Molecular characteristics of malignant gliomas and their future perspectives – a review

Markus Mieskolainen[1]

Malignant glial tumours are the most common primary brain tumours and the most aggressive and difficult to treat. Gliomas present heterogeneity histologically, genetically and in their outcome. Despite decades of intense research in this field, there has been little improvement in mortality rates because of the tumours' inter- and intra-tumoural heterogeneity and predisposition of the cancerous cells to infiltrate normal parenchyma of the brain. Gliomas have been traditionally categorized based on their histopathological features, but it has become obvious that this classification alone has its limitations. Recently, expression studies have indicated that by using molecular signatures, malignant glial tumours can be categorized into subclasses that can more effectively predict patient outcome. The use of molecular markers that carry both prognostic and diagnostic information on tumours with histologically reminiscent appearance adds another level of complexity, reduces inter-observer variability, and allows better characterization of novel tumour variants and entities. In modern neuro-oncology, molecular markers have become essential in tumour evaluation, and molecular marker status of a glioma now guides clinical decision-making. This review will discuss general aspects of malignant gliomas, their grading and staging, genetic information and relevant molecular markers, as well as future perspectives in the treatment and classification of these gliomas.

[1] student, Faculty of Medicine, University of Tartu, Estonia

Supervisors:
Toomas Asser[1, 2],
Tambet Teesalu[3]
[1] Department of Neurology and Neurosurgery, University of Tartu, Estonia,
[2] Neurology Clinic, Tartu University Hospital, Estonia,
[3] Laboratory of Cancer Biology, Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia

Correspondence to:
Markus Mieskolainen
markus.mieskolainen@gmail.com

INTRODUCTION

Malignant gliomas are the most common primary malignant brain tumours. In contrast to primary brain tumours, the term secondary brain tumour refers to a metastatic tumour, i.e. cancer that has spread to the brain from its point of origin. Current treatment strategies for primary malignant brain tumours and in particular malignant gliomas include maximally safe surgical resection of the tumour followed by adjuvant therapies like radiation- and chemotherapy. Because these tumours present a highly malignant character, they are very difficult to eliminate completely, despite the above aggressive therapeutic interventions (1).

Difficulties in treatment of malignant gliomas stem from their diffusely infiltrative growth pattern, that follows no clear boundaries, and their indiscriminate proliferation into surrounding tissue, factors that both notably limit the extent of safe surgical resection and make a complete resection of the tumour very challenging. Additionally, these tumours are often also resistant to adjuvant therapies like chemotherapy. After diagnosis, the overall survival of a patient with malignant glioma can be as low as 12-14 months despite contemporary therapeutic interventions. Currently used treatments are for the most part merely delaying the recurrence and prolonging of patient survival (1, 2).

Traditionally, gliomas have been categorized on their histopathological features (3). World Health Organization (WHO) released an updated and anticipated edition of the Classification of Tumours of the Central Nervous System in 2016. In contrast to the previous 2007 edition, molecular parameters are used for the first time, in addition to histology, to define tumour entities, thus formulating a concept of how diagnoses of tumours of the central nervous system (CNS) should be structured in the molecular era (4).

WHAT ARE MALIGNANT GLIOMAS?

Glioma is a general term used to describe any tumour in the CNS that emerges from glial cells. Glial cells, also referred to as neuroglia or glia, are non-neuronal supportive cells of the nervous system. Glia keep neurons in place and assist their functioning. Glial cells in the CNS include astrocytes, ependymal cells, oligodendrocytes and microglia. Consequently, gliomas are classified according to the respective cell type involved in the tumour, i.e. into oligodendroglioma, astrocytoma, medulloblastoma, ependymoma and mixed glioma (2).

Patients with malignant gliomas are usually highly symptomatic. These symptoms vary by tumour type but also by rate of growth and size and location of tumour effects. Some of the most common symptoms include headaches, seizures, nausea, vomiting, neurocognitive dysfunctions, motor deficits, and vision problems like blurred vision, double vision or loss of peripheral vision (5). Glioma diagnosis can be made by means of magnetic resonance imaging (MRI), computed tomography scan (CT scan), biopsy or angiogram, with contrast enhanced MRI being the most common method used. Because gliomas are characterized by invasiveness, diffused cells invariably exist beyond the imaged tumour. Visualization of all cells in a given tumour by common imaging techniques is therefore nearly impossible (6). Prognosis and treatment modalities depend, for example, on the localization of the tumour, its degree of malignancy, proliferation activity, as well as the genetic profile and patient age (7).

Invasive gliomas have high recurrence rate and low cure rate despite the combining of surgery and adjuvant therapies, currently used for their treatment (1). One of the problems concerning treatment is that chemo- and radiation therapies target tumour cells in their growth phase, but fail to affect quiescent glioma stem cells, i.e. subsets of tumour cells driving tumorigenesis by their potential for self-renewal (2, 8). The blood-brain barrier in the brain is an additional complicating factor to effective therapy because it inhibits adequate therapeutic concentration of most chemotherapeutic agents in tumour mass and peritumoural area, leading to suboptimal therapeutic response (3, 9). Only a few known agents are able to penetrate the blood-brain barrier and affect the brain tissue. These include agents like temozolomide (TMZ) and nitrosurease that are highly lipid soluble and have low molecular weight (9). An additional factor that complicats the use of adjuvant therapies for treatment of malignant gliomas is the intrinsic heterogeneity of the tumour microenvironment and its cell populations. Regarding the overall result, adjuvant treatments have often been merely able to delay tumour recurrence and prolong patient survival (1, 2).

GRADING AND STAGING

A numerical grade of II, III or IV is used to describe the extent of the tumour's morphological features (illustrative pictures 1, 2 and 3), for example, its microvascular proliferation found at pathological evaluation (10, 11). The current histopathological WHO classification also includes grade I tumours, for example, pilocytic astrocytoma, which, due to their potential for malignant transformation are also considered low-grade gliomas. The purpose of the grading is to identify the aggressiveness or the malignancy of the tumour and to reflect its predicted biological behaviour. A higher grade indicates a more malignant tumour and, as a rule, patients have a better prognosis with a lower grade tumour (11). It should be noted that high grade gliomas (grades III, IV) can develop from a low grade tumour that was previously present. Tumour
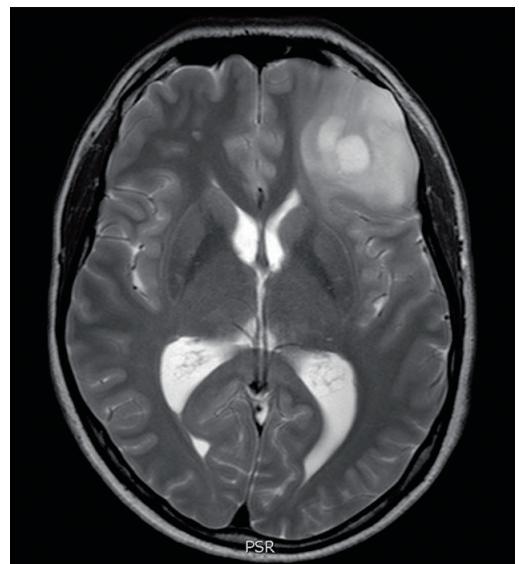
Picture 1. Grade II *oligodendroglioma*, IDH-mutant.
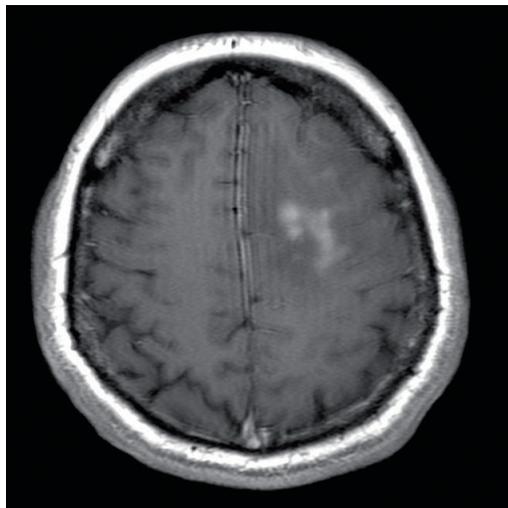
**Picture 2.** Grade III *astrocytoma anaplasticum*.

progression from a lower to a higher grade cannot be predicted, but approximately two-thirds of all low-grade gliomas advance to higher grade tumours. Staging indicates whether the tumour has spread or not. CNS tumour staging is typically performed based on MRI images, CT scans or by an examination of cerebrospinal fluid (12).

Grade IV glioblastomas typically have histo-morphological features that include intra-tumoural necrosis, increased proliferation, i.e. increased mitotic rate, nuclear atypia/pleomorphism, and microvascular proliferation (13).

GENETIC INFORMATION AND MOLECULAR MARKERS OF GLIOMAS

Significant progress has been made in the last decade to illuminate the underlying genetic causes of gliomas (14). The resulting molecular and genetic markers have been shown to be important in supplementing histological analyses for a more precise definition of distinct disease entities. Molecular classifications of gliomas have become seminal also in terms of patient survival and prognosis, since classification of gliomas according to their molecular features is presumed to better reflect their clinical outcome and behaviour (1). Delivery of highest quality treatment requires therefore an understanding of these molecular characteristics of malignant gliomas (2).

Important molecular markers for malignant gliomas include 1p19q co-deletion, O^6-methylguanine- DNA methyltransferase (MGMT) promoter methylation, isocitrate
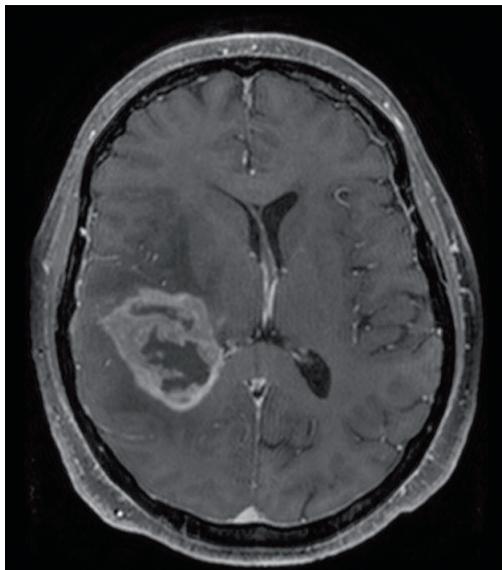
Picture 3. Grade IV *glioblastoma multiforme*, 1p19q co-deleted.

Source of MRI pictures: Tartu University Hospital

dehydrogenase (IDH) encoding gene mutation, Ki-67, and epidermal growth factor receptor variant III (EGFRvIII) amplification (2). The above markers are described in more detail below.

It should be noted that currently no single molecular marker expresses the condition of all gliomas. Instead, a combination of molecular markers is used to evaluate the prognosis and response to treatment in glioma patients. For example, 1p19q deletion often exhibits also IDH mutations. A combination of MGMT promoter methylation, IDH mutation and 1p19q co-deletion is thought to greatly increase the survival rates of patients (2).

1p19q co-deletion

The so called 1p19q co-deletion occurs at the short arm of chromosome 1 (1p) and at the long arm of chromosome 19 (19q). Gliomas that have a prominent oligodendroglial component like grade II oligodendrogliomas,often involve allelic loss in 1p. In mixed gliomas and oligodendroglial tumours, the incidence of allelic loss in 19q is notably high (2).

In patients with 1p19q co-deletion, the rate of response to chemotherapy with temozolomide (TMZ) has been shown to be better compared to the patients without it, and the growth rate of the tumour measured by its diameter was found to be

lower compared with other patients. 1p19q co-deletion may also delay the development of TMZ resistance. Additionally, 1p19q co-deletion serves as an important indicator of chemo-sensitivity and prognosis of low-grade gliomas. For example, among grade II oligodendroglioma patients, 5-year survival rates with 1p19q co-deletion were found to be considerably higher than among patients without the deletion (2).

O⁶-methylguanine-DNA methyltransferase promoter methylation

O^6-methylguanine-DNA methyltransferase (MGMT) is an omnipresent DNA repair enzyme that plays a crucial role in cellular DNA damage resistance. DNA damage is induced by alkylating agents, for example TMZ, that remove the alkyl group from the O^6-atom of guanine. Because MGMT protein acts as methyl acceptor and methyltransferase for inactivation of MGMT during methylation, the process does not require co-factors. It has been proven in clinical trials that methylation of MGMT promoter is a positive prognostic marker that results in the tumours being more sensitive to radiation therapy (15). According to evidence, MGMT methylation is a positive predictive marker for the responsiveness to alkylating agents of newly diagnosed gliomas. In glioma patients with MGMT promoter methylation, TMZ chemotherapeutic sensitivity has been found to be greater than in patients without the methylation, and is consequently, a sign of better prognosis (2).

Isocitrate dehydrogenase encoding gene mutation

Isocitrate dehydrogenase (IDH) is one of the major players in human metabolism and has two variants, IDH1 and IDH2. Isocitrate dehydrogenase encodes IDH that catalyses the oxidative decarboxylation process that yields α-ketoglutarate and carbon dioxide. NADP+ is used as a co-factor for IDH1 and IDH2 for catalysing the reaction. More than 70% of malignant gliomas exhibit IDH mutations with the IDH1 variant being the most frequent mutation (in over 95% of cases). The mutation occurs at R132H, i.e. in the single amino acid residue of IHD1 active site, and it leads to the inability of the enzyme to catalyse the conversion of isocitrate to α-ketoglutarate. Instead,

IDH1 mutant catalyses α-ketoglutarate to 2-hydroxy glutaric acid that is associated with malignant transformations. IDH1 mutation is an early molecular marker in the prognosis and diagnosis of glioma patients because it is the earliest and most frequent genetic change in a glioma (2).

Ki-67

Ki-67 is a cell proliferation nuclear antigen and a marker of cell division. Malignancy of tumour cells and their proliferation may be reflected objectively by Ki-67 levels (16). Ki-67 is also an important marker for differentiation of malignant and benign tumours. This is so because the overexpression of Ki-67 results in increased invasiveness and proliferation, which in turn leads to a higher grade of malignancy, recurrence of the tumour and dismal prognosis for glioma patients (17, 18).

Epidermal growth factor receptor variant III amplification

Epidermal growth factor belongs to the receptor tyrosine kinase (RTK) family, a membrane receptor superfamily that exhibits protein tyrosine kinase activity. A particular mutation of epidermal growth factor receptor (EGFR), named EGFR variant III (EGFRvIII), is often found in conjunction with glioblastomas. In 25–30% of patients with malignant gliomas, active mutant EGFRvIII is known to be present with EGFR overexpression or amplification (19, 20). By activating other RTKs, EGFRvIII plays a role in tumorigenesis (2). Greenall *et al* (21) have shown that in U87MG type glioma cells, EGFRvIII activity is directly proportional to MET transactivation. In a mouse model, targeting of both transactivated RTKs and EGFRvIII simultaneously led to notably better survival of the test subject. This indicates that by blocking both EGFRvIII and transactivated RTK may be an effective treatment option for EGFRvIII–positive glioma conditions (2).

FUTURE PERSPECTIVES

Understanding of the genetic basis of tumorigenesis has increased markedly thanks to studies conducted over the past two decades. This has challenged traditional tumour classifications that have been devised on the basis of histogenetic descriptions of microscopic similarities and that have relied on ultrastructural characteriza-

tions, immunohistochemical expressions of lineage-associated proteins, and descriptions of hematoxylin & eosin –stained sections of light-miscroscopic analyses. In the WHO Classification of Tumours of the Central Nervous System 2007, the use of genetic alterations already provided predictive or prognostic data within the diagnostic categories established on a histological basis. In 2016, WHO released an updated edition of the Classification of Tumours of the Central Nervous System that finally used molecular parameters for the first time in addition to histology in defining tumour entities, thus formulating a concept specifying how CNS tumour diagnoses should be structured in the future (4).

Use of integrated geno- and phenotypic parameters for the classification of CNS tumours has the potential for providing a new level of objectivity, compared to the diagnostic processes used in the past. This new level of objectivity stems from more narrowly defined and biologically homogenous diagnostic entities, and will hopefully lead to improved patient management, greater accuracy in diagnostics, and more accurate determination of treatment response and prognosis. It is noteworthy that the diagnostic use of both molecular genetic and histological features can potentially lead to contradictory results in which case (according to the new WHO classification) the genotype surpasses the histological phenotype. Consequently, it can be asked whether in the future, classification could proceed without the use of histology, i.e. based solely on the genotype. At this point an exclusively genotypic classification is not possible because it is still required to make a diagnosis of diffuse gliomas to understand the clinical and nosological meaning of certain genetic changes. Also, WHO grades are still determined on the basis of histological criteria. Additionally, individual tumours exist that do not fit into a more narrowly defined geno- and phenotype criteria, which emphasizes the need to retain the phenotype as a basis for classification (4).

The new WHO framework is a "layered diagnosis", which combines molecular pathology with histology:
- Layer 1. Integrated diagnosis
- Layer 2. Histological diagnosis
- Layer 3. WHO grade
- Layer 4. Molecular information

Layer 1 represents the final "integrated diagnosis", but information from all lower layers must first be gained. As mentioned above, as a general rule, molecular information surpasses histology. The principle of NOS i.e. "not otherwise specified" was also presented in the WHO 2016 classification, meant to be used for example in situations where the sample does not allow testing (22, 23).

Increasing the understanding of malignant glioma biology and behaviour to the level where targeted curative therapy could be developed is crucial in the future. A direct fallout is that genomic technology needs to become more available in clinics in order administer more personalized precision therapies like tailored treatments based on molecular information on various gliomas, tissue sequencing, and analysis of data for individual therapeutic recommendations. Undoubtedly, this requires a multidisciplinary approach in tackling the complexity presented by malignant tumours. Nevertheless, a significant amount of information already exists on, for example, gliomagenesis –related genetic pathways and biological and clinical behaviour of malignant tumours. Integrating this type of multidisciplinary information is essential in gaining a comprehensive view on the genetic and cellular mechanisms underlying tumour biology, and in developing and providing effective targeted therapies (24, 25).

The ability to routinely perform molecular characterizations of gliomas, advances in imaging and advanced targeting therapies will improve the management of gliomas in the future. A major issue in the successful treatment of gliomas lies in gaining a broader understanding of the molecularly defined subsets that could differentiate various gliomas into different disease entities. This could have the potential to develop more effective targeted therapies addressing driver mutations. Advances in mechanisms of delivery of drugs to tumours will also improve patient outcomes (24).

SUMMARY

Today when precision medicine is becoming a norm in treatment of gliomas, a specific diagnosis that integrates histological information, tumour grade and molecular data is required. Owing to advances in molecular biotechnologies in recent years, molecular

characterization of gliomas has improved significantly, leading to attempts at personalized medicine and molecularly targeted therapies. These are thought to be the future breakthroughs also in glioma therapy. Molecular classification of gliomas will likely be performed routinely in the future to guide different therapeutic options and to develop novel drugs that will likely also improve therapeutic outcomes. All things considered, immense hope exists that the latest WHO Classification of Tumours of the Central Nervous System 2016 will facilitate the epidemiological, experimental and clinical studies leading to improvements in the lives of brain tumour patients.

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