an individual glioma and between different gliomas, and is of great importance in tumour grading, assessing response to therapies and understanding therapeutic resistance. The heterogeneous nature of GBM is one of the challenges faced in treating the disease because some tumour cells within an individual tumour may respond to a particular therapy, whereas other tumour cells may not. Various theories, reviewed by Bonavia and colleagues, (**Bonavia *et al.*, 2011**) explained how tumor heterogeneity occurs and is maintained. Heterogeneity could arise from clonal evolution, in which the more adaptive clones survive under selective pressures (eg, drug treatment) and produce subsequent mutations. Heterogeneity could also arise from CSCs that divide indefinitely and give rise to cells that differentiate heterogeneously. Acquired CSC mutations may generate a heterogeneous population of these cells. Furthermore, CSCs are thought to be drug resistant; thus, surviving cells may act as a reservoir for tumor recurrence (**Bonavia *et al.*, 2011**). Primary and secondary GBMs are morphologically indistinguishable but genetically dissimilar. Because of their genetic differences, it is hypothesized that

primary and secondary GBMs will respond differently to targeted therapies.

Classification and diagnosis of GBM

There are currently no tools for screening or detecting GBMs before clinical presentation, and no specific tumour markers have been identified. Diagnosis of GBM is achieved through tomographic imaging techniques, with magnetic resonance imaging (MRI) being the gold standard. Newer techniques include diffusion weighted imaging (DWI), perfusion-weighted imaging (PWI or perfusion MR), dynamic contrast-enhanced T1 permeability imaging (T1P), diffusion-tensor imaging (DTI), and MR spectroscopy. If patients present symptoms that suggest a brain tumour, they usually undergo a Magnetic Resonance Imaging (MRI) scan, which produces a detailed picture of the brain, enabling any abnormalities to be seen. Diagnosis is confirmed by a biopsy, where sample tissue is taken from the suspected lesion. Biopsy of a brain tumour must be undertaken with caution to limit damage to normal brain function. Brain tumour classification and grading is defined by the World Health Organization (WHO) classification of nervous system tumours. There are four grades of brain tumours which are classified on a scale according to the presence of certain criteria, such as growth rate and cell differentiation

- Grade I tumours are slow growing, non-malignant and are associated with long-term survival
- Grade II tumours are slow growing but generally return more frequently than grade I tumours
- Grade III lesions are malignant, fast growing and poorly differentiated
- Grade IV tumours are the fastest growing, highly malignant and are poorly differentiated.

GBM is classified as a grade IV brain tumour.

Limitations of the WHO classification system include the following: it cannot predict therapeutic response of individual tumours within the same histologic grade; it cannot precisely guide the choice of therapy, particularly those targeting molecular or genetic pathways; and it does not account for anatomical size or location. Nonetheless, the WHO system is the primary means for guiding therapy and assessing overall prognosis in patients with brain tumours. As our understanding of the role of targeted therapies and the genetic and molecular abnormalities associated with GBM increases, it may be particularly valuable to classify GBMs on the basis of these underlying abnormalities. Recently, following an integrated genomic analysis, Verhaak and colleagues (**Verhaak *et al.*, 2010**) described a system for classifying high-grade gliomas into four distinct tumor types according to genetic and molecular features. Furthermore, Young has suggested that incorporating advanced tumour imaging techniques into a classification system may provide advantages over theexisting histopathological WHO classification system.

EPIDEMIOLOGY OF GBM: PAST AND THE PRESENT

Incidence and mortality

Worldwide, there are an estimated 240,000 cases of brain and nervous system tumours per year – GBM is the most common, and the most lethal, of these tumours. In the USA alone, approximately 18,000 people are diagnosed with GBM eeach year. GBM accounts for around 13,000 cancer deaths in the US annually. In most European countries, new cases of GBM occur in approximately 2-3 people in every 100,000 each year (**Varhaak *et al*., 2010**).

Age and Sex of Patients

For all primary brain tumors, the patient's average age at onset is about 54 years. For glioblastoma and meningioma, the average age at onset is 62 years. Age distributions differ by tumour site and histology type, suggesting the likelihood of many different etiologic factors for the different histologic types. For example, Increase in age indicates an increase in glioma incidence, except for a slight decline in the 85 years and older age group. Conversely, astrocytoma and glioblastoma peak in incidence at age 65 to 74 years, and oligodendroglioma at age 35 to 44 years. Some of this variation may reflect differing diagnostic practices and access to diagnosis in different age groups. It is likely that the duration of exposure required for malignant transformation, the number of genetic alterations required to produce clinical disease, or poorer immune surveillance with advancing age may account for those tumour types that increase in incidence with age. An intriguing and as yet vaguely understood characteristic feature of brain tumour epidemiology is a peak in incidence in young children, some, but not all, of which is attributable to medulloblastoma and other tumours of primitive neuroectodermal origin. A recent study from New York state showed that the sex differential (greater incidence in males) in glioblastoma began to be evident around the age of menarche, was greatest around the age of menopause, and decreased thereafter, suggesting that female hormones may have a protective effect. Any comprehensive theory of the distribution and causes of brain tumours should explain the biologic and social factors that account for these consistently observed sex differences.

Geographic and Ethnic Variations

Interpretation of geographic and ethnic variations in the incidence of brain tumours is confounded not only by ascertainment bias but also by inconsistent reporting. The incidence rate for malignant brain tumours in East Asia is less than half that in Northern Europe. In the U.S., glioma affects more whites than blacks, but the incidence of meningioma is nearly equal among blacks and whites. The absolute variation in brain tumour incidence rates from high-risk to low-risk areas in both the U.S. and the world is about 4- to 5-fold. In contrast, 20-fold differences have been observed for lung cancer and 150-fold differences for melanoma. As with international comparisons, interpretation of these geographic differences is complicated by variations in diagnostic and reporting practices. **Chen *et al*. (2001)** showed that, among adults with astrocytic gliomas-GBM, anaplastic astrocytoma, and astrocytoma—diagnosed in the Bay Area between 1991 and 1994, whites were less likely than non-whites to have tumours containing mutations in *TP53* gene exons 5-8 (13% versus 42%). Whites were much more likely than non-whites to have tumours that accumulated p53 protein in the absence of

demonstrable *TP53* mutation (74% versus 50%) and were somewhat more likely to have tumours that neither accumulated p53 protein nor had mutations in the *TP53* gene (13% versus 8%). Age- and sex-adjusted comparisons were statistically significant. This was the first such report and clearly requires replication. For example, it is possible, although it seems unlikely, that the diagnosis—rather than the occurrence—of different molecular subtypes varies by ethnicity. However, the findings, combined with the intriguing findings of a much lower occurrence of *CDKN2A/p16ink4a* deletion and mutation among Japanese patients with glioma, compared with American and European white patients, clearly suggest that further research into ethnic differences in molecular subtypes of gliomas is warranted.

Symptoms

As GBM is an aggressive disease that progresses rapidly, patients can deteriorate quickly. The symptoms of GBM varies depending on the size and location of the tumour in the brain. The following are common symptoms (Medscape, 2012) Increased intracranial pressure (pressure build-up in the head) manifesting as headaches, nausea and vomiting, Cognitive impairment or slowing of cognitive function (e.g. losing the ability to speak or think clearly), Changes in personality, mood or concentration, Visual impairment, Seizures, Motor dysfunction such as paralysis, Sensory loss e.g. numbness, weakness. The symptoms of GBM are often distressing to patients and their caregivers as they significantly and negatively impact on quality of life as well as ability to carry out activities of daily living. Because of this, symptom management can be as important as treatment of the disease.

Prognosis

Cancer statistics often use an 'overall 5-year survival rate' to give a better idea of the longer term outlook for people with a particular cancer. It is almost impossible to predict how long an individual patient might live, but 5-year survival rates can give an approximate range. As GBM is a 'high grade' and advanced disease, the average 5-year survival rate for patients is particularly poor, at less than 3%.2 The majority of GBM patients do not live over a year.

However, the prognosis of GBM does vary depending on the age of the patient, the tumour size and location, the amount of tumour that can be removed during surgery and the neurological performance status of the patient which may impact on their treatment options (i.e. the ability for patients to live a 'normal' life and carry out day-today tasks).

Survival Factors

Molecular and genetic markers within the tumours also may have prognostic value For example, in an earlier study (**Simmons *et al*., 2001**) it was showed that a complex relationship of survival with the patient's age and the p53 and EGFR characteristics of the tumour in 110 patients with GBM. However, 5-year survival for patients with primitive neuroectodermal brain tumours expressing high levels of neurotrophin receptor TrkC mRNA was 89% compared with 47% when low or no levels of neurotrophin receptor TrkC mRNA were expressed (**Grotzer *et al*., 2000**).

According to **James *et al*. (2002)**, the only tumour types for which there is suffciently convincing data to propose specific alterations responsible for progression from lower to higher stage are those from astrocytoma to anaplastic astrocytoma to glioblastoma; p53 modifications are inversely related to stage, whereas changes in *p14arf*, *EGFR*, *CDKN2A*, and *PTEN* are more common in higher-stage tumours. Clearly, there is still enormous work to be done to systematically characterize the molecular alterations in primary brain tumours and to study the relationships of important modifications to etiology, progression, and prognosis.

Prognostic and predictive biomarkers:PDGF, PDGFR, and EGFR

Overexpression of PDGF and its receptor (PDGFR) is associated with low-grade astrocytomas and secondary GBM. Although the predictive relevance of PDGF and PDGFR is unclear, one study in 101 patients with recurrent GBM linked PDGFR-_ expression and phosphorylation to shorter survival (*P* _ .028 and *P* _ .030, respectively). This suggests that the biomarker may have prognostic relevance (**Paulsson *et al*., 2011**). A prognostic role for PDGF is also supported by the finding of increased expression of PDGF A- and B-chains in higher-grade versus lower grade gliomas. Aberrations of EGFR have been found in several cancers, especially gliomas. More than 40% of GBMs have shown *EGFR* gene amplification,which has been associated with poor prognosis in some but not all studies.

MGMT

MGMT (O6-methylguanine–DNA methyltransferase) is a DNA repair enzyme that protects cells against damage from ionizing radiation and alkylating agents.When the MGMT promoter is methylated, MGMT is not synthesised and so the cell is unable to repair DNA damage properly. Importantly, a methylated MGMT promoter is present in approximately 40% to 45% of GBMs, and it is unclear whether this alteration is differentially distributed among different types of glioma (**Weller *et al*., 2011; Jha *et al*., 2010**). The standard method for MGMT testing is a methylation specific polymerase chain reaction (MS-PCR), although immunohistochemical methods are also used (**Von Deimling *et al*., 2011**). MGMT promoter hyper methylation has been identified in several studies as a potent predictor of response to the alkylating agent temozolomide in patients with GBM. In a study of GBM in which 573 patients were treated with radiotherapy plus temozolomide or radiotherapy alone, patients in a representative subgroup (N _ 206) with a methylated MGMT promoter who received combination therapy showed longer median overall survival than those without methylation (23.4 months *v* 12.6 months, respectively). The methylation status of the MGMT promoter is currently considered as the strongest predictor of outcome and benefit to temozolomide treatment. According to Gorlia and colleagues, adjuvant and recurrent GBM trials using alkylating agents should stratify patients according to MGMT promoter methylation status, an assertion

that is supported by von Deimling and colleagues (**Von Deimling *et al.*, 2011**). However, they point out that in the absence of alternative treatments in the clinical setting, temozolomide is often used as first-line therapy, even in patients without a methylated MGMT promoter, as these patients have shown benefit from the drug (**Von Deimling *et al.*, 2011**).

IDH1

The mutated form of the metabolic enzyme IDH1 (isocitrate dehydrogenase 1) has recently been implicated as having a role in cancer.44 *IDH1* mutations appear to occur with similar frequency in WHO grade II and III anaplastic gliomas and secondary GBM, but are rare in primary GBM.41 *IDH1* status may also be of use in distinguishing oligodendroglial tumors, in which *IDH1* mutations are common, from other conditions that characteristically do not have mutations in this gene. (**Von Deimling *et al.*, 2011**) In addition to their potential role in diagnosis and classification of gliomas, *IDH1* mutations may be of value as a prognostic marker for GBM. In a randomized phase III trial of 318 patients with anaplastic gliomas, multivariate analyses demonstrated that mutated *IDH1* was prognostic for significant improvements in time to-treatment failure compared with wild-type *IDH1* (hazard ratio, 2.0; 95% confidence interval [CI], 1.2– 3.3; *P* _ .0128). In addition, a study of 301 patients with newly diagnosed GBM showed prolonged progression- free survival (relative risk [RR], 0.42; 95% CI, 0.19–0.91; *P* _ .028) and a trend toward longer overall survival (RR, 0.43; 95% CI, 0.15–1.19; *P* _ .10) in patients with *IDH1* mutations. The precise role by which mutated *IDH1* contributes to tumorogenicity is unclear. One theory is that mutated *IDH1* converts _-ketoglutarate to 2-hydroxyglutarate, which may block a variety of enzymes, thereby contributing to tumour growth (**Von Deimling *et al.*, 2011; Garber *et al.*,2010**).Nonetheless, the discovery of mutated *IDH1* in gliomas raises the possibility of new metabolic targets and prognostic factors relevant to GBM.

***BRAF* Fusions**

BRAF alterations have been frequently found in pilocytic astrocytomas (WHO grade I glioma) (**Von Deimling *et al.*, 2011**). Although its prognostic significance is unknown, *BRAF* may represent a novel therapeutic target for inhibitionof the mitogen-activated protein kinase (MAPK) cascade, which ultimately regulates substrates involvedin cell differentiation, proliferation, and apoptosis (**Von Deimling *et al.*, 2011**). It has been proposed that successful treatment of low-grade gliomas may prevent their progression to GBM.

GATA4 Regulator

The transcription factor GATA4 is a negative regulator of normal astrocyte proliferation and is believed to have tumour suppressive effects. Agnihotri and colleagues demonstrated the following (1) re-expression of GATA4 sensitizes GBM cells to temozolomide treatment, irrespective of MGMT status (2) GATA4 suppresses GBM transformation in vitro and in vivo; and (3) GATA4 is lost in the majority of human GBM specimens (94/163). Although further studies are needed to validate *GATA4* as a tumour-suppressor gene and identify its downstream targets, the finding that re-expression of GATA4 conferred sensitivity of GBM cells to temozolomide. This suggests that it could potentially function as a predictive biomarker.

Treatment options

Glioma Chemotherapy: TMZ and Gliadel

TMZ is an acid-stable orally administered alkylating drug that crosses the BBB (**Zhang *et al.*, 2012**). It has excellent uptake and distribution behaviour, and there is direct evidence of tumour localization. TMZ is a pro-drug, and its aqueous chemistry is typical of imidazotetrazine compounds. It undergoes hydrolytic ring opening at neutral or alkaline pH under purely chemical control, and the first significant intermediate is the open-chain triazene MTIC. Gliadel is a biodegradable polifeprosan 20 wafer impregnated with carmustine, a small lipophilic alkylating and inter strand crosslinking nitrosourea. There are strong parallels between the mechanisms of prodrug activation and action of carmustine and TMZ.Gliadel wafers are implanted in the cranial resection cavity prior to IR treatment. The Gliadel wafers produce high local concentrations of carmustine directly into the tumour bed after surgery when the tumour burden is low. Furthermore, the wafers release carmustine for several weeks. In contrast, systemically administered carmustine persists only for a few hours. Clinical trials demonstrated that Gliadel wafers are safe for both newly diagnosed and recurring GBMs.IR plus Gliadel showed greater overall survival (OS) than IR alone. However, the combination of IR, TMZ and Gliadel did not show any significant increase in survival over IR and TMZ statistically. Therefore IR and TMZ continue to be the standard therapy for GBMs.

DNA Damage Repair

Methyl Guanine Methyl Transferase (MGMT)

The best-documented mechanism of resistance to TMZ is mediated by the DNA repair protein MGMT, which removes methyl groups from *O6*-MeG lesions that arise from TMZ treatment (**Park *et al.*, 2012**). In a new retrospective study, (**Lalezari *et al.*, 2013**) focused on 418 patients with newly diagnosed GBMs, of whom 410 were treated with IR and TMZ. Tumors were analyzed for MGMT protein expression via IHC, promoter methylation by methylation-specific PCR (MSP), and individual CpG sites were analyzed by bisulfite sequencing (BiSEQ). Low MGMT protein expression (<30% positive cells) and high promoter methylation individually correlated with OS and progression-free survival (PFS). MGMT MSP correlated with MGMT IHC, and IHC status stratified outcome in the methylated group. This data was further validated by BiSEQ analysis of 24 CpG sites within the differentially-methylated region 2 (DMR2) of the MGMT promoter. Protein levels inversely correlated with methylation density in the DMR2 and showed that hypermethylation (≥3 CpG sites) was correlated with higher OS and PFS. Combining analyses of protein expression and promoter methylation offers superior prognosis than individual analyses of these factors

and was recommended for testing of newly diagnosed GBMs (**Lalezari *et al*., 2013**).

Autophagy

Autophagy is a self degradative process that is important for balancing the sources of energy at critical times in development and in response to nutrient stress. During this process, a double-membrane cytosolic vesicle, known as the autophagosome, envelopes macromolecules and even whole organelles. Autophagosomes fuse with lysosomes to form autolysosomes, resulting in the degradation of cellular contents. Autophagy occurs in cells at a basal level and is required for homeostasis (as reviewed by (**Maes *et al*., 2013; Kimura *et al*., 2013**). In the context of glioma cells, autophagy acts as a mechanism following chemotherapy treatment for both cell survival (**Firat *et al*., 2012; Knizhnik *et al*., 2013**) and cell death (**Zhuang *et al*., 2012; Wang *et al*., 2013**) the role of autophagy in resistance to therapy is unusually complex because autophagy can enhance cell death or survival, often depending on the cell identity and the details of the treatment. Additional laboratory studies and clinical trials are therefore necessary to determine and predict whether autophagy can be manipulated to enhance cancer therapy.

Development of Novel TMZ-like Drugs

TMZ is a successful drug with oral administration, manageable side effects and enhanced survival for patients with glioblastomas.However, its most toxic product, *O6*-MeG, is readily reversed by MGMT, and methylation of DNA at other sites is reversed by BER. A drug with less readily repaired products would enhance therapy. However, TMZ may reach brain tumors and react with DNA more effectively as well as efficiently than these new compounds. TMZ and related compounds have been studied extensively and this information will facilitate design of TMZ-like drugs with increased anticancer activity and good pharmacokinetics.

Drugs Directed Against Isocitrate Dehydrogenase

Using large-scale sequencing, several novel and exciting glioblastoma-associated mutations were identified. They found that 50%–80% of low-grade gliomas carried mutations of isocitrate dehydrogenase 1 (IDH1) or isocitrate dehydrogenase 2 (IDH2). Later studies showed that 5% of primary glioblastomas and 60-90% of secondary glioblastomas express mutant IDH proteins (**Prensner *et al*., 2011; Zhang *et al*.,2013**). A natural question arises whether IDHs are targets for therapy. Although IDH is expressed universally, the unique IDH mutations could be targeted specifically thereby lowering the levels of 2-HG and hopefully retarding tumour growth. This is particularly appealing for low-grade gliomas for which there are few appealing treatment possibilities. In two recent studies, promising IDH inhibitors were described (**Wang *et al*., 2013; Rohle *et al*., 2013**). Both the IDH1 and IDH2 inhibitors showed marked preferences for the cancer-mutated IDH enzymes. Wang *et al.* inhibited the mutated IDH2 enzyme in leukemia cells, slowing cell proliferation and inducing differentiation. (**Rohle *et al*., 2013**) used the IDH1 inhibitor to slow proliferation of glioblastoma cells, induce demethylation of histones and enhance astroglial differentiation. For example, a mutated IDH inhibitor with low toxicity will favour to wards delaying the progression of low-grade to high-grade neoplasms.

Hypoxia Response Element [HRE]–Controlled Cancer Treatments

A fundamental problem for GBM cancer therapy is the lack of a tumour-selective delivery system. One approach to overcome this, at least in part, is to develop tumour-specific gene expression systems. A HRE is a short segment of nucleotides within a gene's promoter region that is recognized by transcription factors of the HIF family. The HREs from mouse phosphoglycerate kinase and human *VEGF* or erythropoietin genes have been used to control the expression of marker and therapeutic genes in vitro and in vivo. Multimerized HREs coupled to minimal viral promoters have increased the efficiency of these systems. This approach has been used to increase apoptosis, produce an enzyme necessary for prodrug activation, and rescue postischemic neurons (**Shibata *et al*., 2002**). Post and Van Meir106 have developed novel HIF-activated systems for cancer therapy, including a bidirectional hypoxia/HIF-responsive expression vector to target gene expression to hypoxic cells. They showed that these vectors have moderate to high inducibility at 1% O2 but maintain tight regulation under normoxic conditions. With this system, they have produced a conditionally replicative oncolytic virus that can specifically lyse hypoxic tumour cells. There is convincing evidence that Hypoxia Responsive Molecules such as HIF-1a, VEGF, carbonic anhydrase IX, or glucose transporter–1, may play a role in the tumorigenesis and angiogenesis of a number of malignancies, but their role is unclear in the case of malignant gliomas.

Summary and conclusion

Although the prognosis of patients with GBM remains poor, there has been progress recently in developing more effective therapies. Ultimately, truly effective therapies will result from the use of complementary combinations of targeted agents or the combination of targeted agents with other treatment modalities such as RT and chemotherapy. There is increasing interest in the administration of therapeutic agents into the tumour cavity or by convection-enhanced delivery. These include cytotoxic agents, mAbs, immunotoxins, radionuclides and viral vectors. Progress is also being made in the development of more effective gene therapies and immunotherapies. Recently, there have been important technologic advances in surgery and RT, increasing the safety of these therapies. Although both of these therapies have a role in patients with GBM, the infiltrative nature of these tumors makes it unlikely that local treatments will significantly improve prognosis. Only therapies that effectively treat both the infiltrating cells as well as the main tumour mass will prolong survival.

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