Świątek et al.
Medical Journal of Cell Biology 2024
DOI: 10.2478/acb-2024-0001
Received: 15.02.2024
Accepted: 10.04.2024

Glioblastoma: a molecular insight into current discoveries and treatment directions

Wiktor Świątek[1], Olgierd Kłodziński[1], Michał Ciesielski[1], Zuzanna Adamkiewicz[1], Marcin Podolak[1], Paul Edward Mozdziak[2,3], Wiesława Kranc[1]

Abstract

Glioblastoma is a highly aggressive and complex pathology that has garnered significant interest among researchers and clinicians due to its high mortality rates. This research article provides a systematic analysis of key aspects related to glioblastoma, offering comprehensive insights into its underlying complexities. The text explores the epidemiological patterns, etiological factors, and genetic and molecular foundations underlying the development of GBM. It also examines the interplay between the immune system and the tumor, identifying specific immune markers with potential diagnostic value. The article describes the complex processes involved in tumor growth, including its interaction with surrounding tissues, the development of the tumor microenvironment, and the role of stem cells. It also provides an analysis of current treatment options and the challenges they face, particularly in relation to tumor resistance. The article concludes with a thorough examination of the changing landscape of diagnostic and therapeutic approaches. It highlights notable recent research findings and provides insight into potential advancements that could shape the future of medical interventions for glioblastoma.

Running title: Glioblastoma: current discoveries and treatments

Keywords: glioblastoma, GBM, IDH, EGFR, GSC, TME

[1]Department of Anatomy, Poznan University of Medical Sciences, Poznan, Poland
[2]Graduate Physiology Program, North Carolina State University, Raleigh, USA
[3]Prestage Department of Poultry Sciences, North Carolina State University, Raleigh, North Carolina, USA
***Correspondence:** wkranc@ump.edu.pl
Full list of author information is available at the end of article

sciendo

Introduction

Glioblastoma (GBM) is the most common glioma, constituting 14.2% of all intracranial neoplasms and 50.9% of malignant brain tumors. While it can occur in patients of any age, it predominantly affects older adults, with a peak incidence observed in individuals aged 56-72 years. In the pediatric population, glioblastoma contributes to 2.6% of all central nervous system (CNS) tumors. The male-to-female (M:F) ratio for glioblastoma is reported as 1.4:1 in the United States [1]. Annual age-adjusted incidence rates for glioblastoma have increased in recent years to 3-6 cases per 100 0000 people, as documented in reports from the USA, Canada, England, and Australia [1–4]. The observed rise cannot be solely attributed to advancements in diagnostic techniques and lifestyle modifications; environmental factors might be potential contributors [3,5–7]. Glioblastoma is less common in Asian and African countries, phenomenon potentially linked to differences in age distribution and, in part, to underascertainment [8,9].

The reason behind the majority of glioblastomas remains unknown [10]. A small fraction of these tumors occurs in multiple family members or is inherited within genetic tumor syndromes, such as Lynch syndrome, constitutional mismatch repair deficiency syndrome, Li-Fraumeni syndrome, and neurofibromatosis type 1 [11]. Through genome-wide association studies, genomic variants in *TERT*, *EGFR*, *CCDC26*, *CDKN2B*, *PHLDB1*, *TP53*, and *RTEL1* have been linked to an elevated glioma risk [11–13]. Additionally, certain single nucleotide polymorphisms have been identified, specifically associated with an increased susceptibility to gliomas, distinct from those in patients with other types of brain tumors [10,14,15]. The incidence of glioblastoma appears to be on the rise, hinting at potential roles of environmental factors in its development [16]. The sole validated risk factor identified is ionizing radiation to the head and neck [10,17]. Notably, individuals treated for acute lymphoblastic leukemia (ALL) face a heightened risk of developing glioblastoma [18–20], as well as survivors of atomic bomb irradiation. Conversely, there is no elevated risk associated with diagnostic irradiation [21]. Furthermore, individuals with a history of allergies or atopic diseases appear to have a decreased risk of GBM [11].

Experiments using mouse models suggest that various primary cell types within the CNS can undergo transformation into malignant cells, exhibiting characteristics reminiscent of glioblastoma. These cell types encompass oligodendrocyte precursor cells [22], neural precursor cells [23], astrocytes [23], and neurons [24]. The likelihood of transformation appears to decrease with lineage restriction [25]. Deep genetic sequencing studies on human glioblastomas propose that a neural precursor in the subventricular zone is a probable origin for these cells [26]. This proposition finds support in the concurrent anatomical location of neural precursor cells in the subventricular zone and the identification of stem cell-like cells directly within glioblastomas [27–29]. Nevertheless, the question of whether stem cell-like cells in glioblastoma result from the transformation of a neural precursor in the CNS or arise through dedifferentiation of a lineage-restricted cell type remains unsettled. Genetic events steering the development of glioblastoma may influence cellular lineages, and the potential for cellular plasticity poses a challenge in identifying the definitive cells of origin [30].

Genomic and molecular findings

Cytogenetics and chromosome alterations

Trisomy 7 and monosomy 10 stand out as the most prevalent numerical chromosome alterations observed in glioblastoma, frequently co-occurring [31–35]. Less commonly, alterations are confined to gains in 7q and/or losses in 10q. A predominant gene amplification involves the *EGFR* locus at 7p11.2 [31]. Additional numerical chromosome alterations frequently observed in IDH-wildtype glioblastomas include losses on 9p, encompassing the homozygous deletion of the *CDKN2A* and/or *CDKN2B* locus at 9p21, as well as alterations in 13q, 22q, and the sex chromosomes [36,37]. Gains of chromosomes 19 and 20 are also noted. Gene amplifications often present cytogenetically as extrachromosomal double-minute chromosomes and can be identified in up to 50% of glioblastoma karyotypes [38]. Gene fusions in IDH-wildtype glioblastoma are mostly confined to members of the RTK family and recurrently involve *EGFR* (6-13%); *FGFR3* (-3%); *MET* (1-4%); or *NTRK1*, *NTRK2*, or *NTRK3* (1-2%) [39–42].

EGFR

The receptor tyrosine kinase (RTK) EGFR frequently undergoes alterations in IDH-wildtype glioblastoma. Approximately 60% of tumors exhibit indications of *EGFR* amplification, mutation, rearrangement, or altered splicing [37]. The most prevalent among these modifications is *EGFR* amplification [43], observed in about 40% of all IDH wildtype glioblastomas [31,44] and up to 60% of glioblastomas in the DNA methylation group RTK2. However, this alteration is found in only about 25% of RTK1 and mesenchymal glioblastomas [31,45]. In most cases, *EGFR* amplifications are linked with a secondary EGFR alteration, such as mutations in the extracellular domain or in-frame intragenic deletions that encode either *EGFRvIII* or other alternative transcripts [46–48].

PI3K-AKT-mTOR

The regulation of cell growth relies significantly on the PI3K pathway [49]. Signaling within this pathway is initiated by RTKs and/or RAS and is co-

unteracted by PTEN. In IDH-wildtype glioblastoma, alterations in RTK genes, PI3K pathway genes, and *PTEN* are identified in approximately 90% of cases [37,50]. Amplifications, truncations, and fusions of RTK genes are notably prevalent, with *EGFR* (approximately 60%), *PDGFRA* (10-15%), *MET* (2-5%), or *FGFR3* (around 3%) frequently involved [51,52]. NF1 mutations, which activate the PI3K pathway by reducing RAS inhibition, are present in about 10% of cases [53]. PTEN, the central inhibitor of the pathway, experiences mutation/deletion in about 40% of glioblastomas, while mutations in *PIK3CA*, *PIK3R1*, or other PI3K pathway genes are observed in 25-30% of cases [54,55]. Alterations in RTKs may co-occur with changes in PI3K or *PTEN* in approximately 40% of cases, whereas mutations in PI3K and mutations/deletions in *PTEN* appear to be mutually exclusive [56,57].

p14ARF-MDM2-MDM4-p53

The p53 pathway plays a central role in inducing DNA repair, cell cycle arrest, and apoptosis [58]. Genetic dysregulation of this pathway is prevalent, occurring in nearly 90% of glioblastomas [37]. Both MDM2 and MDM4 act as inhibitors of p53, and their activation through amplification is observed in about 15% of glioblastomas [59]. MDM2 is itself counteracted by p14ARF, an alternate reading frame protein encoded by the CDKN2A locus. This locus undergoes deletion in approximately 60% of glioblastomas, leading to the inactivation of the p53 pathway and activation of the RB1 cell-cycle pathway [50]. TP53 undergoes mutation or deletion in 20-25% of IDH-wildtype glioblastomas [60]. Genetic alterations affecting CDKN2A (p14ARF), MDM2 or MDM4, and TP53 are largely exclusive of one another [55].

CDK4/6-CDKN2A/B-RB1

CDK4 and CDK6 play a role in catalyzing the phosphorylation of RB1, leading to the release of E2F transcription factors [61]. These factors, in turn, trigger the expression of genes involved in the transition from G1 to S phase of the cell cycle. p16, encoded by *CDKN2A*, acts as an inhibitor of CDK4 [62]. Genetic alterations in the CDK4/6-RB1 cell cycle pathway are evident in approximately 80% of glioblastomas [37,50]. The most common alterations include *CDKN2A* deletions, often involving nearby CDKN2B (around 60%), amplifications of *CDK4* or *CDK6* (about 15%), and mutations/deletions of *RB1* (approximately 8%) [63,64]. *CDKN2A* deletion and *RB1* alterations are mutually exclusive [65], while *CDKN2A* deletion and *CDK4* amplification may occasionally coexist [63]. In rare instances, the cell cycle pathway may be alternatively activated by the deletion of *CDKN2C* or amplifications of *CDK6* or *CCND2* [37,50].

Epigenetics, chromatin and promoter methylation

A crucial role of chromatin regulation is to uphold inactive regions of the genome within repressive structures. Canonical repressive states include heterochromatin distinguished by H3 p.K28me3 (K27me3), a mark deposited by PRC2 and its catalytic subunit, EZH2. In IDH-wildtype glioblastoma and various other cancer types, EZH2 is overexpressed, likely contributing to the silencing of key tumor suppressor genes [66]. Interestingly, loss-of-function mutations of *EZH2* in IDH-wildtype glioblastoma are rare (<1%), but inhibition of EZH2 enzymatic activity can occur through mutation of H3 genes in diffuse midline gliomas, leading to p.K28M (K27M)-mutant protein [67,68]. Mutations in *ATRX*, responsible for depositing H3.3 in pericentromeric and subtelomeric regions as a chromatin remodeler, were observed in 13 (4.5%) of 291 IDH-wildtype glioblastomas [37]. Within chromatin, actively transcribed gene bodies bear the mark H3 p.K37me3 (K36me3), deposited by the methyltransferase SETD2. *SETD2* mutations occur in about 2% of IDH-wildtype glioblastomas, with a higher incidence in pediatric and IDH-mutant gliomas [69]. Epigenetic gene silencing via DNA methylation is another common mechanism for gene inactivation [70–72]. The *MGMT* gene, encoding a DNA repair protein [73], is transcriptionally silenced by promoter methylation in approximately 40-50% of IDH-wildtype glioblastomas [37,74–76]. MGMT specifically removes promutagenic alkyl groups from the 06 position of guanine in DNA, diminishing the treatment effects of alkylating agents [77,78]. *MGMT* promoter methylation is predictive of the benefit from therapy with alkylating agents like temozolomide, present in 40-50% of glioblastomas [37,74–76]. A higher frequency (>75%) of *MGMT* promoter methylation is associated with tumors exhibiting the glioma CpG island methylator phenotype (G-CIMP), characteristic of IDH-mutant gliomas [79,80].

Molecular diagnostics

IDH-wildtype glioblastomas are characterized by the absence of mutations in *IDH1* codon 132 and *IDH2* codon 172, as well as the lack of H3 p.K28 (K27) or H3 p.G35 (G34) mutations. In the absence of immunoreactivity for IDH1 p.R132H, a diagnosis of IDH-wildtype glioblastoma is sufficient, especially in patients aged > 55 years with a histologically classic glioblastoma not located in midline structures and no history of a pre-existing lower-grade glioma [81]. This practical diagnostic approach is feasible because the likelihood of a non-canonical IDH mutation is < 1% in glioblastomas from patients aged > 55 years [82]. Frequent and diagnostically significant molecular alterations in IDH-wildtype glioblastomas include *TERT* promoter mutations,

EGFR gene amplification, and a +7/-10 genotype [31]. Although the *BRAF* p.V600E mutation is infrequent in IDH-wildtype glioblastoma [83], it can be detected in as many as 50% of glioblastomas with epithelioid histology [84].

The role of the immune system in glioblastoma

Molecular markers

So far, many molecular and non-molecular features of glioblastoma related to the immune system have been characterized, and these features have the potential to be utilized in clinical practice (**Fig. 1**) [85]. The most numerous group among the discussed properties are immunologic markers that allow predicting the progression or remission of the tumor. A potential marker indicating an unfavorable prognosis is the high expression of the MARCO receptor, which has been previously identified as a prognostic factor for melanoma and non-small cell lung cancer. The discussed receptor is present on tumor-associated macrophages (TAMs) and is mainly associated with the mesenchymal subtype of glioblastoma [85]. Among other potential molecular prognostic markers, PD-1 protein (programmed cell death receptor) stands out, particularly associated with lymphocytes and involved in suppressing the immune response. A correlation has been observed between increased expression of the PD-1 coding gene and lower OS among glioblastoma patients [86]. Additionally, the detection and assessment of the quantity of T lymphocytes with phosphorylated PD-1 receptors may indicate the progression of immunosuppression [87]. Lower OS have also been observed in patients with increased expression of the CHI3L2 gene. Its product is mainly observed in astrocytes and CD4+ T cells and is associated with tumor metabolic pathways [88]. Higher expression of the EFEMP2 gene, encoding an endothelial growth factor, has been noted in GBM and the mesenchymal subtype and its association with TAMs has been demonstrated. This suggests that the level of EFEMP2 gene expression may serve as a marker of tumor malignancy and a predictive factor for its progression and patient prognosis [89]. A similar role in OS assessment may be played by the PDIA5 gene, as its abnormal expression has been correlated with poor prognoses [90]. Elevated levels of interleukin 10 (IL-10) and CD39 enzyme, molecules that inhibit the immune response through their anti-inflammatory properties, have been observed in patients with a more severe course and worse prognosis in GBM [91]. This suggests their role as markers for evaluating disease progression. The protein CCL22, a chemokine produced by antigen-presenting cells and macrophages, exhibits an inverse relationship with GBM risk and OS with low serum concentrations of CCL22 negatively predicting outcomes. Furthermore, its serum level is correlated with CD4+ cell count, suggesting its potential use

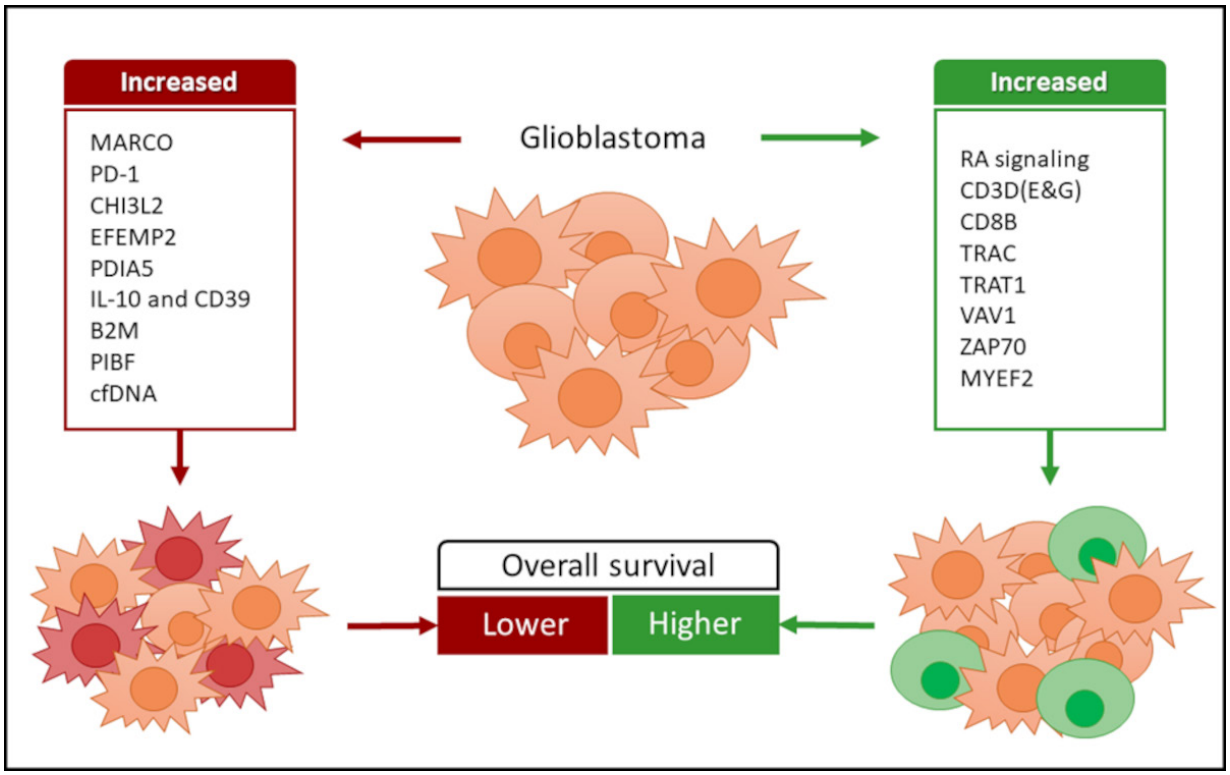


FIGURE 1 Increase in certain genes expression related to specific immune system response can lead to different outcomes in GBM progression and patient prognosis

as a marker indicating immunosuppression in GBM [92]. Another interesting discovery is the observation of higher levels of β2-microglobulin (B2M), a subunit of MHC class I protein, expressed on glioma cells. Increased levels of this protein has been associated with the induction of TGFβ (transforming growth factor β) secretion, an increased number of TAMs and B2M may also contribute to the growth of the tumor itself [93]. The PIBF gene undergoes expression to an immunomodulating protein involved in immunosuppression, and its increased levels have been found in rapidly progressing cases of GBM and several other cancers [94].

Cell count markers

Indicators of an unfavorable prognosis in GBM may also include features associated with the immune system cells count, such as an increased proportion of TAMs [95]. The presence of senescent CD4+CD28-CD57+ T cells has also been linked to lower overall survival (OS) [96]. Neutrophils, playing a significant role in the glioma microenvironment, may also suggest the prognosis of disease progression. An elevated ratio of neutrophils to leukocytes and platelets to leukocytes in the blood of patients has been associated with worse prognoses. Moreover, the level of cfDNA, short fragments of extracellular DNA released by neutrophils, may serve as a marker for the survival of GBM patients [97].

Indicators of improvement

On the other hand, several potential markers indicating an improvement in GBM patients performance have been described. A link between reduced retinoic acid signaling and increased expression of genes related to the immune system, including CD3D, CD3E, CD3G, CD8B, TRAC, TRAT1, VAV1, and ZAP70, whose proteins are produced in T cells, leads to higher GBM survival [98]. Increased MYEF2 gene expression, encoding an CNS RNA-binding protein, correlated with a lower proportion of exhausted CD8+ T cells, may also indicate better patient outcomes. It has also been shown that decreased expression of this gene is associated with the migration and proliferation of cancer cells observed in patients with poorer prognoses [99].

Diagnostic possibilities

Currently, in clinical practice, there is no significant diagnostic biomarker for GBM. However, there are studies indicating the potential use of GBM-associated T lymphocytes (GBMAT) and T lymphocytes associated with GBM stem cells (GSCAT). Their high specificity and sensitivity in diagnosing GBM have been demonstrated, with both parameters reaching nearly 100% for both cell types[100] Moreover, increase in the expression of factors related to the regulation of the immune response, such as IDO, PD-L1, CD11c, Tregs and a decrease in the number of CD8+ T cells progressing with aging, with a peak at age 60-69– group with the highest GBM incidence [101]. This observed relationship may suggest a new direction in GBM diagnostics, but more importantly, it highlights the significant role of the immune system in counteracting the development of GBM. It may indicate its crucial role in combating this tumor, with any immunosuppressive factors potentially worsening prognosis.

Immunosuppression in GBM

The mentioned immunosuppression in the course of GBM is induced by numerous mechanisms, among which are those also occurring in a healthy organism. In GBM, these mechanisms are exploited to create an immunosuppressive tumor microenvironment, including the blood-brain barrier, immunomodulatory cytokines (TGFβ, IL-10, VEGF, and others), and immune system cells [102,103]. Issues related to immune cells and cytokines in this context are closely interconnected, as illustrated by the stimulation of type II NK cells by GBM. As a consequence, these cells release IL-13 and TGFβ, contributing to anti-tumor response inhibition [102]. GBM cells utilize various mechanisms to establish immunosuppression, such as tumor microenvironment hypoxia, induction of T and NK cell dysfunction, stimulation of Tregs, TAMs, and MDSCs (myeloid-derived suppressor cells), as well as increased expression of surface factors PD-L1 and Fas-L [96] [104]. The protein CD200 is also associated with immunosuppression in GBM, as its elevated levels coincide with MDSC expansion. Additionally, inhibiting the activity of this molecule has been shown to limit MDSC development [105] It is noteworthy that chemotherapy and radiotherapy contribute to the genesis of immunosuppression in GBM patients. Both forms of treatment can result in lymphopenia, which evidently weakens the immune system [106] [107]. The negative impact of GBM on the immune system poses a challenge in the context of dendritic cell-based vaccine therapy, and issues have been described with the application of such vaccines in the immunosuppressive tumor microenvironment [108] Treatment methods based on utilizing T lymphocytes have also encountered resistance within the tumor's internal habitat [109]

Aggressive nature of GBM

GBM is characterized by progressive growth, diffuse invasiveness, and frequent resistance to chemotherapy [110]. These properties epitomize the aggressive behavior of GBM within both its proximate and distal environments, directly stemming from the manipulation of the tumor microenvironment (TME) (**Fig. 2**) [111,112]. TME describes active tumor milieu and in GBM it comprises glioma-associated microglia/macrophages, monocytes, dendritic cells, tumor-associated neutrophils, myeloid-derived sup-

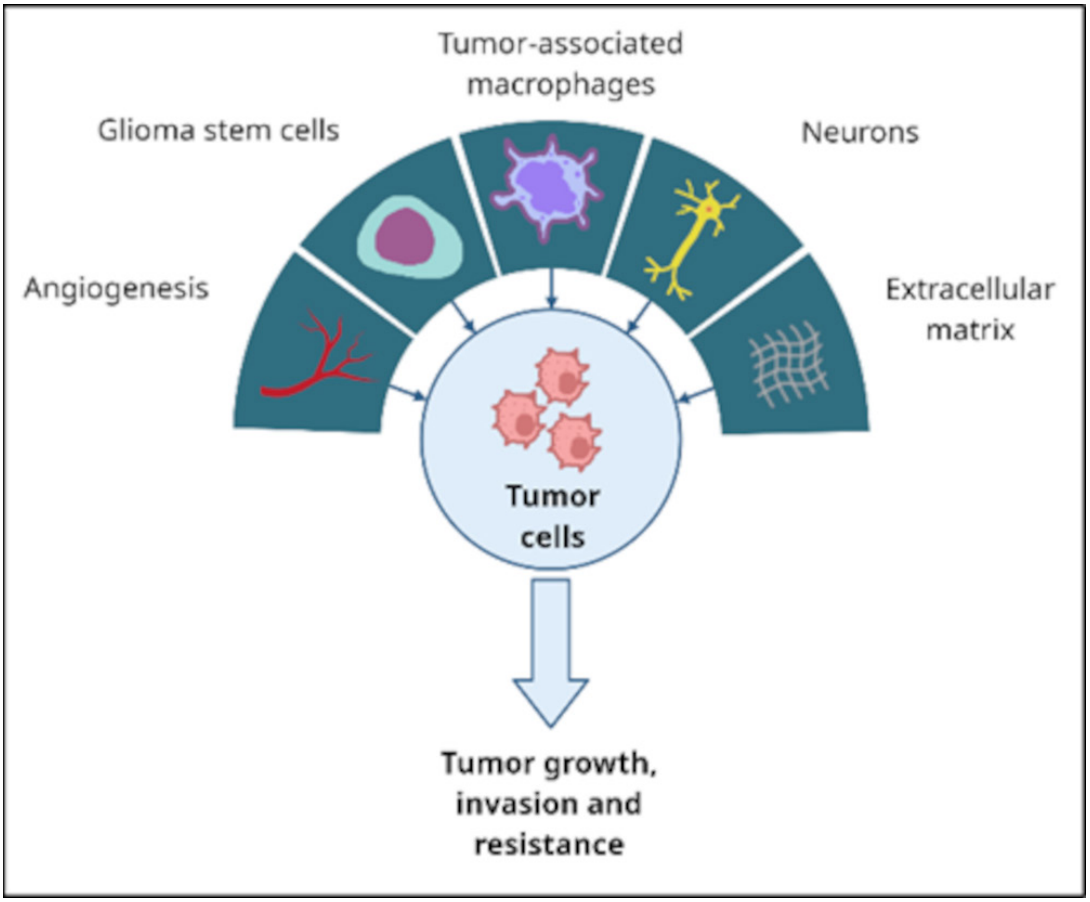


FIGURE 2 Overview of the interplay between glioblastoma and the tumor microenvironment

pressor cells, and normal and reactive astrocytes and vascular pericytes [113–119] . This dynamic interplay within the TME fuels tumor development by releasing a diverse array of cytokines, chemokines, growth factors, and other critical molecules [120,121].

Angiogenesis

Vascular proliferation is a pathological hallmark of gliomas and a sine qua non for tumor growth and progression [122]. The tumor possesses a highly abnormal, dysfunctional vasculature system that differs from normal brain vessels in morphology, functionality, and molecular characteristics [123,124]. During tumor growth new blood vessels are formed through angiogenesis, which involves proliferation, migration, and differentiation of vascular endothelial cells (ECs) [125]. Hypoxia, being the fundamental stimulus responsible for angiogenesis [126,127], is a result of tumor cells hyperproliferation; causing formation of avascular zones [128], leading to limited oxygen diffusion [129]. Hypoxia promotes invasion through the activation of hypoxia inducible factor 1 (HIF-1) and other hypoxia-inducible transcription factors [130,131], and glioblastoma stem cells (GSCs) respond to hypoxia by increasing HIF-1-dependent expression of alarmin receptors [132] and additional factors associated with cell motility [133,134]. Recent insights reveal a new role of HIF-1 in the circadian regulation of angiogenesis by CLOCK, which directs the expression of olfactomedin like 3 (OLFML3), leading to HIF1-mediated transcriptional upregulation of periostin. Subsequently, the secreted periostin acts as a key player in tumor angiogenesis, activating the TANK-binding kinase 1 signaling pathway in ECs [135]. Importantly, a plethora of other molecules and signaling pathways exist that both promote and inhibit angiogenesis, exerting significant influence on the progression of GBM. Non-coding RNAs (ncRNAs) have been discovered as novel factors with a wide ability to modulate the expression of many genes involved in angiogenesis [136]. One intriguing way that ncRNAs can affect the angiogenesis process is by being secreted inside exosomes from GBM cells, and then taken up by neighboring ECs where they can affect gene expression of pro-angiogenic factors [137,138]. Jiang et al. identified that circKIF18A, circular RNA found in exosomes produced by TAMs, participates in GBM angiogenesis via targeting FOXC2 [139]. Exosomal transport can be further facilitated by integrin α3β1-promoted calcium influx during nascent lumen formation [140].

Glioma stem cells

Glioma stem cells (GSCs) represent a mainstay in the pathogenesis of treatment resistance and recurrences [141]. Interactions with surrounding normal cells, such as endothelial cells or with tumor-activated macrophages (TAMs), are instrumental to promote and maintain a stemness phenotype [142]. The closest and most important interactions occur between the endothelium and GSCs via various molecular messengers and pathways, such as nitric oxide, cyclic guanosine monophosphate, and, most importantly, Notch signaling [143–146]. While ligands such as Delta-like ligand 4 (DLL4) and Jagged1 (JAG1) for the Notch pathway are expressed on the endothelium, the expression of Notch-1 and Notch-2 is observed on GSCs. Activation of the Notch pathway by these ligands induces the activation of target genes Hes1 and Hey1, representing one of the mechanisms promoting GSCs [147]. Consequently, GSCs contribute to endothelial proliferation by producing vascular endothelial growth factor (VEGF), facilitating angiogenesis, mediating the homing of bone marrow-derived endothelial precursor cells, and inducing the transdifferentiation of GSCs into pericytes [141,147]. Similarly, GSCs contribute to immune cell infiltration and the formation of a protumorigenic inflammatory environment through the secretion of cytokines and growth factors, such as inhibitory cytokine-1 (MIC-1), transforming growth factor (TGF-β), soluble colony-stimulating factor (sCSF) [148]. In this context, GSCs induce TAMs to adopt an immunosuppressive phenotype, whereas TAMs secrete factors contributing to GSC maintenance [149]. Therefore, the TME appears to be a key regulator of stemness, whereas GSCs affect the microenvironment. This complex dynamic is instrumental for tumor aggressiveness and, thus, its targeting could improve therapeutic strategies [150].

Tumor-associated macrophages

GBM cells recruit immune cells and change their phenotype to support tumor growth [151]. Among the most prevalent non-neoplastic cells within the TME are TAMs, which are part of the innate immune system [152]. TAMs can account for up to 40% of the tumor mass and include both brain-resident microglia and bone marrow-derived myeloid cells [153–155]. TAMs are highly plastic cells that are attracted to and reprogrammed by glioma cells via the secretion of chemokines and other soluble factors to promote invasion, angiogenesis, and immunosuppression [156]. Recent breakthroughs have unveiled previously unknown mechanisms through which TAMs induce immunosuppression. Notably, bone marrow-derived macrophages engage in the phagocytosis of GBM cells, giving rise to double-positive cells that express both macrophage and tumor signatures. These cells subsequently transform into M2-like macrophages, driving an immunosuppressive milieu within the TME [157]. Pan et al. showed that this transformation can also be achieved through stabilization of insulin-like growth factor 2 mRNA binding protein 3 by circNEIL3, novel circular RNA, which is overexpressed in glioma cells and transmitted to TAMs through exosomes [158]. Furthermore, the TME state is non-indifferent to exosomal transportation. A comparative analysis between normoxic glioma-derived exosomes (N-GDEs) and hypoxic glioma-derived exosomes (H-GDEs) revealed that H-GDEs markedly enhanced autophagy and M2-like macrophage polarization when compared to N-GDEs [159]. Another mechanism by which TAMs mediate immunosuppression is enhancement of ferroptosis, a type of programmed cell death dependent on iron, which induces activation and infiltration of immune cells but attenuates antitumor cytotoxic killing [160].

Neuronal signaling and hyperactivity

Recent research on glioblastoma multiforme (GBM) underscores a bidirectional signaling mechanism between tumors and neurons, creating a self-perpetuating cycle of tumor proliferation, synaptic integration, and brain hyperactivity [161–166]. This intricate communication is mediated through synapses, neurotransmitters, ion channels, gap junctions, tumor microtubes (TMs), and neuronal molecules [167], with glutamate, an excitatory neurotransmitter, playing a crucial role [168–170]. Neurons form synapses with glioma cells, promoting their growth by activating α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors on the glioma cells [166,171]. This novel synaptic input induces coordinated calcium transients that propagate through glioma cells interconnected by TMs [172], leading to a calcium-dependent increase in glioma invasiveness and proliferation. Venkataramani et al. revealed that even glioma cell subpopulations lacking TM connections with other tumor cells can drive extensive brain colonization [173]. This recent discovery necessitated a shift from focusing solely on localized interactions, as it was demonstrated that callosal projection neurons in the hemisphere contralateral to the primary GBM significantly contribute to tumor progression and extensive infiltration via the axon guidance gene SEMA4F. SEMA4F is believed to facilitate activity-dependent cell migration and enable bidirectional communication with neurons by remodeling synapses adjacent to the tumor, thus contributing to increased brain network hyperactivity [174]. During gliomagenesis, this hyperactivity is driven by secreted glypican family members, which are selectively expressed in tumors with specific PIK3CA variants [161].

Extracellular matrix proteolysis and tumor invasion

The unique composition of the GBM extracellular matrix (ECM) contributes to angiogenesis, invasion,

and therapeutic resistance of GBM [175]. Essentially all the common components of ECM including proteoglycans and proteins (laminins, fibronectin, or tenascins) influence the behavior of glioma cells [176]. Glioma cells exhibit high expression of various ECM constituents, such as hyaluronic acid, brevican, tenascin C, fibronectin, and thrombospondin [177–181]. Notably, among these, Tenascin C promotes malignant properties, such as neovascularization, proliferation, invasiveness, and immunomodulation [182]. Additionally, glioma cells upregulate specific integrins and other receptors (CD44, BEHAB/brevican, and N-CAM) [178,183,184] that interact with ECM components, fostering adhesion and migration of glioma cells [185]. The second key interaction with the ECM is proteolysis. An increased capacity for ECM degradation via protease secretion correlates well with increased invasion [186]. GBM cells interact within the TME via the expression and activity of a variety of proteolytic enzymes, such as metzincins peptidases (MMPs, ADAMs, ADAMTS), KLKs and cathepsins [187]. MMPs degrade various ECM proteins, including fibronectin, laminins and collagen, promoting cell migration and releasing activated proteins through cleavage [188]. Contrary to the initial expectation that proteases, whether derived from cancer or non-cancerous cells, primarily contribute to ECM degradation in the TME [189–191], recent insights highlight a broader role, as these proteases modulate other pericellular events, in particular the activity and bioavailability of growth factors, cytokines, cell surface receptors, and adhesion molecules, as well as intracellular signaling pathways [189,192,193]. Membrane-type MMPs are a subgroup of metalloproteinases that are membrane-associated and have cytoplasmic domains, which may be important in cellular signaling. MT1-MMP was found to be involved in the epithelial-to-mesenchymal-transition of GBM cells through pathway signaling, which involves TGF-β and SNAIL [194]. Similarly, other studies found a correlation between the expression of the MT1-MMP, Beta1-integrin, YAP1 pathways and the grading of gliomas [195].

Therapeutic targeting and resistance

Current standard of care

GBM composes a 14.2% of all primary CNS tumors and 50% of all malignant primary CNS tumors [196] and poses serious challenge for doctors and patients. Current standard of care comprise maximal safe resection, adjuvant radio and chemotherapy and subsequent six months of chemotherapy with Temozolomide (TMZ), an oral alkylating agent[197]. Despite years of research current median OS is between 12-14 months[198] which drives the further treatment development. TMZ chemotherapy has been especially beneficial for patients with MGMT promoter methylation[197,199] , IDH1/2 mutation or 1p19q codeletion providing prolonged progression-free survival, coexistence of MGMT promotor methylation and IDH1/2 mutation results in even better prognosis in TMZ therapy [200]. Among the recurrence treatment, the most commonly used in clinical trials was Iomustine[201], monofunctional alkylating agent which alkylates DNA and RNA and can cross-link DNA and thus acts in a cell cycle-dependent and independent manner[202].

Resistance to treatment

Conventional therapies resistance is often associated with GBM microenvironment, high heterogeneity of cancer cells, existence of GSCs, issue with blood-brain barrier (BBB) permeability restriction[203–207]. The BBB is of non-fenestrated physical barrier composed of tight junctions consisting of claudins, occludins and junctional adhesion molecules. Additionally BBB is supported by complex of astrocytic endfeet, pericytes, and intermittent ends of neurons which enhances molecular impenetrability. The BBB allows only lipophilic, small (<500 Da and <400 nm) to diffuse across. Rest of molecules are transported through receptor- or carried mediator related transcytosis, pinocytosis or solute-carrier-protein mechanisms[208,209]. Built-in vessel wall protein transporters (multidrug resistance-1 MDR1, P-glycoprotein, breast cancer resistance protein) prevent the penetration of various drugs into the brain, thus hindering brain drug concentration. Attempts to influence these proteins did not bring satisfactory results [207,210,211]. Slowly dividing and therapy resistant GSCs are known cause of minimal residual disease and recurrence. Through neighbouring cells alternation they induce microenvironment which maintain CNS population. CNS resides in the perivascular hypoxic niches of the brain. High heterogeneity of GBM cells follows high molecular locational and cellular genetic variability. This characteristic of GBM makes it futile to find a universal drug for every patient, which is why histological, molecular and immunological analysis of the tumor are important.

Radiotherapy (RT)

The benefits of combining RT with chemotherapy or surgery vs. RT alone are well known. In the Stupp [197] study, an increase in two-year survival rate of 26.5% using TMZ with RT compared to 10.4% with monotherapy was observed [197,212]. The role of irradiation in adjunctive therapy is essential at it seems that TMZ promotes dsDNA breaks in a radiation-induced pro-apoptotic environment[213]. MGMT promoter methylation in some GBM(s) has shown better response to radiotherapy than unmethylated MGMT tumors that actually progress during radiation treatment [214]. Properly targeted irradiation with marked tumor cells may increase the effectiveness of radiotherapy, in clinical trials researchers are using 3,4-dihydroxy-6[18F]-fluoro-

-L-phenylalanine (18F-DOPA) positron emission tomography (PET) which is sensitive and specific for identifying regions of lesion + MRI to assess scope of cancerous infiltration that are normally not visible in MRI alone [215,216].

Tumor treating fields (TTFs)

TTFs is emerging therapy that influence charged macromolecules and organelles and inhibits cancer cell division by alternating electric fields of specific frequency and intensity [217]. It also disrupts prophase of mitotic cells causing instability and blistering of the plasma membrane causing apoptosis [217–219]. Only proliferating cells seems to be affected according to models. TTFs interferes with formation of tubulin, function of cytokinetic furrow and induce cell death through activation of caspase pathway in p53 dependent manner. TTFs combined with RT synergistically enhances PARP (poly ADP ribose polymerase) and caspage-3 clevage [217–219]. RT results in DNA damage in cancers cells and TTFs delays its repair. NovoTAL software utilizes MRI in order to properly locate the tumor, its size and optimal dosage of field. Treatment is delivered by four transducer arrays. The TTFs device was approved as adjuvant therapy for newly diagnosed GBM in 2015. The effectiveness of this therapy is particularly noticeable in combination with chemotherapy and in phase three trials it was characterized by prolonged OS and PFS [220,221]. Increase in the quality of life was also observed [222].

Tumor specific therapy

Developments in immunology have become a driving force for new research on targeted therapy for GBM which would be free from side effects of chemotherapy and radiotherapy. Vaccines are an example of such therapy. Rindopepimut CDX 110 contains unique EGFRvIII peptide sequence that stimulates the immune system to produce antibodies against the EGFRvIII [223–225]. EGFRvIII is present in 30% of primary glioblastoma and are linked with poor long term survival[226]. Oncolytic viruses are also emerging as an attractive targeted therapy for GBM. Genetically modified viruses would combine with specific GBM antigens and cause lysis of tumor cells and stimulate the immune system response. The therapy is under development and involves certain issues, such as the selection of the appropriate virus, the route of virus administration, immunization, and the potential for carcinogenesis due to incorrect incorporation of virus's genetic material [227]. Chimeric antigen receptor (CAR) T cell therapy is a candidate for personalized form of adoptive T cell therapy (**Fig. 3**). Patient's own T cells are modified to express CARs which target GBM cells. CAR T cell therapy has been tested in refractory ALL and DLBCL with success. Potential variants of glioblastoma CAR T include EGFRvIII, IL13Ralfa2 and HER2 mutants. IL13Ralfa2 antigen overexpression is present within approximately 58% GBM tumors and is associated with poor prognosis which makes it a proper target for immunotherapy in general [228].

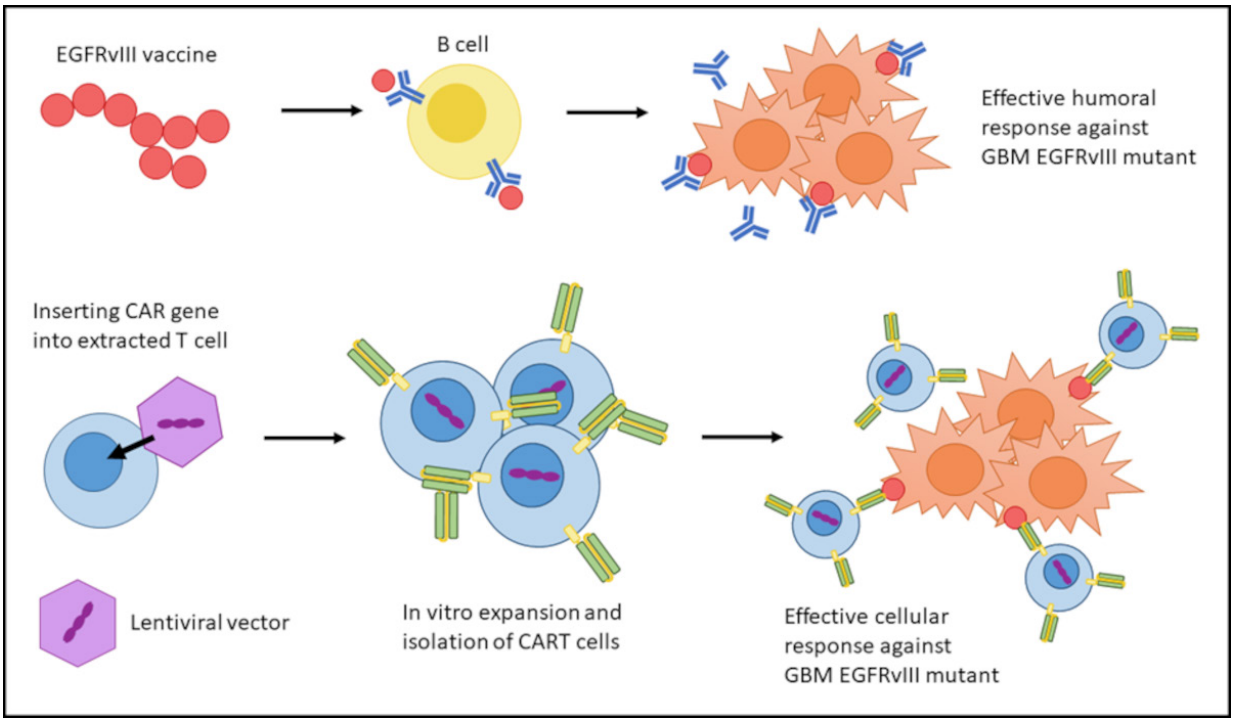


FIGURE 3 Glioblastoma EGFRvIII mutant targeted therapy including Rindopepimut CDX 110 vaccine and personalized CAR (chimeric antigen receptor) T cell therapy

Efficacy of CART T cells for solid tumors in clinical trials are limited due to poor tumor infiltration, off--target effects [225,229]. To minimize unsuccessful therapy it is speculated that CART T cells should target more than one specific antigens. To assess effectiveness of immunotherapy more studies are required [225,229–233].

Future directions

Improved preclinical models

Human-derived cell lines

Understanding the biology of GBM cells and their interactions with environment play a significant role in the development of therapeutic possibilities, driving a constant search for new preclinical models [234]. Commonly used human-derived GBM cell lines, such as U-251 and U-87, both fail to represent certain characteristics of a human brain tumor. The U-251 line presents a diffuse, infiltration growth pattern similar to human GBM, however does not invade the white matter tracks and remains responsive to radiotherapy as well as TMZ [234]. On the contrary, the U-87 cell line tumors display well-demarcated borders, differing from the typical GBM growth pattern, yet featuring vasculature which can be a target point for anti-angiogenic testing [235]. Individual cell lines do not represent the heterogeneity found in GBM tumors and hence the need to examine several patient-derived samples [236]. Acquiring specimen from a group of patients and classifying them into preneural, classical, mesenchymal and neural cells pointed to preneural type having the highest proliferative capacity paired with short OS in mice [236]. In the same study, tumors induced by certain cell lines, namely U3047MG, U3021MG and U3020MG demonstrated evident histopathological similarity to patient GBM, offering hope for more representative future research conducted on the established biobank. Patient-derived xenografts (PDX) in rodents, however, do not represent the tumor microenvironment and require immunosuppression of the mice, thus cannot reflect antitumor immunological response [237]. One solution appears to be zebrafish blastomere xenograft emerging as a clinically relevant model suitable for large-scale drug testing [238].

3D-models

Considering the limitations of 2D cultures & PDX models, another solution appears to be 3D-models including spherical cancer models, organoid models, scaffolds and 3D bioprinting [239]. Using induced pluripotent stem cells as a scaffold for patient-derived GBM cells, enabled establishing a model in which microtube formation and changes in gene expression could be observed [240]. The study suggests increased network formation and invasion as a result of cell to cell communication which should be further investigated. Another research team managed to obtain a “miniature brain” model with high level of structural development consisting of neurons, glial cells and synapses [241]. Coculturing with patient-derived glial stem cells (GSC) revealed a rise in resistance to TMZ and radiation as a consequence of intercellular interactions that indicates the importance of 3d-modeling development. In order to obtain the possibly most well-defined 3D structure and enable customization bioprinting was introduced. This technique allows not only cell-ECM and cell-cell interaction studies but also clinically relevant drug testing. Meticulous design paired with biomaterial advances allows research into specific niches occurring in a heterogenous GBM tumor and gives hope for accurate blood-brain barrier models [242]. Models using hyaluronic acid- enriched biomaterial and varying stiffness patterns not only elucidated the influence of biophysical properties on developing certain GBM subtypes but also highlighted endothelial cell ability to enhance drug resistance [243].

Microfluidics

Another state-of-the-art method appears to be microfluidics, providing precise control over cell and fluid manipulation [235]. Microfluidic chips containing patient-derived GBM cells in a biomaterial facilitate research in dose-dependent drug response, combination therapy outcomes and drug penetration into tumor. Single-cell analysis of treatment-resistant GBM could provide insight into the molecular and physiological changes occurring in cells, which paired with drug testing could potentially facilitate tailored therapy change for specific patient. Moreover, the technique enables exploration of the dynamic tumor microenvironment [244].

As mentioned above, ongoing research into development of new preclinical models remains crucial for comprehending the intricate interactions among GBM and GSC cells, surrounding cells and ECM [245]. Considering the limitations of current models, including insufficient resemblance to human tumors, imperfect biomaterials as well as the absence of vasculature and BBB [234], it is evident that there is still a lot to be achieved in the field of tumor modeling [246].

Accurate diagnosis and emerging biomarkers

Biopsy and intraoperative diagnostics

Following the research done in the field of GBM genetics and metabolism traits, a few diagnostic and prognostic markers have been introduced. While effective, traditional tissue biopsy required to obtain a specimen for genetic tests captures only a fraction of the tumor’s heterogenous tissue. Thus, the new perspective for biomarker search seems to be liquid biopsy [247–249]. A novel approach – Stimulated Raman Histology has shown accuracy comparable to commonly employed frozen section histology.

However, with greatly enhanced time to diagnosis it holds the promise for rapid and successful surgical resections [250]. Compared to other intraoperative pathologic diagnosis methods, namely MRI, ultrasound or fluorescence-guided microsurgery, Raman spectroscopy displays superior sensitivity, less environmental requirements and is not affected by intraoperative drift [251]. A study performed on fresh tissue samples revealed that the technique paired with machine learning algorithm successfully distinguishes malignancy from normal tissue [252] and thus, could be employed in neurosurgery [253].

Blood and CSF

Blood and cerebrospinal fluid (CSF) samples, being easily obtainable and in direct contact with many tumor cells, serve as a rich source of metabolites, nucleic acids and proteins [248]. So called circulatory biomarkers include miRNA which may act as a regulatory factor in gene expression influencing proliferation, angiogenesis and metastasis [254]. Several studies have proven a change in miR-21 level after tumor resection, thus it may reflect the tumor regrowth after incomplete resection. miR-21 and miR-155 have also been linked to poorer OS [247,254]. The up and downregulation of miRNA in GBM patients and their possible prognostic value underscores the need of comprehensive research including entire miRNA panel identification in bodily fluids [249]. Another tumor-derived compound found in plasma and CSF is circulating tumor DNA (ctDNA). Since ctDNA concentration is generally low in plasma, increased sensitivity of detection method is demanded. Nevertheless, collecting ctDNA and gene sequencing could provide information about specific mutations without the need of traditional biopsy [247]. Long non-coding RNA (lncRNA) emerges as another promising circulating biomarker [255]. It has been proven that certain lncRNA correlate with poor OS [256,257] and a team of researchers was able to identify six lncRNA that could divide patients into high and low-risk groups in terms of OS: AC005013.5, UBE2R2-AS1, ENTPD1-AS1, RP11-89C21.2, AC073115.6 and XLOC_004803 [258]. Extracellular vesicle (EV) isolation seems to be another approach, as GBM cells produce EVs containing proteins (enzymes, receptors, signaling molecules), lipids and genetic material that take part in intercellular communication, acquiring treatment resistance and immunosuppression [248]. EVs may hence be a source of biomarkers including EGFRvIII, mutated IDH1, miR-151a and miR-21, but further research needs to be conducted in order to delineate GBM specific markers allowing differentiation from other cell-derived EV [259].

Imaging

Beyond insight into the metabolic and genetic characteristics, imaging remains an important factor in GBM diagnosis and treatment. Positron emission tomography (PET) and magnetic resonance imaging (MRI) are techniques frequently used in tumor monitoring, prognostic and assessment. In general, radiotracers used in traditional PET can be divided to glucose metabolism tracers and amino acid transport tracers [260]. Due to the high uptake of glucose metabolism tracers like F-2-fluoro-2-deoxy-D-glucose in normal brain tissue, it lacks the demanded sensitivity, thus the need to use amino acid tracers, namely C-methyl-methionine (MET), O-(2-[18F]-fluoroethyl)-L-tyrosine (F-FET) and 3,4-dihydroxy-6-[18F]-fluoro-L-phenylalanine (F-DOPA). In several studies F-FET and F-DOPA markers revealed a larger tumor volume and extent compared to MRI [261]. It has also been suggested that both F-FET PET and C-MET PET show potential in differentiating patients with pseudoprogression from true responders after completion of therapy [262]. FET-PET has also shown utility in distinguishing brain lesions previously undefined by MRI [263] which holds promise for introducing proper treatment sooner in patients. Furthermore, ongoing research points to sigma receptors (SR) as an alternative target for radiotracers [264]. Both sigma 1 receptor and sigma 2 receptor are expressed in various tumor cells reflecting invasiveness and proliferation, respectively [265]. However, identification of a radioligand with sufficient pharmacokinetics remains a subject for further research, with [18F](S)-Fluspidine being the most promising [264,265]. On the other hand, IDH-sensitive radioligands emerge as alternative prognostic factor, considering the fact that IDH mutations in GBM patients are linked to better OS [266].

Arising therapeutic possibilities

Focused ultrasound

One of the obstacles accounting for GBM treatment resistance is BBB, which significantly exacerbates drug penetration and accumulation in malignant tissue. Focused ultrasound (FUS) has been proposed as a new, non-invasive approach to this problem. Low-intensity FUS (LIFU) paired with administration of microbubbles has proven effective in BBB disruption with the advantage of minimal temperature increase, high penetration of ultrasonic waves and no damage to brain tissue [267]. FUS has also been linked with efflux-pump inhibition leading to higher drug concentration in tumor [268]. BBB opening resulted in enriched signal of liquid biopsy markers as well [269]. Transcranial magnetic resonance-guidedFUS (MRgFUS) provides hope for clinical usage of the technique improving permeability of nanoparticles [270] which could potentially carry therapeutic agents directly to GBM cells with tumor-targeted ligands. On the contrary, high-intensity FUS (HIFUS) bears the potential of causing tumor tissue coagulative necrosis by indu-

cing hyperthermia [271]. Another application of ultrasound may be sonodynamic therapy (SDT) in which non-toxic agents, called sonosensitizers, accumulate in tumor tissue and display cytotoxicity after exposure to LIFUS. Several sonosensitizers, including 5-Aminolevulinic acid and porphyrin derivatives have proven successful in preclinical trials [272]. Effective BBB disturbance has encouraged search for targeted nanoparticles capable of drug delivery directly to tumor cells. Due to the high iron uptake of GBM cells, liposomes with attached transferrin, targeted at transferrin receptor could serve as such [273]. In a study combining FUS and liposome carriers, greater accumulation of therapeutic agents has been found in tumor tissue [274]. Another receptor overly expressed in GBM cells and also present in BBB cells is low-density lipoprotein receptor (LDLR) with apoB/apoE ligand, which has been effectively used as a targeted radiosensitizer [275] as well as combination therapy carrier [276]. These findings give hope for drug delivery with limited side-effects on healthy tissue combined with direct and aggressive tumor-targeted chemotherapy, which could potentially prevent later treatment resistance, common among tumors recurring from leftover GSC [277].

MRI guided radiotherapy

The search for novel therapies for GBM includes trials with techniques that have previously been used for other tumors [278]. Magnetic Resonance image guided Radiation Therapy (MRgRT), applied mainly to malignancies located in the chest and abdomen, holds the potential of providing anatomic adaptive radiotherapy in tumors displaying pseudoprogression. Another aim is to provide physiologic adaptive radiotherapy using multiparametric MRI (mpMRI) [279]. Quantitative multi-parametric mapping, diffusion weighted imaging as well as proton magnetic resonance spectroscopy provide information about tumor progression, cellular density and concentration of tumor-derived particles in the brain [280][281] that paired with general knowledge about GBM heterogeneity could prompt radiotherapy modulation on tumor parts displaying resistance that can be assessed using presented techniques [279].

Other

Constant research providing insights into cell metabolism points to autophagy regulation as a new way of treatment. This cellular process has been linked with cell survival as it enables recycling essential elements, but also with cell death induction. A few studies have shown opposite effects of autophagy blockage/induction in terms of treatment susceptibility and cell apoptosis and thus the need for further research in order to develop a proper strategy in different GBM types [282]. For instance, autophagy elicited by valproic acid has proved to prompt tumor cell lines apoptosis [283]. Valproic acid, acting as a histone acetylase inhibitor, may as well influence signaling pathways in cells and therefore have an effect on therapy sensitivity and cell growth [284]. It has also been linked with reduced vascular endothelial growth factor secretion and thus angiogenesis inhibition [285]. Another recent approach to GBM treatment involves cold atmospheric plasma and plasma activated liquids combined with traditional chemotherapy, which proved successful enhancing the cytotoxic therapeutic effect [286–288].

In conclusion, despite many recent discoveries and ongoing trials, there is still room for improvement in glioblastoma treatment. Taking into account the limitations of constantly emerging techniques and aggressiveness of this malignancy, introducing new therapeutic agents remains a challenge for scientific community. Nevertheless, the perspective of ameliorating patients' condition and doing what now seems impossible, drives incessant search for possibilities.

Ethical approval

The conducted research is not related to either human or animal use.

Acknowledgments

Not applicable.

Corresponding author

Wiesława Kranc, Department of anatomy, Poznan University of Medical Sciences, Poznań, Poland, email: wkranc@ump.edu.pl.

Conflicts of interest

The authors declare they have no conflict of interest.

References

1. Ostrom QT, Price M, Neff C, Cioffi G, Waite KA, Kruchko C, Barnholtz-Sloan JS. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2016-2020. Neuro Oncol. 2023;25:iv1-99; DOI:10.1093/NEUONC/NOAD149.
2. Walker E V., Davis FG, Shaw A, Louchini R, Shack L, Woods R, Kruchko C, Spinelli J, Guiot MC, Perry J, Melin B, Barnholtz-Sloan J, Turner D, King MJ, Hannah H, Bryant H. Malignant primary brain and other central nervous system tumors diagnosed in Canada from 2009 to 2013. Neuro Oncol. 2019;21:360-9; DOI:10.1093/NEUONC/NOY195.
3. Philips A, Henshaw DL, Lamburn G, O'Carroll MJ. Brain tumours: rise in glioblastoma multiforme incidence in England 1995-2015 suggests an adverse environmental or lifestyle factor. J Environ Public Health. 2018;2018; DOI:10.1155/2018/7910754.
4. Dobes M, Khurana VG, Shadbolt B, Jain S, Smith SF, Smee R, Dexter M, Cook R. Increasing incidence of glioblastoma multiforme and meningioma, and decreasing incidence of Schwannoma (2000-2008): findings of a multicenter Australian study. Surg Neurol Int. 2011;2; DOI:10.4103/2152-7806.90696.
5. Davis FG, Smith TR, Gittleman HR, Ostrom QT, Kruchko C, Barnholtz-Sloan JS. Glioblastoma incidence rate trends in Canada and the United States compared with England, 1995-2015. Neuro Oncol. 2020;22:301-2; DOI:10.1093/NEUONC/NOZ203.
6. Andersen ZJ, Pedersen M, Weinmayr G, Stafoggia M, Galassi C, Jørgensen JT, Sommar JN, Forsberg B, Olsson D, Oftedal B, Aasvang GM, Schwarze P, Pyko A, Pershagen G, Korek M, Faire U De, Östenson CG, Fratiglioni L, Eriksen KT, Poulsen AH, Tjønneland A, Braüner EV, Peeters PH, Bueno-De-Mesquita B, Jaensch A, Nagel G, Lang A, Wang M, Tsai MY, Grioni S, Marcon A, Krogh V, Ricceri F, Sacerdote C, Migliore E, Vermeulen R, Sokhi R, Keuken M, De Hoogh K, Beelen R, Vineis P, Cesaroni G, Brunekreef B, Hoek G, Raaschou-Nielsen O. Long-term exposure to ambient

air pollution and incidence of brain tumor: the European Study of Cohorts for Air Pollution Effects (ESCAPE). Neuro Oncol. 2018;20:420-32; DOI:10.1093/NEUONC/NOX163.
7. Villeneuve PJ, Agnew DA, Johnson KC, Mao Y, Paulse B, Dewar R, Dryer D, Kreiger N, Kliewer E, Robson D, Fincham S, Le N. Brain cancer and occupational exposure to magnetic fields among men: results from a Canadian population-based case-control study. Int J Epidemiol. 2002;31:210-7; DOI:10.1093/IJE/31.1.210.
8. Dho Y-S, Jung K-W, Ha J, Seo Y, Park C-K, Won Y-J, Yoo H. An updated nationwide epidemiology of primary brain tumors in Republic of Korea, 2013. Brain Tumor Res Treat. 2017;5:16; DOI:10.14791/BTRT.2017.5.1.16.
9. Manoharan N, Julka PK, Rath GK. Descriptive epidemiology of primary brain and CNS tumors in Delhi, 2003-2007. Asian Pac J Cancer Prev. 2012;13:637-40; DOI:10.7314/APJCP.2012.13.2.637.
10. Ostrom QT, Gittleman H, Stetson L, Virk SM, Barnholtz-Sloan JS. Epidemiology of gliomas. Cancer Treat Res. 2015;163:1-14; DOI:10.1007/978-3-319-12048-5_1.
11. Ostrom QT, Bauchet L, Davis FG, Deltour I, Fisher JL, Langer CE, Pekmezci M, Schwartzbaum JA, Turner MC, Walsh KM, Wrensch MR, Barnholtz-Sloan JS. The epidemiology of glioma in adults: a state of the science review. Neuro Oncol. 2014;16:896-913; DOI:10.1093/neuonc/nou087.
12. Wrensch M, Jenkins RB, Chang JS, Yeh RF, Xiao Y, Decker PA, Ballman K V., Berger M, Buckner JC, Chang S, Giannini C, Halder C, Kollmeyer TM, Kosel ML, Lachance DH, McCoy L, O'Neill BP, Patoka J, Pico AR, Prados M, Quesenberry C, Rice T, Rynearson AL, Smirnov I, Tihan T, Wiemels J, Yang P, Wiencke JK. Variants in the CDKN2B and RTEL1 regions are associated with high grade glioma susceptibility. Nat Genet. 2009;41:905; DOI:10.1038/NG.408.
13. Shete S, Hosking FJ, Robertson LB, Dobbins SE, Sanson M, Malmer B, Simon M, Marie Y, Boisselier B, Delattre JY, Hoang-Xuan K, Hallani S El, Idbaih A, Zelenika D, Andersson U, Henriksson R, Bergenheim AT, Feychting M, Lönn S, Ahlbom A, Schramm J, Linnebank M, Hemminki K, Kumar R, Hepworth SJ, Price A, Armstrong G, Liu Y, Gu X, Yu R, Lau C, Schoemaker M, Muir K, Swerdlow A, Lathrop M, Bondy M, Houlston RS. Genome-wide association study identifies five susceptibility loci for glioma. Nat Genet. 2009;41:899; DOI:10.1038/NG.407.
14. Kinnersley B, Houlston RS, Bondy ML. Genome-wide association studies in Glioma. Cancer Epidemiol Biomarkers Prev. 2018;27:418-28; DOI:10.1158/1055-9965.EPI-17-1080.
15. Melin BS, Barnholtz-Sloan JS, Wrensch MR, Johansen C, Il'yasova D, Kinnersley B, Ostrom QT, Labreche K, Buring J, et al. Genome-wide association study of glioma subtypes identifies specific differences in genetic susceptibility to glioblastoma and non-glioblastoma tumors. Nat Genet. 2017;49:789-94; DOI:10.1038/ng.3823.
16. Grech N, Dalli T, Mizzi S, Meilak L, Calleja N, Zrinzo A. Rising incidence of glioblastoma multiforme in a well-defined population. Cureus. 2020;12(5):e8195; DOI:10.7759/cureus.8195.
17. Wrensch M, Minn Y, Chew T, Bondy M, Berger MS. Epidemiology of primary brain tumors: current concepts and review of the literature. Neuro Oncol. 2002;4(4):278-99; DOI:10.1093/neuonc/4.4.278.
18. Shapiro S, Mealey J Jr. Late anaplastic gliomas in children previously treated for acute lymphoblastic leukemia. Pediatr Neurosci. 1989;15(4):176-80; DOI:10.1159/000120465.
19. Fontana M, Stanton C, Pompili A, Amadori S, Mandelli F, Meloni G, Riccio A, Rubinstein LJ. Late multifocal gliomas in adolescents previously treated for acute lymphoblastic leukemia. Cancer. 1987;60(7):1510-8; DOI:10.1002/1097-0142(19871001)60:7<1510::aid-cncr2820600718>3.0.co;2-v.
20. Salvati M, Frati A, Russo N, Caroli E, Polli FM, Minniti G, Delfini R. Radiation-induced gliomas: Report of 10 cases and review of the literature. Surg Neurol. 2003;60:60-7; DOI:10.1016/S0090-3019(03)00137-X.
21. Wingren C, James P, Borrebaeck CAK. Radiation-induced gliomas. Expert Rev Neurother. 2009;9:1511-7; DOI:10.1002/PMIC.200800802.
22. Liu C, Sage JC, Miller MR, Verhaak RGW, Hippenmeyer S, Vogel H, Foreman O, Bronson RT, Nishiyama A, Luo L, Zong H. Mosaic analysis with double markers reveals tumor cell of origin in glioma. Cell. 2011;146:209-21; DOI:10.1016/j.cell.2011.06.014.
23. Holland EC, Hively WP, DePinho RA, Varmus HE. A constitutively active epidermal growth factor receptor cooperates with disruption of G1 cell-cycle arrest pathways to induce glioma-like lesions in mice. Genes Dev. 1998;12(23):3675-85; DOI:10.1101/gad.12.23.3675.
24. Friedmann-Morvinski D, Bushong EA, Ke E, Soda Y, Marumoto T, Singer O, Ellisman MH, Verma IM. Dedifferentiation of neurons and astrocytes by oncogenes can induce gliomas in mice. Science (1979). 2012;338:1080-4; DOI:10.1126/science.1226929.
25. Alcantara Llaguno S, Sun D, Pedraza AM, Vera E, Wang Z, Burns DK, Parada LF. Cell-of-origin susceptibility to glioblastoma formation declines with neural lineage restriction. Nat Neurosci. 2019;22:545-55; DOI:10.1038/s41593-018-0333-8.
26. Lee JH, Lee JE, Kahng JY, Kim SH, Park JS, Yoon SJ, Um JY, Kim WK, Lee JK, Park J, Kim EH, Lee JH, Lee JH, Chung WS, Ju YS, Park SH, Chang JH, Kang SG, Lee JH. Human glioblastoma arises from subventricular zone cells with low-level driver mutations. Nature. 2018;560:243-7; DOI:10.1038/s41586-018-0389-3.
27. Piccirillo SGM, Reynolds BA, Zanetti N, Lamorte G, Binda E, Broggi G, Brem H, Olivi A, Dimeco F, Vescovi AL. Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. Nature. 2006;444:761-5; DOI:10.1038/nature05349.
28. Gimple RC, Bhargava S, Dixit D, Rich JN. Glioblastoma stem cells: lessons from the tumor hierarchy in a lethal cancer. 2019; DOI:10.1101/gad.324301.
29. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. Nature. 2004;432:396-401; DOI:10.1038/NATURE03128.
30. Neftel C, Laffy J, Filbin MG, Hara T, Shore ME, Rahme GJ, Richman AR, Silverbush D, Shaw ML, Hebert CM, Dewitt J, Gritsch S, Perez EM, Gonzalez Castro LN, Lan X, Druck N, Rodman C, Dionne D, Kaplan A, Bertalan MS, Small J, Pelton K, Becker S, Bonal D, Nguyen QD, Servis RL, Fung JM, Mylvaganam R, Mayr L, Gojo J, Haberler C, Geyeregger R, Czech T, Slavc I, Nahed BV, Curry WT, Carter BS, Wakimoto H, Brastianos PK, Batchelor TT, Stemmer-Rachamimov A, Martinez-Lage M, Frosch MP, Stamenkovic I, Riggi N, Rheinbay E, Monje M, Rozenblatt-Rosen O, Cahill DP, Patel AP, Hunter T, Verma IM, Ligon KL, Louis DN, Regev A, Bernstein BE, Tirosh I, Suvà ML. An Integrative model of cellular states, plasticity, and genetics for glioblastoma. Cell. 2019;178(4):835-49.e21; DOI:10.1016/j.cell.2019.06.024.
31. Stichel D, Ebrahimi A, Reuss D, Schrimpf D, Ono T, Shirahata M, Reifenberger G, Weller M, Hänggi D, Wick W, Herold-Mende C, Westphal M, Brandner S, Pfister SM, Capper D, Sahm F, von Deimling A. Distribution of EGFR amplification, combined chromosome 7 gain and chromosome 10 loss, and TERT promoter mutation in brain tumors and their potential for the reclassification of IDHwt astrocytoma to glioblastoma. Acta Neuropathol. 2018;136:793-803; DOI:10.1007/s00401-018-1905-0.
32. Balesaria S, Brock C, Bower M, Clark J, Nicholson SK, Lewis P, De Sanctis S, Evans H, Peterson D, Mendoza N, Glaser MG, Newlands ES, Fisher RA. Loss of chromosome 10 is an independent prognostic factor in high-grade gliomas. Br J Cancer. 1999;81:1371; DOI:10.1038/SJ.BJC.6693403.
33. Lopez-Gines C, Cerda-Nicolas M, Gil-Benso R, Pellin A, Lopez-Guerrero JA, Callaghan R, Benito R, Roldan P, Piquer J, Llacer J, Barbera J. Association of chromosome 7, chromosome 10 and EGFR gene amplification in glioblastoma multiforme. Clin Neuropathol. 2005;24(5):209-18.
34. Liu EM, Shi ZF, Li KKW, Malta TM, Chung NYF, Chen H, Chan JYT, Poon MFM, Kwan JSH, Chan DTM, Noushmehr H, Mao Y, Ng HK. Molecular landscape of IDH -wild type, pTERT -wild type adult glioblastomas. Brain Pathology. 2022;32; DOI:10.1111/BPA.13107.
35. Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, Barretina J, Boehm JS, Dobson J, Urashima M, Mc Henry KT, Pinchback RM, Ligon AH, Cho YJ, Haery L, Greulich H, Reich M, Winckler W, Lawrence MS, Weir BA, Tanaka KE, Chiang DY, Bass AJ, Loo A, Hoffman C, Prensner J, Liefeld T, Gao Q, Yecies D, Signoretti S, Maher E, Kaye FJ, Sasaki H, Tepper JE, Fletcher JA, Tabernero J, Baselga J, Tsao MS, Demichelis F, Rubin MA, Janne PA, Daly MJ, Nucera C, Levine RL, Ebert BL, Gabriel S, Rustgi AK, Antonescu CR, Ladanyi M, Letai A, Garraway LA, Loda M, Beer DG, True LD, Okamoto A, Pomeroy SL, Singer S, Golub TR, Lander ES, Getz G, Sellers WR, Meyerson M. The landscape of somatic copy-number alteration across human cancers. Nature. 2010;463(7283):899-905; DOI:10.1038/nature08822.
36. Boisselier B, Dugay F, Belaud-Rotureau MA, Coutolleau A, Garcion E, Menei P, Guardiola P, Rousseau A. Whole genome duplication is an early event leading to aneuploidy in IDH-wild type glioblastoma. Oncotarget. 2018;9:36017-28; DOI:10.18632/ONCOTARGET.26330.
37. Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, Salama SR, Zheng S, Chakravarty D, Sanborn JZ, Berman SH, Beroukhim R, Bernard B, Wu CJ, Genovese G, Shmulevich I, Barnholtz-Sloan J, Zou L, Vegesna R, Shukla SA, Ciriello G, Yung WK, Zhang W, Sougnez C, Mikkelsen T, Aldape K, Bigner DD, Van Meir EG, Prados M, Sloan A, Black KL, Eschbacher J, Finocchiaro G, Friedman W, Andrews DW, Guha A, Iacocca M, O'Neill BP, Foltz G, Myers J, Weisenberger DJ, Penny R, Kucherlapati R, Perou CM, Hayes DN, Gibbs R, Marra M, Mills GB, Lander E, Spellman P, Wilson R, Sander C, Weinstein J, Meyerson M, Gabriel S, Laird PW, Haussler D, Getz G, Chin L; TCGA Research Network. The somatic genomic landscape of glioblastoma. Cell. 2013;155(2):462-77; DOI:10.1016/j.cell.2013.09.034.
38. Bigner SH, Mark J, Burger PC, Mahaley MS Jr, Bullard DE, Muhlbaier LH, Bigner DD. Specific chromosomal abnormalities in malignant human gliomas. Cancer Res. 1988;48(2):405-11.
39. Tian L, Li Y, Edmonson MN, Zhou X, Newman S, McLeod C, Thrasher A, Liu Y, Tang B, Rusch MC, Easton J, Ma J, Davis E, Trull A, Michael JR,

Szlachta K, Mullighan C, Baker SJ, Downing JR, Ellison DW, Zhang J. CICERO: A versatile method for detecting complex and diverse driver fusions using cancer RNA sequencing data. Genome Biol. 2020;21; DOI:10.1186/s13059-020-02043-x.
40. Parker BC, Annala MJ, Cogdell DE, Granberg KJ, Sun Y, Ji P, Li X, Gumin J, Zheng H, Hu L, Yli-Harja O, Haapasalo H, Visakorpi T, Liu X, Liu CG, Sawaya R, Fuller GN, Chen K, Lang FF, Nykter M, Zhang W. The tumorigenic FGFR3-TACC3 gene fusion escapes miR-99a regulation in glioblastoma. J Clin Invest. 2013;123(2):855-65; DOI: 10.1172/JCI67144.
41. Bao ZS, Chen HM, Yang MY, Zhang CB, Yu K, Ye WL, Hu BQ, Yan W, Zhang W, Akers J, Ramakrishnan V, Li J, Carter B, Liu YW, Hu HM, Wang Z, Li MY, Yao K, Qiu XG, Kang CS, You YP, Fan XL, Song WS, Li RQ, Su XD, Chen CC, Jiang T. RNA-seq of 272 gliomas revealed a novel, recurrent PTPRZ1-MET fusion transcript in secondary glioblastomas. Genome Res. 2014;24:1765-73; DOI:10.1101/gr.165126.113.
42. Frattini V, Trifonov V, Chan JM, Castano A, Lia M, Abate F, Keir ST, Ji AX, Zoppoli P, Niola F, Danussi C, Dolgalev I, Porrati P, Pellegatta S, Heguy A, Gupta G, Pisapia DJ, Canoll P, Bruce JN, McLendon RE, Yan H, Aldape K, Finocchiaro G, Mikkelsen T, Privé GG, Bigner DD, Lasorella A, Rabadan R, Iavarone A. The integrated landscape of driver genomic alterations in glioblastoma. Nat Genet. 2013;45:1141-9; DOI:10.1038/ng.2734.
43. Fuller GN, Bigner SH. Amplified cellular oncogenes in neoplasms of the human central nervous system. Mutation Research/Reviews in Genetic Toxicology. 1992;276:299-306; DOI:10.1016/0165-1110(92)90016-3.
44. Inda MDM, Bonavia R, Mukasa A, Narita Y, Sah DWY, Vandenberg S, Brennan C, Johns TG, Bachoo R, Hadwiger P, Tan P, DePinho RA, Cavenee W, Furnari F. Tumor heterogeneity is an active process maintained by a mutant EGFR-induced cytokine circuit in glioblastoma. Genes Dev. 2010;24:1731; DOI:10.1101/GAD.1890510.
45. Sturm D, Witt H, Hovestadt V, Khuong-Quang DA, Jones DT, Konermann C, Pfaff E, Tönjes M, Sill M, Bender S, Kool M, Zapatka M, Becker N, Zucknick M, Hielscher T, Liu XY, Fontebasso AM, Ryzhova M, Albrecht S, Jacob K, Wolter M, Ebinger M, Schuhmann MU, van Meter T, Frühwald MC, Hauch H, Pekrun A, Radlwimmer B, Niehues T, von Komorowski G, Dürken M, Kulozik AE, Madden J, Donson A, Foreman NK, Drissi R, Fouladi M, Scheurlen W, von Deimling A, Monoranu C, Roggendorf W, Herold-Mende C, Unterberg A, Kramm CM, Felsberg J, Hartmann C, Wiestler B, Wick W, Milde T, Witt O, Lindroth AM, Schwartzentruber J, Faury D, Fleming A, Zakrzewska M, Liberski PP, Zakrzewski K, Hauser P, Garami M, Klekner A, Bognar L, Morrissy S, Cavalli F, Taylor MD, van Sluis P, Koster J, Versteeg R, Volckmann R, Mikkelsen T, Aldape K, Reifenberger G, Collins VP, Majewski J, Korshunov A, Lichter P, Plass C, Jabado N, Pfister SM. Hotspot Mutations in H3F3A and IDH1 Define Distinct Epigenetic and Biological Subgroups of Glioblastoma. Cancer Cell. 2012;22:425-37; DOI:10.1016/j.ccr.2012.08.024.
46. Sugawa N, Ekstrand AJ, James CD, Collins VP. Identical splicing of aberrant epidermal growth factor receptor transcripts from amplified rearranged genes in human glioblastomas. Proc Natl Acad Sci USA. 1990;87(21):8602-6; DOI:10.1073/pnas.87.21.8602.
47. Schwechheimer K, Huang S, Cavenee WK. EGFR gene amplification-rearrangement in human glioblastomas. Int J Cancer. 1995;62(2):145-8; DOI: 10.1002/ijc.2910620206.
48. Cho J, Pastorino S, Zeng Q, Xu X, Johnson W, Vandenberg S, Verhaak R, Cherniack AD, Watanabe H, Dutt A, Kwon J, Chao YS, Onofrio RC, Chiang D, Yuza Y, Kesari S, Meyerson M. Glioblastoma-derived epidermal growth factor receptor (EGFR) carboxyl-terminal deletion mutants are transforming and are sensitive to EGFR-directed therapies. Cancer Res. 2011;71:7587; DOI:10.1158/0008-5472.CAN-11-0821.
49. Fadhal E. A Comprehensive analysis of the PI3K/AKT pathway: unveiling key proteins and therapeutic targets for cancer treatment. Cancer Inform. 2023;22; DOI:10.1177/11769351231194273.
50. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008;455(7216):1061-8; DOI: 10.1038/nature07385.
51. Singh D, Chan JM, Zoppoli P, Niola F, Sullivan R, Castano A, Liu EM, Reichel J, Porrati P, Pellegatta S, Qiu K, Gao Z, Ceccarelli M, Riccardi R, Brat DJ, Guha A, Aldape K, Golfinos JG, Zagzag D, Mikkelsen T, Finocchiaro G, Lasorella A, Rabadan R, Iavarone A. Transforming fusions of FGFR and TACC genes in human glioblastoma. Science (1979). 2012;337:1231-5; DOI:10.1126/science.1220834.
52. Carlotto BS, Trevisan P, Provenzi VO, Soares FP, Rosa RFM, Varella-Garcia M, Zen PRG. PDGFRA, KIT, and KDR gene amplification in glioblastoma: heterogeneity and clinical significance. Neuromolecular Med. 2023;25(3):441-50; DOI:10.1007/s12017-023-08749-y.
53. Verhaak RGW, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, Alexe G, Lawrence M, O'Kelly M, Tamayo P, Weir BA, Gabriel S, Winckler W, Gupta S, Jakkula L, Feiler HS, Hodgson JG, James CD, Sarkaria JN, Brennan C, Kahn A, Spellman PT, Wilson RK, Speed TP, Gray JW, Meyerson M, Getz G, Perou CM, Hayes DN. An integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR and NF1. Cancer Cell. 2010;17:98; DOI:10.1016/J.CCR.2009.12.020.
54. Tay Y, Kats L, Salmena L, Weiss D, Tan SM, Ala U, Karreth F, Poliseno L, Provero P, Di Cunto F, Lieberman J, Rigoutsos I, Pandolfi PP. Coding-independent regulation of the tumor suppressor PTEN by competing endogenous mRNAs. Cell. 2011;147:344; DOI:10.1016/J.CELL.2011.09.029.
55. Ciriello G, Cerami E, Sander C, Schultz N. Mutual exclusivity analysis identifies oncogenic network modules. Genome Res. 2012;22:398; DOI:10.1101/GR.125567.111.
56. Snuderl M, Fazlollahi L, Le LP, Nitta M, Zhelyazkova BH, Davidson CJ, Akhavanfard S, Cahill DP, Aldape KD, Betensky RA, Louis DN, Iafrate AJ. Mosaic amplification of multiple receptor tyrosine kinase genes in glioblastoma. Cancer Cell. 2011;20:810-7; DOI:10.1016/J.CCR.2011.11.005.
57. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature. 2012;490(7418):61-70; DOI: 10.1038/nature11412.
58. Ozaki T, Nakagawara A. Role of p53 in cell death and human cancers. Cancers (Basel). 2011;3:994; DOI:10.3390/CANCERS3010994.
59. Crespo I, Vital AL, Gonzalez-Tablas M, Patino MDC, Otero A, Lopes MC, De Oliveira C, Domingues P, Orfao A, Tabernero MD. Molecular and genomic alterations in glioblastoma multiforme. Am J Pathol. 2015;185:1820-33; DOI:10.1016/J.AJPATH.2015.02.023.
60. Zacher A, Kaulich K, Stepanow S, Wolter M, Köhrer K, Felsberg J, Malzkorn B, Reifenberger G. Molecular diagnostics of gliomas using next generation sequencing of a glioma-tailored gene panel. Brain Pathology. 2017;27:146-59; DOI:10.1111/bpa.12367.
61. Fassl A, Geng Y, Sicinski P. CDK4 and CDK6 kinases: from basic science to cancer therapy. Science. 2022;375:eabc1495; DOI:10.1126/SCIENCE.ABC1495.
62. Chung M, Liu C, Yang HW, Köberlin MS, Cappell SD, Meyer T. Transient hysteresis in CDK4/6 activity underlies passage of the restriction point in G1. Mol Cell. 2019;76:562; DOI:10.1016/J.MOLCEL.2019.08.020.
63. Nishikawa R, Furnari FB, Lin H, Arap W, Berger MS, Cavenee WK, Su Huang HJ. Loss of P16INK4 expression is frequent in high grade gliomas. Cancer Res. 1995;55(9):1941-5.
64. Reifenberger G, Reifenberger J, Ichimura K, Meltzer PS, Collins VP. Amplification of multiple genes from chromosomal region 12q13-14 in human malignant gliomas: preliminary mapping of the amplicons shows preferential involvement of CDK4, SAS, and MDM2. Cancer Res. 1994;54(16):4299-303.
65. Ueki K, Ono Y, Henson JW, Efird JT, von Deimling A, Louis DN. CDKN2/p16 or RB alterations occur in the majority of glioblastomas and are inversely correlated. Cancer Res. 1996;56(1):150-3.
66. deVries NA, Hulsman D, Akhtar W, deJong J, Miles DC, Blom M, vanTellingen O, Jonkers J, VanLohuizen M. Prolonged Ezh2 depletion in glioblastoma causes a robust switch in cell fate resulting in tumor progression. Cell Rep. 2015;10:383-97; DOI:10.1016/j.celrep.2014.12.028.
67. Schwartzentruber J, Korshunov A, Liu XY, Jones DT, Pfaff E, Jacob K, Sturm D, Fontebasso AM, Quang DA, Tönjes M, Hovestadt V, Albrecht S, Kool M, Nantel A, Konermann C, Lindroth A, Jäger N, Rausch T, Ryzhova M, Korbel JO, Hielscher T, Hauser P, Garami M, Klekner A, Bognar L, Ebinger M, Schuhmann MU, Scheurlen W, Pekrun A, Frühwald MC, Roggendorf W, Kramm C, Dürken M, Atkinson J, Lepage P, Montpetit A, Zakrzewska M, Zakrzewski K, Liberski PP, Dong Z, Siegel P, Kulozik AE, Zapatka M, Guha A, Malkin D, Felsberg J, Reifenberger G, von Deimling A, Ichimura K, Collins VP, Witt H, Milde T, Witt O, Zhang C, Castelo-Branco P, Lichter P, Faury D, Tabori U, Plass C, Majewski J, Pfister SM, Jabado N. Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. Nature. 2012;482(7384):226-31; DOI:10.1038/nature10833.
68. Wu G, Broniscer A, McEachron TA, Lu C, Paugh BS, Becksfort J, Qu C, Ding L, Huether R, Parker M, Zhang J, Gajjar A, Dyer MA, Mullighan CG, Gilbertson RJ, Mardis ER, Wilson RK, Downing JR, Ellison DW, Zhang J, Baker SJ. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. Nat Genet. 2012;44:251-3; DOI:10.1038/ng.1102.
69. Fontebasso AM, Schwartzentruber J, Khuong-Quang DA, Liu XY, Sturm D, Korshunov A, Jones DTW, Witt H, Kool M, Albrecht S, Fleming A, Hadjadj D, Busche S, Lepage P, Montpetit A, Staffa A, Gerges N, Zakrzewska M, Zakrzewski K, Liberski PP, Hauser P, Garami M, Klekner A, Bognar L, Zadeh G, Faury D, Pfister SM, Jabado N, Majewski J. Mutations in SETD2 and genes affecting histone H3K36 methylation target hemispheric high-grade gliomas. Acta Neuropathol. 2013;125:659-69; DOI:10.1007/s00401-013-1095-8.
70. Portela A, Esteller M. Epigenetic modifications and human disease. Nat Biotechnol. 2010;28:1057-68; DOI:10.1038/nbt.1685.

71. Lambiv WL, Vassallo I, Delorenzi M, Shay T, Diserens AC, Misra A, Feuerstein B, Murat A, Migliavacca E, Hamou MF, Sciuscio D, Burger R, Domany E, Stupp R, Hegi ME. The Wnt inhibitory factor 1 (WIF1) is targeted in glioblastoma and has a tumor suppressing function potentially by induction of senescence. Neuro Oncol. 2011;13:736-47; DOI:10.1093/neuonc/nor036.
72. Götze S, Wolter M, Reifenberger G, Müller O, Sievers S. Frequent promoter hypermethylation of Wnt pathway inhibitor genes in malignant astrocytic gliomas. Int J Cancer. 2010;126:2584-93; DOI:10.1002/ijc.24981.
73. Ciriello G, Miller ML, Aksoy BA, Senbabaoglu Y, Schultz N, Sander C. Emerging landscape of oncogenic signatures across human cancers. Nat Genet. 2013;45:1127-33; DOI:10.1038/ng.2762.
74. Quillien V, Lavenu A, Karayan-Tapon L, Carpentier C, Labussière M, Lesimple T, Chinot O, Wager M, Honnorat J, Saikali S, Fina F, Sanson M, Figarella-Branger D. Comparative assessment of 5 methods (methylation-specific polymerase chain reaction, methylight, pyrosequencing, methylation-sensitive high-resolution melting, and immunohistochemistry) to analyze O6-methylguanine-DNA- methyltranferase in a series of 100 glioblastoma patients. Cancer. 2012;118:4201-11; DOI:10.1002/cncr.27392.
75. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, Bromberg JE, Hau P, Mirimanoff RO, Cairncross JG, Janzer RC, Stupp R. MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med. 2005;352(10):997-1003; DOI:10.1056/NEJMoa043331.
76. Esteller M, Hamilton SR, Burger PC, Baylin SB, Herman JG. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. Cancer Res. 1999;59(4):793-7.
77. Gerson SL. MGMT: its role in cancer aetiology and cancer therapeutics. Nat Rev Cancer. 2004;4:296-307; DOI:10.1038/nrc1319.
78. Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V, Baylin SB, Herman JG. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. N Engl J Med. 2000;343(19):1350-4; DOI:10.1056/NEJM200011093431901.
79. Ohgaki H, Kleihues P. The definition of primary and secondary glioblastoma. Clinical Cancer Research. 2013;19:764-72; DOI:10.1158/1078-0432.CCR-12-3002.
80. Bady P, Sciuscio D, Diserens AC, Bloch J, Van Den Bent MJ, Marosi C, Dietrich PY, Weller M, Mariani L, Heppner FL, Mcdonald DR, Lacombe D, Stupp R, Delorenzi M, Hegi ME. MGMT methylation analysis of glioblastoma on the Infinium methylation BeadChip identifies two distinct CpG regions associated with gene silencing and outcome, yielding a prediction model for comparisons across datasets, tumor grades, and CIMP-status. Acta Neuropathol. 2012;124:547-60; DOI:10.1007/s00401-012-1016-2.
81. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P, Ellison DW. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 2016;131:803-20; DOI:10.1007/s00401-016-1545-1.
82. Chen L, Voronovich Z, Clark K, Hands I, Mannas J, Walsh M, Nikiforova MN, Durbin EB, Weiss H, Horbinski C. Predicting the likelihood of an isocitrate dehydrogenase 1 or 2 mutation in diagnoses of infiltrative glioma. Neuro Oncol. 2014;16:1478-83; DOI:10.1093/neuonc/nou097.
83. Schindler G, Capper D, Meyer J, Janzarik W, Omran H, Herold-Mende C, Schmieder K, Wesseling P, Mawrin C, Hasselblatt M, Louis DN, Korshunov A, Pfister S, Hartmann C, Paulus W, Reifenberger G, Von Deimling A. Analysis of BRAF V600E mutation in 1,320 nervous system tumors reveals high mutation frequencies in pleomorphic xanthoastrocytoma, ganglioglioma and extra-cerebellar pilocytic astrocytoma. Acta Neuropathol. 2011;121:397-405; DOI:10.1007/s00401-011-0802-6.
84. Kleinschmidt-Demasters BK, Aisner DL, Birks DK, Foreman NK. Epithelioid GBMs show a high percentage of BRAF V600E mutation. Am J Surg Pathol. 2013;37:685-98; DOI:10.1097/PAS.0b013e31827f9c5e.
85. Chen AX, Gartrell RD, Zhao J, Upadhyayula PS, Zhao W, Yuan J, Minns HE, Dovas A, Bruce JN, Lasorella A, Iavarone A, Canoll P, Sims PA, Rabadan R. Single-cell characterization of macrophages in glioblastoma reveals MARCO as a mesenchymal pro-tumor marker. Genome Med. 2021;13:88; DOI:10.1186/s13073-021-00906-x.
86. Miao Y, Wang J, Li Q, Quan W, Wang Y, Li C, Wu J, Mi D. Prognostic value and immunological role of PDCD1 gene in pan-cancer. Int Immunopharmacol. 2020;89:107080; DOI:10.1016/j.intimp.2020.107080.
87. Bardhan K, Aksoylar H-I, Le Bourgeois T, Strauss L, Weaver JD, Delcuze B, Charest A, Patsoukis N, Boussiotis VA. Phosphorylation of PD-1-Y248 is a marker of PD-1-mediated inhibitory function in human T cells. Sci Rep. 2019;9:17252; DOI:10.1038/s41598-019-53463-0.
88. Qian W, Wang Q, Zhang C, Zhu J, Zhang Q, Luo C. M2 macrophage marker CHI3L2 could serve as a potential prognostic and immunological biomarker in glioma by integrated single-cell and bulk RNA-Seq analysis. J Gene Med. 2023;25:e3523; DOI:10.1002/jgm.3523.
89. Huang L, Wang Z, Chang Y, Wang K, Kang X, Huang R, Zhang Y, Chen J, Zeng F, Wu F, Zhao Z, Li G, Huang H, Jiang T, Hu H. EFEMP2 indicates assembly of M0 macrophage and more malignant phenotypes of glioma. Aging. 2020;12:8397-412; DOI:10.18632/aging.103147.
90. Chen Y, He J, Chen R, Wang Z, Dai Z, Liang X, Wu W, Luo P, Zhang J, Peng Y, Zhang N, Liu Z, Zhang L, Zhang H, Cheng Q. Pan-cancer analysis of the immunological role of PDIA5: a potential target for immunotherapy. Front Immunol. 2022;13:881722; DOI:10.3389/fimmu.2022.881722.
91. Mostafa H, Pala A, Högel J, Hlavac M, Dietrich E, Westhoff MA, Nonnenmacher L, Burster T, Georgieff M, Wirtz CR, Schneider EM. Immune phenotypes predict survival in patients with glioblastoma multiforme. J Hematol Oncol. 2016;9:77; DOI:10.1186/s13045-016-0272-3.
92. Zhou M, Bracci PM, McCoy LS, Hsuang G, Wiemels JL, Rice T, Zheng S, Kelsey KT, Wrensch MR, Wiencke JK. Serum macrophage-derived chemokine/CCL22 levels are associated with glioma risk, CD4 T cell lymphopenia and survival time. Int J Cancer. 2015;137:826-36; DOI:10.1002/ijc.29441.
93. Li D, Zhang Q, Li L, Chen K, Yang J, Dixit D, Gimple RC, Ci S, Lu C, Hu L, Gao J, Shan D, Li Y, Zhang J, Shi Z, Gu D, Yuan W, Wu Q, Yang K, Zhao L, Qiu Z, Lv D, Gao W, Yang H, Lin F, Wang Q, Man J, Li C, Tao W, Agnihotri S, Qian X, Shi Y, You Y, Zhang N, Rich JN, Wang X. β2-Microglobulin maintains glioblastoma stem cells and induces M2-like polarization of tumor-associated macrophages. Cancer Res. 2022;82:3321-34; DOI:10.1158/0008-5472.CAN-22-0507.
94. Kyurkchiev D, Naydenov E, Tumangelova-Yuzeir K, Ivanova-Todorova E, Belemezova K, Bochev I, Minkin K, Mourdjeva M, Velikova T, Nachev S, Kyurkchiev S. Cells isolated from human glioblastoma multiforme express progesterone-induced blocking factor (PIBF). Cell Mol Neurobiol. 2014;34:479-89; DOI:10.1007/s10571-014-0031-3.
95. Belghali MY, Admou B, Brahimi M, Khouchani M, Ba-M'hamed S. Immune tumoral microenvironment in gliomas: focus on CD3+ T cells, Vδ1+ T cells, and microglia/macrophages. Immunol Res. 2022;70:224-39; DOI:10.1007/s12026-022-09260-5.
96. Huff WX, Kwon JH, Henriquez M, Fetcko K, Dey M. The evolving role of CD8+CD28- immunosenescent T cells in cancer immunology. Int J Mol Sci. 2019;20; DOI:10.3390/ijms20112810.
97. Jarmuzek P, Kozlowska K, Defort P, Kot M, Zembron-Lacny A. Prognostic values of systemic inflammatory immunological markers in glioblastoma: a systematic review and meta-analysis. Cancers (Basel). 2023;15; DOI:10.3390/cancers15133339.
98. Czapski B, Baluszek S, Herold-Mende C, Kaminska B. Clinical and immunological correlates of long term survival in glioblastoma. Contemp Oncol (Pozn). 2018;22(1A):81-5; DOI:10.5114/wo.2018.73893.
99. Zhang Y, Wen Y, Nie J, Wang T, Wang G, Gao Q, Cao Y, Wang H, Qi S, Xie S. MYEF2: an immune infiltration-related prognostic factor in IDH--wild-type glioblastoma. Aging. 2023;15:7760-80; DOI:10.18632/aging.204939.
100. Dhinakaran AK, Dharmalingam P, Ganesh S, Venkatakrishnan K, Das S, Tan B. Molecular crosstalk between T cells and tumor uncovers GBM-Specific T cell signatures in blood: noninvasive GBM diagnosis using immunosensors. ACS Nano. 2022;16:14134-48; DOI:10.1021/acsnano.2c04160.
101. Ladomersky E, Scholtens DM, Kocherginsky M, Hibler EA, Bartom ET, Otto-Meyer S, Zhai L, Lauing KL, Choi J, Sosman JA, Wu JD, Zhang B, Lukas R V, Wainwright DA. The coincidence between increasing age, immunosuppression, and the incidence of patients with glioblastoma. Front Pharmacol. 2019;10:200; DOI:10.3389/fphar.2019.00200.
102. Kamran N, Chandran M, Lowenstein PR, Castro MG. Immature myeloid cells in the tumor microenvironment: implications for immunotherapy. Clin Immunol. 2018;189:34-42; DOI:10.1016/j.clim.2016.10.008.
103. Perng P, Lim M. Immunosuppressive mechanisms of malignant gliomas: parallels at non-CNS sites. Front Oncol. 2015;5:153; DOI:10.3389/fonc.2015.00153.
104. Grabowski MM, Sankey EW, Ryan KJ, Chongsathidkiet P, Lorrey SJ, Wilkinson DS, Fecci PE. Immune suppression in gliomas. J Neurooncol. 2021;151:3-12; DOI:10.1007/s11060-020-03483-y.
105. Moertel CL, Xia J, LaRue R, Waldron NN, Andersen BM, Prins RM, Okada H, Donson AM, Foreman NK, Hunt MA, Pennell CA, Olin MR. CD200 in CNS tumor-induced immunosuppression: the role for CD200 pathway blockade in targeted immunotherapy. J Immunother Cancer. 2014;2:46; DOI:10.1186/s40425-014-0046-9.
106. Campian JL, Ghosh S, Kapoor V, Yan R, Thotala S, Jash A, Hu T, Mahadevan A, Rifai K, Page L, Lee BH, Ferrando-Martinez S, Wolfarth AA, Yang SH, Hallahan D, Chheda MG, Thotala D. Long-acting recombi-

nant human interleukin-7, NT-I7, increases cytotoxic CD8 T cells and enhances survival in mouse glioma models. Clin Cancer Res. 2022;28:1229-39; DOI:10.1158/1078-0432.CCR-21-0947.
107. Ghosh S, Huang J, Inkman M, Zhang J, Thotala S, Tikhonova E, Miheecheva N, Frenkel F, Ataullakhanov R, Wang X, DeNardo D, Hallahan D, Thotala D. Radiation-induced circulating myeloid-derived suppressor cells induce systemic lymphopenia after chemoradiotherapy in patients with glioblastoma. Sci Transl Med. 2023;15:eabn6758; DOI:10.1126/scitranslmed.abn6758.
108. Chistiakov DA, Chekhonin I V, Gurina OI, Bobryshev Y V, Chekhonin VP. Approaches to improve efficiency of dendritic cell-based therapy of high grade gliomas. Curr Pharm Des. 2016;22:5738-51; DOI:10.2174/1381612822666160719110618.
109. Yang F, Zhang D, Jiang H, Ye J, Zhang L, Bagley SJ, Winkler J, Gong Y, Fan Y. Small-molecule toosendanin reverses macrophage-mediated immunosuppression to overcome glioblastoma resistance to immunotherapy. Sci Transl Med. 2023;15:eabq3558; DOI:10.1126/scitranslmed.abq3558.
110. Omuro A, DeAngelis LM. Glioblastoma and other malignant gliomas: A Clinical Review. JAMA. 2013;310:1842-50; DOI:10.1001/JAMA.2013.280319.
111. Manini I, Caponnetto F, Bartolini A, Ius T, Mariuzzi L, Loreto C Di, Beltrami AP, Cesselli D. Role of microenvironment in glioma invasion: what we learned from in vitro models. Int J Mol Sci. 2018;19(1):147; DOI:10.3390/ijms19010147.
112. Klemm F, Maas RR, Bowman RL, Kornete M, Soukup K, Nassiri S, Brouland JP, Iacobuzio-Donahue CA, Brennan C, Tabar V, Gutin PH, Daniel RT, Hegi ME, Joyce JA. Interrogation of the microenvironmental landscape in brain tumors reveals disease-specific alterations of immune cells. Cell. 2020;181:1643-1660.e17; DOI:10.1016/J.CELL.2020.05.007.
113. Dapash M, Hou D, Castro B, Lee-Chang C, Lesniak MS. The interplay between glioblastoma and its microenvironment. Cells. 2021;10(9):2257; DOI:10.3390/cells10092257.
114. Schiffer D, Annovazzi L, Casalone C, Corona C, Mellai M. Glioblastoma: microenvironment and niche concept. Cancers (Basel). 2018;11(1):5; DOI:10.3390/cancers11010005.
115. Liang J, Piao Y, Holmes L, Fuller GN, Henry V, Tiao N, de Groot JF. Neutrophils promote the malignant glioma phenotype through S100A4. Clin Cancer Res. 2014;20(1):187-98; DOI:10.1158/1078-0432.CCR-13-1279.
116. Kohanbash G, Okada H. Myeloid-derived suppressor cells (MDSCs) in gliomas and glioma-development. Immunol Invest. 2012;41:658-79; DOI:10.3109/08820139.2012.689591.
117. Feng X, Szulzewsky F, Yerevanian A, Chen Z, Heinzmann D, Rasmussen RD, Alvarez-Garcia V, Kim Y, Wang B, Tamagno I, Zhou H, Li X, Kettenmann H, Ransohoff RM, Hambardzumyan D. Loss of CX3CR1 increases accumulation of inflammatory monocytes and promotes gliomagenesis. Oncotarget. 2015;6:15077; DOI:10.18632/ONCOTARGET.3730.
118. Ho IAW, Shim WSN. Contribution of the microenvironmental niche to glioblastoma heterogeneity. Biomed Res Int. 2017;2017; DOI:10.1155/2017/9634172.
119. Mineharu Y, Castro MG, Lowenstein PR, Sakai N, Miyamoto S. Dendritic cell-based immunotherapy for glioma: multiple regimens and implications in clinical trials. Neurol Med Chir (Tokyo). 2013;53(11):741-54; DOI:10.2176/nmc.ra2013-0234.
120. DeCordova S, Shastri A, Tsolaki AG, Yasmin H, Klein L, Singh SK, Kishore U. Molecular heterogeneity and immunosuppressive microenvironment in glioblastoma. Front Immunol. 2020;11:1402; DOI:10.3389/fimmu.2020.01402.
121. Perus LJM, Walsh LA. Microenvironmental heterogeneity in brain malignancies. Front Immunol. 2019;10:2294; DOI:10.3389/fimmu.2019.02294.
122. Kane JR. The role of brain vasculature in glioblastoma. Mol Neurobiol. 2019;56:6645-53; DOI:10.1007/S12035-019-1561-Y.
123. Hardee ME, Zagzag D. Mechanisms of glioma-associated neovascularization. Am J Pathol. 2012;181:1126-41; DOI:10.1016/J.AJPATH.2012.06.030.
124. Dimberg A. The glioblastoma vasculature as a target for cancer therapy. Biochem Soc Trans. 2014;42:1647-52; DOI:10.1042/BST20140278.
125. Dudley AC, Griffioen AW. Pathological angiogenesis: mechanisms and therapeutic strategies. Angiogenesis. 2023;26:313-47; DOI:10.1007/S10456-023-09876-7.
126. Yang L, Lin C, Wang L, Guo H, Wang X. Hypoxia and hypoxia-inducible factors in glioblastoma multiforme progression and therapeutic implications. Exp Cell Res. 2012;318:2417-26; DOI:10.1016/J.YEXCR.2012.07.017.
127. Monteiro AR, Hill R, Pilkington GJ, Madureira PA. The role of hypoxia in glioblastoma invasion. Cells. 2017;6(4):45; DOI:10.3390/cells6040045.
128. Thomlinson RH, Gray LH. The histological structure of some human lung cancers and the possible implications for radiotherapy. Br J Cancer. 1955;9:539-49; DOI:10.1038/BJC.1955.55.
129. Park JH, Lee HK. Current understanding of hypoxia in glioblastoma multiforme and its response to immunotherapy. Cancers (Basel). 2022;14(5):1176; DOI:10.3390/cancers14051176.
130. Kaur B, Khwaja FW, Severson EA, Matheny SL, Brat DJ, Van Meir EG. Hypoxia and the hypoxia-inducible-factor pathway in glioma growth and angiogenesis. Neuro Oncol. 2005;7:134-53; DOI:10.1215/S1152851704001115.
131. Zagzag D, Lukyanov Y, Lan L, Ali MA, Esencay M, Mendez O, Yee H, Voura EB, Newcomb EW. Hypoxia-inducible factor 1 and VEGF upregulate CXCR4 in glioblastoma: implications for angiogenesis and glioma cell invasion. Lab Invest. 2006;86:1221-32; DOI:10.1038/labinvest.3700482.
132. Papale M, Buccarelli M, Mollinari C, Russo MA, Pallini R, Ricci-Vitiani L, Tafani M. Hypoxia, Inflammation and necrosis as determinants of glioblastoma cancer stem cells progression. Int J Mol Sci. 2020;21(8):2660; DOI:10.3390/ijms21082660.
133. Brat DJ, Castellano-Sanchez AA, Hunter SB, Pecot M, Cohen C, Hammond EH, Devi SN, Kaur B, Van Meir EG. Pseudopalisades in glioblastoma are hypoxic, express extracellular matrix proteases, and are formed by an actively migrating cell population. Cancer Res. 2004;64:920-7; DOI:10.1158/0008-5472.CAN-03-2073.
134. Rong Y, Durden DL, Van Meir EG, Brat DJ. "Pseudopalisading" necrosis in glioblastoma: a familiar morphologic feature that links vascular pathology, hypoxia, and angiogenesis. J Neuropathol Exp Neurol. 2006;65:529-39; DOI:10.1097/00005072-200606000-00001.
135. Pang L, Dunterman M, Xuan W, Gonzalez A, Lin Y, Hsu WH, Khan F, Hagan RS, Muller WA, Heimberger AB, Chen P. Circadian regulator CLOCK promotes tumor angiogenesis in glioblastoma. Cell Rep. 2023;42(2):112127; DOI:10.1016/j.celrep.2023.112127.
136. Dalmay T, Edwards DR. MicroRNAs and the hallmarks of cancer. Oncogene. 2006;25:6170-5; DOI:10.1038/SJ.ONC.1209911.
137. Balandeh E, Mohammadshafie K, Mahmoudi Y, Hossein Pourhanifeh M, Rajabi A, Bahabadi ZR, Mohammadi AH, Rahimian N, Hamblin MR, Mirzaei H. Roles of non-coding RNAs and angiogenesis in glioblastoma. Front Cell Dev Biol. 2021;9:716462; DOI:10.3389/FCELL.2021.716462.
138. Li D, Zhang Z, Xia C, Niu C, Zhou W. Non-coding RNAs in Glioma microenvironment and angiogenesis. Front Mol Neurosci. 2021;14:763610; DOI:10.3389/FNMOL.2021.763610.
139. Jiang Y, Zhao J, Xu J, Zhang H, Zhou J, Li H, Zhang G, Xu K, Jing Z. Glioblastoma-associated microglia-derived exosomal circKIF18A promotes angiogenesis by targeting FOXC2. Oncogene. 2022;41:3461-73; DOI:10.1038/S41388-022-02360-4.
140. Bae E, Huang P, Müller-Greven G, Hambardzumyan D, Sloan AE, Nowacki AS, Marko N, Carlin CR, Gladson CL. Integrin α3β1 promotes vessel formation of glioblastoma-associated endothelial cells through calcium-mediated macropinocytosis and lysosomal exocytosis. Nat Commun. 2022;13(1):4268; DOI:10.1038/S41467-022-31981-2.
141. Carruthers RD, Ahmed SU, Ramachandran S, Strathdee K, Kurian KM, Hedley A, Gomez-Roman N, Kalna G, Neilson M, Gilmour L, Stevenson KH, Hammond EM, Chalmers AJ. Replication stress drives constitutive activation of the DNA damage response and radioresistance in glioblastoma stem-like cells. Cancer Res. 2018;78(17):5060-71; DOI:10.1158/0008-5472.CAN-18-0569/653606.
142. Biserova K, Jakovlevs A, Uljanovs R, Strumfa I. Cancer stem cells: significance in origin, pathogenesis and treatment of glioblastoma. Cells. 2021;10(3):621; DOI:10.3390/cells10030621.
143. Hambardzumyan D, Becher OJ, Rosenblum MK, Pandolfi PP, Manova-Todorova K, Holland EC. PI3K pathway regulates survival of cancer stem cells residing in the perivascular niche following radiation in medulloblastoma in vivo. Genes Dev. 2008;22(4):436-48; DOI:10.1101/gad.1627008.
144. Schiffer D, Annovazzi L, Mazzucco M, Mellai M. The microenvironment in gliomas: phenotypic expressions. Cancers (Basel). 2015;7(4):2352-9; DOI:10.3390/cancers7040896.
145. Charles NA, Holland EC, Gilbertson R, Glass R, Kettenmann H. The brain tumor microenvironment. Glia. 2012;60(3):502-14; DOI:10.1002/GLIA.21264.
146. Hira VVV, Wormer JR, Kakar H, Breznik B, van der Swaan B, Hulsbos R, Tigchelaar W, Tonar Z, Khurshed M, Molenaar RJ, Van Noorden CJF. Periarteriolar glioblastoma stem cell niches express bone mar-

row hematopoietic stem cell niche proteins. J Histochem Cytochem. 2018;66(3):155-173; DOI:10.1369/0022155417749174.
147. Guardia GDA, Correa BR, Araujo PR, Qiao M, Burns S, Penalva LOF, Galante PAF. Proneural and mesenchymal glioma stem cells display major differences in splicing and lncRNA profiles. NPJ Genom Med. 2020;5:2; DOI:10.1038/s41525-019-0108-5.
148. Deshors P, Toulas C, Arnauduc F, Malric L, Siegfried A, Nicaise Y, Lemarié A, Larrieu D, Tosolini M, Cohen-Jonathan Moyal E, Courtade-Saidi M, Evrard SM. Ionizing radiation induces endothelial transdifferentiation of glioblastoma stem-like cells through the Tie2 signaling pathway. Cell Death Dis. 2019;10(11):816; DOI:10.1038/s41419-019-2055-6.
149. Wu A, Wei J, Kong LY, Wang Y, Priebe W, Qiao W, Sawaya R, Heimberger AB. Glioma cancer stem cells induce immunosuppressive macrophages/microglia. Neuro Oncol. 2010;12(11):1113-25; DOI:10.1093/NEUONC/NOQ082.
150. Bikfalvi A, da Costa CA, Avril T, Barnier JV, Bauchet L, Brisson L, Cartron PF, Castel H, Chevet E, Chneiweiss H, Clavreul A, Constantin B, Coronas V, Daubon T, Dontenwill M, Ducray F, Enz-Werle N, Figarella-Branger D, Fournier I, Frenel JS, Gabut M, Galli T, Gavard J, Huberfeld G, Hugnot JP, Idbaih A, Junier MP, Mathivet T, Menei P, Meyronet D, Mirjolet C, Morin F, Mosser J, Moyal EC, Rousseau V, Salzet M, Sanson M, Seano G, Tabouret E, Tchoghandjian A, Turchi L, Vallette FM, Vats S, Verreault M, Virolle T. Challenges in glioblastoma research: focus on the tumor microenvironment. Trends Cancer. 2023;9(1):9-27; DOI:10.1016/j.trecan.2022.09.005.
151. Broekman ML, Maas SLN, Abels ER, Mempel TR, Krichevsky AM, Breakefield XO. Multidimensional communication in the microenvirons of glioblastoma. Nat Rev Neurol. 2018;14:482-95; DOI:10.1038/S41582-018-0025-8.
152. Khan F, Pang L, Dunterman M, Lesniak MS, Heimberger AB, Chen P. Macrophages and microglia in glioblastoma: heterogeneity, plasticity, and therapy. J Clin Invest. 2023;133; DOI:10.1172/JCI163446.
153. Hambardzumyan D, Bergers G. Glioblastoma: defining tumor niches. Trends Cancer. 2015;1(4):252-65; DOI:10.1016/j.trecan.2015.10.009.
154. Hambardzumyan D, Gutmann DH, Kettenmann H. The role of microglia and macrophages in glioma maintenance and progression. Nat Neurosci. 2016;19(1):20-7; DOI:10.1038/nn.4185.
155. Morantz RA, Wood GW, Foster M, Clark M, Gollahon K. Macrophages in experimental and human brain tumors. Part 1: studies of the macrophage content of experimental rat brain tumors of varying immunogenicity. J Neurosurg. 1979;50(3):298-304; DOI:10.3171/jns.1979.50.3.0298.
156. Gutmann DH, Kettenmann H. Microglia/brain macrophages as central drivers of brain tumor pathobiology. Neuron. 2019;104:442-9; DOI:10.1016/j.neuron.2019.08.028.
157. Wu M, Wu L, Wu W, Zhu M, Li J, Wang Z, Li J, Ding R, Liang Y, Li L, Zhang T, Huang B, Cai Y, Li K, Li L, Zhang R, Hu B, Lin F, Wang X, Zheng S, Chen J, You Y, Jiang T, Zhang J, Chen H, Wang Q. Phagocytosis of glioma cells enhances the immunosuppressive phenotype of bone marrow-derived macrophages. Cancer Res. 2023;83:771-85; DOI:10.1158/0008-5472.CAN-22-1570.
158. Pan Z, Zhao R, Li B, Qi Y, Qiu W, Guo Q, Zhang S, Zhao S, Xu H, Li M, Gao Z, Fan Y, Xu J, Wang H, Wang S, Qiu J, Wang Q, Guo X, Deng L, Zhang P, Xue H, Li G. EWSR1-induced circNEIL3 promotes glioma progression and exosome-mediated macrophage immunosuppressive polarization via stabilizing IGF2BP3. Mol Cancer. 2022;21(1):16; DOI:10.1186/S12943-021-01485-6.
159. Xu J, Zhang J, Zhang Z, Gao Z, Qi Y, Qiu W, Pan Z, Guo Q, Li B, Zhao S, Guo X, Qian M, Chen Z, Wang S, Gao X, Zhang S, Wang H, Guo X, Zhang P, Zhao R, Xue H, Li G. Hypoxic glioma-derived exosomes promote M2-like macrophage polarization by enhancing autophagy induction. Cell Death Dis. 2021;12(4):373; DOI:10.1038/S41419-021-03664-1.
160. Liu T, Zhu C, Chen X, Guan G, Zou C, Shen S, Wu J, Wang Y, Lin Z, Chen L, Cheng P, Cheng W, Wu A. Ferroptosis, as the most enriched programmed cell death process in glioma, induces immunosuppression and immunotherapy resistance. Neuro Oncol. 2022;24:1113-25; DOI:10.1093/NEUONC/NOAC033.
161. Yu K, Lin CCJ, Hatcher A, Lozzi B, Kong K, Huang-Hobbs E, Cheng YT, Beechar VB, Zhu W, Zhang Y, Chen F, Mills GB, Mohila CA, Creighton CJ, Noebels JL, Scott KL, Deneen B. PIK3CA variants selectively initiate brain hyperactivity during gliomagenesis. Nature. 2020;578:166-71; DOI:10.1038/s41586-020-1952-2.
162. Krishna S, Choudhury A, Keough MB, Seo K, Ni L, Kakaizada S, Lee A, Aabedi A, Popova G, Lipkin B, Cao C, Nava Gonzales C, Sudharshan R, Egladyous A, Almeida N, Zhang Y, Molinaro AM, Venkatesh HS, Daniel AGS, Shamardani K, Hyer J, Chang EF, Findlay A, Phillips JJ, Nagarajan S, Raleigh DR, Brang D, Monje M, Hervey-Jumper SL. Glioblastoma remodelling of human neural circuits decreases survival. Nature. 2023;617:599-607; DOI:10.1038/S41586-023-06036-1.
163. Pan Y, Hysinger JD, Barron T, Schindler NF, Cobb O, Guo X, Yalçın B, Anastasaki C, Mulinyawe SB, Ponnuswami A, Scheaffer S, Ma Y, Chang KC, Xia X, Toonen JA, Lennon JJ, Gibson EM, Huguenard JR, Liau LM, Goldberg JL, Monje M, Gutmann DH. NF1 mutation drives neuronal activity-dependent initiation of optic glioma. Nature. 2021;594:277-82; DOI:10.1038/s41586-021-03580-6.
164. Curry RN, Aiba I, Meyer J, Lozzi B, Ko Y, McDonald MF, Rosenbaum A, Cervantes A, Huang-Hobbs E, Cocito C, Greenfield JP, Jalali A, Gavvala J, Mohila C, Serin Harmanci A, Noebels J, Rao G, Deneen B. Glioma epileptiform activity and progression are driven by IGSF3-mediated potassium dysregulation. Neuron. 2023;111:682-95; DOI:10.1016/j.neuron.2023.01.013.
165. Venkatesh HS, Johung TB, Caretti V, Noll A, Tang Y, Nagaraja S, Gibson EM, Mount CW, Polepalli J, Mitra SS, Woo PJ, Malenka RC, Vogel H, Bredel M, Mallick P, Monje M. Neuronal activity promotes glioma growth through neuroligin-3 secretion. Cell. 2015;161:803-16; DOI:10.1016/j.cell.2015.04.012.
166. Venkatesh HS, Morishita W, Geraghty AC, Silverbush D, Gillespie SM, Arzt M, Tam LT, Espenel C, Ponnuswami A, Ni L, Woo PJ, Taylor KR, Agarwal A, Regev A, Brang D, Vogel H, Hervey-Jumper S, Bergles DE, Suvà ML, Malenka RC, Monje M. Electrical and synaptic integration of glioma into neural circuits. Nature. 2019;573:539-45; DOI:10.1038/s41586-019-1563-y.
167. Hua T, Shi H, Zhu M, Chen C, Su Y, Wen S, Zhang X, Chen J, Huang Q, Wang H. Glioma-neuronal interactions in tumor progression: mechanism, therapeutic strategies and perspectives (review). Int J Oncol. 2022;61(3):104; DOI:10.3892/IJO.2022.5394.
168. Corsi L, Mescola A, Alessandrini A. Glutamate receptors and glioblastoma multiforme: An old "route" for new perspectives. Int J Mol Sci. 2019;20(7):1796; DOI:10.3390/ijms20071796.
169. Mollazadeh H, Mohtashami E, Mousavi SH, Soukhtanloo M, Vahedi MM, Hosseini A, Afshari AR, Sahebkar A. Deciphering the role of glutamate signaling in glioblastoma multiforme: current therapeutic modalities and future directions. Curr Pharm Des. 2020;26:4777-88; DOI :10.2174/1381612826666200603132456.
170. So J-S, Kim H, Han K-S. Mechanisms of invasion in glioblastoma: extracellular matrix, Ca2+ signaling, and glutamate. Front Cell Neurosci. 2021;15:663092; DOI:10.3389/fncel.2021.663092.
171. Venkataramani V, Tanev DI, Strahle C, Studier-Fischer A, Fankhauser L, Kessler T, Körber C, Kardorff M, Ratliff M, Xie R, Horstmann H, Messer M, Paik SP, Knabbe J, Sahm F, Kurz FT, Acikgöz AA, Herrmannsdörfer F, Agarwal A, Bergles DE, Chalmers A, Miletic H, Turcan S, Mawrin C, Hänggi D, Liu HK, Wick W, Winkler F, Kuner T. Glutamatergic synaptic input to glioma cells drives brain tumour progression. Nature. 2019;573:532-8; DOI:10.1038/S41586-019-1564-X.
172. Osswald M, Jung E, Sahm F, Solecki G, Venkataramani V, Blaes J, Weil S, Horstmann H, Wiestler B, Syed M, Huang L, Ratliff M, Karimian Jazi K, Kurz FT, Schmenger T, Lemke D, Gömmel M, Pauli M, Liao Y, Häring P, Pusch S, Herl V, Steinhäuser C, Krunic D, Jarahian M, Miletic H, Berghoff AS, Griesbeck O, Kalamakis G, Garaschuk O, Preusser M, Weiss S, Liu H, Heiland S, Platten M, Huber PE, Kuner T, Von Deimling A, Wick W, Winkler F. Brain tumour cells interconnect to a functional and resistant network. Nature. 2015;528:93-8; DOI:10.1038/NATURE16071.
173. Venkataramani V, Yang Y, Schubert MC, Reyhan E, Tetzlaff SK, Wißmann N, Botz M, Soyka SJ, Beretta CA, Pramatarov RL, Fankhauser L, Garofano L, Freudenberg A, Wagner J, Tanev DI, Ratliff M, Xie R, Kessler T, Hoffmann DC, Hai L, Dörflinger Y, Hoppe S, Yabo YA, Golebiewska A, Niclou SP, Sahm F, Lasorella A, Slowik M, Döring L, Iavarone A, Wick W, Kuner T, Winkler F. Glioblastoma hijacks neuronal mechanisms for brain invasion. Cell. 2022;185:2899-917.e31; DOI:10.1016/J.CELL.2022.06.054.
174. Huang-Hobbs E, Cheng YT, Ko Y, Luna-Figueroa E, Lozzi B, Taylor KR, McDonald M, He P, Chen HC, Yang Y, Maleki E, Lee ZF, Murali S, Williamson MR, Choi D, Curry R, Bayley J, Woo J, Jalali A, Monje M, Noebels JL, Harmanci AS, Rao G, Deneen B. Remote neuronal activity drives glioma progression through SEMA4F. Nature. 2023;619:844-50; DOI:10.1038/S41586-023-06267-2.
175. Wang C, Zhao Q, Zheng X, Li S, Chen J, Zhao H, Chen F, Cui L, Li W. Decellularized brain extracellular matrix slice glioblastoma culture model recapitulates the interaction between cells and the extracellular matrix without a nutrient-oxygen gradient interference. Acta Biomater. 2023;158:132-150; DOI:10.1016/j.actbio.2022.12.044.
176. Marino S, Menna G, Di Bonaventura R, Lisi L, Mattogno P, Figà F, Bilgin L, D'Alessandris QG, Olivi A, Della Pepa GM. The extracellular matrix in glioblastomas: a glance at its structural modifications in shaping

the tumoral microenvironment– a systematic review. Cancers (Basel). 2023;15(6):1879; DOI:10.3390/cancers15061879.
177. Pibuel MA, Poodts D, Díaz M, Hajos SE, Lompardía SL. The scrambled story between hyaluronan and glioblastoma. J Biol Chem. 2021;296:100549; DOI:10.1016/J.JBC.2021.100549.
178. von Spreckelsen N, Fadzen CM, Hartrampf N, Ghotmi Y, Wolfe JM, Dubey S, Yang BY, Kijewski MF, Wang S, Farquhar C, Bergmann S, Zdioruk M, Wasserburg JR, Scott B, Murrell E, Bononi FC, Luyt LG, DiCarli M, Lamfers MLM, Ligon KL, Chiocca EA, Viapiano MS, Pentelute BL, Lawler SE, Cho CF. Targeting glioblastoma using a novel peptide specific to a deglycosylated isoform of brevican. Adv Ther (Weinh). 2021;4(4):2000244; DOI:10.1002/ADTP.202000244.
179. Angel I, Pilo Kerman O, Rousso-Noori L, Friedmann-Morvinski D. Tenascin C promotes cancer cell plasticity in mesenchymal glioblastoma. Oncogene. 2020;39:6990-7004; DOI:10.1038/S41388-020-01506-6.
180. Wu S, Liu C, Wei X, Nong WX, Lin LN, Li F, Xie XX, Liao XS, Luo B, Zhang QM, Xiao SW. High expression of fibronectin 1 predicts a poor prognosis in glioblastoma. Curr Med Sci. 2022;42(5):1055-65; DOI:10.1007/s11596-022-2638-9.
181. Joseph JV, Magaut CR, Storevik S, Geraldo LH, Mathivet T, Latif MA, Rudewicz J, Guyon J, Gambaretti M, Haukas F, Trones A, Rømo Ystaas LA, Hossain JA, Ninzima S, Cuvellier S, Zhou W, Tomar T, Klink B, Rane L, Irving BK, Marrison J, O'Toole P, Wurdak H, Wang J, Di Z, Birkeland E, Berven FS, Winkler F, Kruyt FAE, Bikfalvi A, Bjerkvig R, Daubon T, Miletic H. TGF-β promotes microtube formation in glioblastoma through thrombospondin 1. Neuro Oncol. 2022;24:541-53; DOI:10.1093/NEUONC/NOAB212.
182. Fu Z, Zhu G, Luo C, Chen Z, Dou Z, Chen Y, Zhong C, Su S, Liu F. Matricellular protein tenascin C: Implications in glioma progression, gliomagenesis, and treatment. Front Oncol. 2022;12:971462; DOI:10.3389/FONC.2022.971462.
183. Mooney KL, Choy W, Sidhu S, Pelargos P, Bui TT, Voth B, Barnette N, Yang I. The role of CD44 in glioblastoma multiforme. J Clin Neurosci. 2016;34:1-5; DOI:10.1016/J.JOCN.2016.05.012.
184. Tews DS. Adhesive and invasive features in gliomas. Pathol Res Pract. 2000;196:701-11; DOI:10.1016/S0344-0338(00)80122-3.
185. Ellert-Miklaszewska A, Poleszak K, Pasierbinska M, Kaminska B. Integrin signaling in glioma pathogenesis: from biology to therapy. Int J Mol Sci. 2020;21(3):888; DOI:10.3390/ijms21030888.
186. Hatoum A, Mohammed R, Zakieh O. The unique invasiveness of glioblastoma and possible drug targets on extracellular matrix. Cancer Manag Res. 2019;11:1843-55; DOI:10.2147/CMAR.S186142.
187. Quesnel A, Karagiannis GS, Filippou PS. Extracellular proteolysis in glioblastoma progression and therapeutics. Biochim Biophys Acta Rev Cancer. 2020;1874(2):188428; DOI:10.1016/J.BBCAN.2020.188428.
188. Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol. 2001;17:463-516; DOI:10.1146/ANNUREV.CELLBIO.17.1.463.
189. Mason SD, Joyce JA. Proteolytic networks in cancer. Trends Cell Biol. 2011;21:228-37; DOI:10.1016/J.TCB.2010.12.002.
190. Vizovisek M, Ristanovic D, Menghini S, Christiansen MG, Schuerle S. The tumor proteolytic landscape: A challenging frontier in cancer diagnosis and therapy. Int J Mol Sci. 2021;22:1-30; DOI:10.3390/IJMS22052514.
191. Mohamed MM, Sloane BF. Cysteine cathepsins: multifunctional enzymes in cancer. Nat Rev Cancer. 2006;6:764-75; DOI:10.1038/NRC1949.
192. Breznik B, Motaln H, Turnšek TL. Proteases and cytokines as mediators of interactions between cancer and stromal cells in tumours. Biol Chem. 2017;398:709-19; DOI:10.1515/HSZ-2016-0283.
193. Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. Cell. 2010;141:52-67; DOI:10.1016/J.CELL.2010.03.015.
194. Djediai S, Gonzalez Suarez N, El Cheikh-Hussein L, Rodriguez Torres S, Gresseau L, Dhayne S, Joly-lopez Z, Annabi B. MT1-MMP cooperates with TGF-β receptor-mediated signaling to trigger SNAIL and induce epithelial-to-mesenchymal-like transition in U87 glioblastoma cells. Int J Mol Sci. 2021;22(23):13006; DOI:10.3390/IJMS222313006.
195. Zhai Y, Sang W, Su L, Shen Y, Hu Y, Zhang W. Analysis of the expression and prognostic value of MT1-MMP, β1-integrin and YAP1 in glioma. Open Med (Wars). 2022;17(1):492-507; DOI:10.1515/med-2022-0449.
196. Kanderi T, Munakomi S, Gupta V. Glioblastoma multiforme [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 [cited 2024 Feb 12]. 36 p. Available from: https://www.ncbi.nlm.nih.gov/books/NBK558954/.
197. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJB, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005;352(10):987-96; DOI:10.1056/nejmoa043330.
198. Koshy M, Villano JL, Dolecek TA, Howard A, Mahmood U, Chmura SJ, Weichselbaum RR, McCarthy BJ. Improved survival time trends for glioblastoma using the SEER 17 population-based registries. J Neurooncol. 2012;107(1):207-12; DOI:10.1007/s11060-011-0738-7.
199. Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V, Baylin SB, Herman JG. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents . N Engl J Med. 2000;343(19):1350-4; DOI:10.1056/nejm200011093431901.
200. Songtao Q, Lei Y, Si G, Yanqing D, Huixia H, Xuelin Z, Lanxiao W, Fei Y. IDH mutations predict longer survival and response to temozolomide in secondary glioblastoma. Cancer Sci. 2012;103(2):269-73; DOI:10.1111/j.1349-7006.2011.02134.x.
201. Weller M, Le Rhun E. How did lomustine become standard of care in recurrent glioblastoma? Cancer Treat Rev. 2020;87:102029; DOI:10.1016/j.ctrv.2020.102029.
202. Kaina B, Christmann M. DNA repair in personalized brain cancer therapy with temozolomide and nitrosoureas. DNA Repair (Amst). 2019;78:128-141; DOI:10.1016/j.dnarep.2019.04.007.
203. Wang EJ, Chen JS, Jain S, Morshed RA, Haddad AF, Gill S, Beniwal AS, Aghi MK. Immunotherapy resistance in glioblastoma. Front Genet. 2021;12:750675; DOI:10.3389/fgene.2021.750675.
204. Lee SY. Temozolomide resistance in glioblastoma multiforme. Genes Dis. 2016;3(3):198-210; DOI:10.1016/j.gendis.2016.04.007.
205. Dymova MA, Kuligina E V., Richter VA. Molecular mechanisms of drug resistance in glioblastoma. Int J Mol Sci. 2021;22(12):6385; DOI:10.3390/ijms22126385.
206. Mattei V, Santilli F, Martellucci S, Monache SD, Fabrizi J, Colapietro A, Angelucci A, Festuccia C. The importance of tumor stem cells in glioblastoma resistance to therapy. Int J Mol Sci. 2021;22(8):3863; DOI:10.3390/ijms22083863.
207. Ou A, Alfred Yung WK, Majd N. Molecular mechanisms of treatment resistance in glioblastoma. Int J Mol Sci. 2021;22(1):351; DOI:10.3390/ijms22010351.
208. Pappenheimer JR, Renkin EM, Borrero LM. Filtration, diffusion and molecular sieving through peripheral capillary membranes; a contribution to the pore theory of capillary permeability. Am J Physiol. 1951;167(1):13-46; DOI:10.1152/ajplegacy.1951.167.1.13.
209. Ueda K, Cornwell MM, Gottesman MM, Pastan I, Roninson IB, Ling V, Riordan JR. The mdrl gene, responsible for multidrug-resistance, codes for P-glycoprotein. Biochem Biophys Res Commun. 1986;141(3):956-62; DOI:10.1016/S0006-291X(86)80136-X.
210. Kizilbash SH, Gupta SK, Chang K, Kawashima R, Parrish KE, Carlson BL, Bakken KK, Mladek AC, Schroeder MA, Decker PA, Kitange GJ, Shen Y, Feng Y, Protter AA, Elmquist WF, Sarkaria JN. Restricted delivery of talazoparib across the blood-brain barrier limits the sensitizing effects of PARP inhibition on temozolomide therapy in glioblastoma. Mol Cancer Ther. 2017;16(12):2735-46; DOI:10.1158/1535-7163.MCT-17-0365.
211. Becker CM, Oberoi RK, McFarren SJ, Muldoon DM, Pafundi DH, Pokorny JL, Brinkmann DH, Ohlfest JR, Sarkaria JN, Largaespada DA, Elmquist WF. Decreased affinity for efflux transporters increases brain penetrance and molecular targeting of a PI3K/mTOR inhibitor in a mouse model of glioblastoma. Neuro Oncol. 2015;17(9):1210-9; DOI:10.1093/neuonc/nov081.
212. Quick A, Patel D, Hadziahmetovic M, Chakravarti A, Mehta M. Current therapeutic paradigms in glioblastoma. Rev Recent Clin Trials. 2010;5(1):14-27; DOI:10.2174/157488710790820544.
213. Chakravarti A, Erkkinen MG, Nestler U, Stupp R, Mehta M, Aldape K, Gilbert MR, Black PML, Loeffler JS. Temozolomide-mediated radiation enhancement in glioblastoma: A report on underlying mechanisms. Clinical Cancer Research. 2006;12(15):4738-46; DOI:10.1158/1078-0432.CCR-06-0596.
214. Rivera AL, Pelloski CE, Gilbert MR, Colman H, De La Cruz C, Sulman EP, Bekele BN, Aldape KD. MGMT promoter methylation is predictive of response to radiotherapy and prognostic in the absence of adjuvant alkylating chemotherapy for glioblastoma. Neuro Oncol. 2010;12(2):116-21; DOI:10.1093/neuonc/nop020.
215. Chen W, Silverman DH, Delaloye S, Czernin J, Kamdar N, Pope W, Satyamurthy N, Schiepers C, Cloughesy T. 18F-FDOPA PET imaging of brain tumors: comparison study with 18F-FDG PET and evaluation of diagnostic accuracy. J Nucl Med. 2006;47(6):904-11.
216. Laack NN, Pafundi D, Anderson SK, Kaufmann T, Lowe V, Hunt C, Vogen D, Yan E, Sarkaria J, Brown P, Kizilbash S, Uhm J, Ruff M, Zakhary M, Zhang Y, Seaberg M, Wan Chan Tseung HS, Kabat B, Kemp B,

Brinkmann D. Initial results of a phase 2 trial of 18F-DOPA PET-guided dose-escalated radiation therapy for glioblastoma. Int J Radiat Oncol Biol Phys. 2021;110(5):1383-95; DOI:10.1016/j.ijrobp.2021.03.032.
217. Kirson ED, Gurvich Z, Schneiderman R, Dekel E, Itzhaki A, Wasserman Y, Schatzberger R, Palti Y. Disruption of cancer cell replication by alternating electric fields. Cancer Res. 2004;64(9):3288-95; DOI:10.1158/0008-5472.can-04-0083.
218. Gera N, Yang A, Holtzman TS, Lee SX, Wong ET, Swanson KD. Tumor treating fields perturb the localization of septins and cause aberrant mitotic exit. PLoS One. 2015;10(5):e0125269; DOI:10.1371/journal.pone.0125269.
219. Kim EH, Kim YJ, Song HS, Jeong YK, Lee JY, Sung J, Yoo SH, Yoon M. Biological effect of an alternating electric field on cell proliferation and synergistic antimitotic effect in combination with ionizing radiation. Oncotarget. 2016;7(38):622267-79; DOI:10.18632/oncotarget.11407.
220. Mrugala MM, Engelhard HH, Dinh Tran D, Kew Y, Cavaliere R, Villano JL, Annenelie Bota D, Rudnick J, Love Sumrall A, Zhu JJ, Butowski N. Clinical practice experience with NovoTTF-100A™ system for glioblastoma: the patient registry dataset (PRiDe). Semin Oncol. 2014;41(Suppl 6):S4-S13; DOI:10.1053/j.seminoncol.2014.09.010.
221. Toms SA, Kim CY, Nicholas G, Ram Z. Increased compliance with tumor treating fields therapy is prognostic for improved survival in the treatment of glioblastoma: a subgroup analysis of the EF-14 phase III trial. J Neurooncol. 2019;141(2):467-73; DOI:10.1007/s11060-018-03057-z.
222. Liu S, Shi W, Zhao Q, Zheng Z, Liu Z, Meng L, Dong L, Jiang X. Progress and prospect in tumor treating fields treatment of glioblastoma. Biomed Pharmacother. 2021;141:111810; DOI:10.1016/j.biopha.2021.111810.
223. Schmittling RJ, Archer GE, Mitchell DA, Heimberger A, Pegram C, Herndon JE, Friedman HS, Bigner DD, Sampson JH. Detection of humoral response in patients with glioblastoma receiving EGFRvIII-KLH vaccines. J Immunol Methods. 2008;339(1):74-81; DOI:10.1016/j.jim.2008.08.004.
224. Padfield E, Ellis HP, Kurian KM. Current therapeutic advances targeting EGFR and EGFRvIII in glioblastoma. Front Oncol. 2015;5:5; DOI:10.3389/fonc.2015.00005.
225. Yu MW, Quail DF. Immunotherapy for glioblastoma: current progress and challenge. Front Immunol. 2021;12:676301; DOI:10.3389/fimmu.2021.676301.
226. Schuster J, Lai RK, Recht LD, Reardon DA, Paleologos NA, Groves MD, Mrugala MM, Jensen R, Baehring JM, Sloan A, Archer GE, Bigner DD, Cruickshank S, Green JA, Keler T, Davis TA, Heimberger AB, Sampson JH. A phase II, multicenter trial of rindopepimut (CDX-110) in newly diagnosed glioblastoma: The ACT III study. Neuro Oncol. 2015;17(6):854-61; DOI:10.1093/neuonc/nou348.
227. Broekman MLD, Nieland L, Hoeben RC. Virotherapy: The next addition to the standard of care for glioblastoma? Cancer Cell. 2022;40(10):1089-91; DOI:10.1016/j.ccell.2022.09.003.
228. Brown CE, Warden CD, Starr R, Deng X, Badie B, Yuan YC, Forman SJ, Barish ME. Glioma IL13Rα2 is associated with mesenchymal signature gene expression and poor patient prognosis. PLoS One. 2013;8(10):e77769; DOI:10.1371/journal.pone.0077769.
229. Patel MA, Kim JE, Ruzevick J, Li G, Lim M. The future of glioblastoma therapy: sSynergism of standard of care and immunotherapy. Cancers (Basel). 2014;6(4):1953-85; DOI:10.3390/cancers6041953.
230. Tomaszewski W, Sanchez-Perez L, Gajewski TF, Sampson JH. Brain tumor microenvironment and host state: implications for immunotherapy. Clin Cancer Res. 2019;25(14):4202-10; DOI:10.1158/1078-0432.CCR-18-1627.
231. Kadiyala P, Li D, Nunez FM, Altshuler D, Doherty R, Kuai R, Yu M, Kamran N, Edwards M, Moon JJ, Lowenstein PR, Castro MG, Schwendeman A. High-Density Lipoprotein-Mimicking Nanodiscs for Chemo-immunotherapy against Glioblastoma Multiforme. ACS Nano. 2019;13(2):1365-84; DOI:10.1021/acsnano.8b06842.
232. Cho DY, Yang WK, Lee HC, Hsu DM, Lin HL, Lin SZ, Chen CC, Harn HJ, Liu CL, Lee WY, Ho LH. Adjuvant immunotherapy with whole-cell lysate dendritic cells vaccine for glioblastoma multiforme: a phase II clinical trial. World Neurosurg. 2012;77(5-6):736-44; DOI:10.1016/j.wneu.2011.08.020.
233. Polivka J, Holubec L, Kubikova T, Priban V, Hes O, Pivovarcikova K, Treskova I. Advances in experimental targeted therapy and immunotherapy for patients with glioblastoma multiforme. Anticancer Res. 2017;37(1):21-33; DOI:10.21873/anticanres.11285.
234. Slika H, Karimov Z, Alimonti P, Abou-Mrad T, De Fazio E, Alomari S, Tyler B. Preclinical models and technologies in glioblastoma research: evolution, current state, and future avenues. Int J Mol Sci. 2023;24(22):16316; DOI:10.3390/ijms242216316.
235. Liu P, Griffiths S, Veljanoski D, Vaughn-Beaucaire P, Speirs V, Brüning-Richardson A. Preclinical models of glioblastoma: limitations of current models and the promise of new developments. Expert Rev Mol Med. 2021;23:e20; DOI:10.1017/erm.2021.20.
236. Xie Y, Bergström T, Jiang Y, Johansson P, Marinescu VD, Lindberg N, Segerman A, Wicher G, Niklasson M, Baskaran S, Sreedharan S, Everlien I, Kastemar M, Hermansson A, Elfineh L, Libard S, Holland EC, Hesselager G, Alafuzoff I, Westermark B, Nelander S, Forsberg-Nilsson K, Uhrbom L. The human glioblastoma cell culture resource: validated cell models representing all molecular subtypes. EBioMedicine. 2015;2(10):1351-63; DOI:10.1016/j.ebiom.2015.08.026.
237. Kijima N, Kanemura Y. Mouse models of glioblastoma. In: De Vleeschouwer S, editor. Glioblastoma [Internet]. Brisbane (AU): Codon Publications; 2017 [cited 2024 Feb 8]. Chapter 7. Available from: 10.15586/codon.glioblastoma.2017.ch7.
238. Pudelko L, Edwards S, Balan M, Nyqvist D, Al-Saadi J, Dittmer J, Almlöf I, Helleday T, Bräutigam L. An orthotopic glioblastoma animal model suitable for high-throughput screenings. Neuro Oncol. 2018;20:1475-84; DOI:10.1093/neuonc/noy071.
239. Ruiz-Garcia H, Alvarado-Estrada K, Schiapparelli P, Quinones-Hinojosa A, Trifiletti DM. Engineering three-dimensional tumor models to study glioma cancer stem cells and tumor microenvironment. Front Cell Neurosci. 2020;14:558381; DOI:10.3389/fncel.2020.558381.
240. Krieger TG, Tirier SM, Park J, Jechow K, Eisemann T, Peterziel H, Angel P, Eils R, Conrad C. Modeling glioblastoma invasion using human brain organoids and single-cell transcriptomics. Neuro Oncol. 2020;22:1138-49; DOI:10.1093/neuonc/noaa091.
241. Linkous A, Balamatsias D, Snuderl M, Edwards L, Miyaguchi K, Milner T, Reich B, Cohen-Gould L, Storaska A, Nakayama Y, Schenkein E, Singhania R, Cirigliano S, Magdeldin T, Lin Y, Nanjangud G, Chadalavada K, Pisapia D, Liston C, Fine HA. Modeling patient-derived glioblastoma with cerebral organoids. Cell Rep. 2019;26:3203-11.e5; DOI:10.1016/j.celrep.2019.02.063.
242. Tang M, Rich JN, Chen S. Biomaterials and 3D Bioprinting Strategies to Model Glioblastoma and the Blood-Brain Barrier. Advanced Materials. 2021;33(5):e2004776; DOI:10.1002/adma.202004776.
243. Tang M, Tiwari SK, Agrawal K, Tan M, Dang J, Tam T, Tian J, Wan X, Schimelman J, You S, Xia Q, Rana TM, Chen S. Rapid 3D bioprinting of glioblastoma model mimicking native biophysical heterogeneity. Small. 2021;17(15):e2006050; DOI:10.1002/smll.202006050.
244. Logun M, Zhao W, Mao L, Karumbaiah L. Microfluidics in Malignant Glioma Research and Precision Medicine. Adv Biosyst. 2018;2(5):1700221; DOI:10.1002/adbi.201700221.
245. Mohiuddin E, Wakimoto H. Extracellular matrix in glioblastoma: opportunities for emerging therapeutic approaches. Am J Cancer Res. 2021;11(8):3742-54.
246. Gómez-Oliva R, Domínguez-García S, Carrascal L, Abalos-Martínez J, Pardillo-Díaz R, Verástegui C, Castro C, Nunez-Abades P, Geribaldi-Doldán N. Evolution of experimental models in the study of glioblastoma: toward finding efficient treatments. Front Oncol. 2021;10:614295; DOI:10.3389/fonc.2020.614295.
247. Śledzińska P, Bebyn MG, Furtak J, Kowalewski J, Lewandowska MA. Prognostic and predictive biomarkers in gliomas. Int J Mol Sci. 2021;22(19):10373; DOI:10.3390/ijms221910373.
248. Silantyev AS, Falzone L, Libra M, Gurina OI, Kardashova KS, Nikolouzakis TK, Nosyrev AE, Sutton CW, Panayioti M, Tsatsakis A. Current and future trends on diagnosis and prognosis of glioblastoma: from molecular biology to proteomics. Cells. 2019;8(8):863; DOI:10.3390/cells8080863.
249. Gatto L, Franceschi E, Di Nunno V, Tosoni A, Lodi R, Brandes AA. Liquid biopsy in glioblastoma management: from current research to future perspectives. Oncologist. 2021;26:865-78; DOI:10.1002/onco.13858.
250. Di L, Eichberg DG, Huang K, Shah AH, Jamshidi AM, Luther EM, Lu VM, Komotar RJ, Ivan ME, Gultekin SH. Stimulated Raman histology for rapid intraoperative diagnosis of gliomas. World Neurosurg. 2021;150:e135-43; DOI:10.1016/j.wneu.2021.02.122.
251. Zhang Y, Yu H, Li Y, Xu H, Yang L, Shan P, Du Y, Yan X, Chen X. Raman spectroscopy: a prospective intraoperative visualization technique for gliomas. Front Oncol. 2023;12:1086643; DOI:10.3389/fonc.2022.1086643.
252. Riva M, Sciortino T, Secoli R, D'amico E, Moccia S, Fernandes B, Nibali MC, Gay L, Rossi M, De Momi E, Bello L. Glioma biopsies classification using Raman spectroscopy and machine learning models on fresh tissue samples. Cancers (Basel). 2021;13:1-14; DOI:10.3390/cancers13051073.
253. Desroches J, Jermyn M, Pinto M, Picot F, Tremblay M-A, Obaid S, Marple E, Urmey K, Trudel D, Soulez G, Guiot M-C, Wilson BC, Petrecca K, Leblond F. A new method using Raman spectroscopy for in vivo tar-

geted brain cancer tissue biopsy. Sci Rep. 2018;8:1792; DOI:10.1038/s41598-018-20233-3.
254. Wu J, Al-Zahrani A, Beylerli O, Sufianov R, Talybov R, Meshcheryakova S, Sufianova G, Gareev I, Sufianov A. Circulating miRNAs as diagnostic and prognostic biomarkers in high-grade gliomas. Front Oncol. 2022;12:898537; DOI:10.3389/fonc.2022.898537.
255. Wu X, Yang L, Wang J, Hao Y, Wang C, Lu Z. The involvement of long non-coding RNAs in glioma: from early detection to immunotherapy. Front Immunol. 2022;13:897754; DOI:10.3389/fimmu.2022.897754.
256. Chen X-D, Zhu M-X, Wang S-J. Expression of long non-coding RNA MAGI2-AS3 in human gliomas and its prognostic significance. Eur Rev Med Pharmacol Sci. 2019;23:3455-60; DOI:10.26355/eurrev_201904_17710.
257. Shang F, Du S-W, Ma X-L. Up-regulation of lncRNA PXN-AS1-L is associated with unfavorable prognosis in patients suffering from glioma. Eur Rev Med Pharmacol Sci. 2019;23:8950-5; DOI:10.26355/eurrev_201910_19293.
258. Zhou M, Zhang Z, Zhao H, Bao S, Cheng L, Sun J. An immune-related six-lncRNA signature to improve prognosis prediction of glioblastoma multiforme. Mol Neurobiol. 2018;55(5):3684-97; DOI10.1007/s12035-017-0572-9.
259. Musatova OE, Rubtsov YP. Effects of glioblastoma-derived extracellular vesicles on the functions of immune cells. Front Cell Dev Biol. 2023;11:1060000; DOI:10.3389/fcell.2023.1060000.
260. Verger A, Langen KJ. PET Imaging in glioblastoma: use in clinical practice. In: De Vleeschouwer S, editor. glioblastoma [Internet]. Brisbane (AU): Codon Publications; 2017 [cited 2024 Feb 10]. Chapter 9. Available from: 10.15586/codon.glioblastoma.2017.ch9.
261. Filss CP, Galldiks N, Stoffels G, Sabel M, Wittsack HJ, Turowski B, Antoch G, Zhang K, Fink GR, Coenen HH, Shah NJ, Herzog H, Langen KJ. Comparison of 18F-FET PET and perfusion-weighted MR imaging: a PET/MR imaging hybrid study in patients with brain tumors. J Nucl Med. 2014;55(4):540-5; DOI:10.2967/jnumed.113.129007.
262. Galldiks N, Dunkl V, Stoffels G, Hutterer M, Rapp M, Sabel M, Reifenberger G, Kebir S, Dorn F, Blau T, Herrlinger U, Hau P, Ruge MI, Kocher M, Goldbrunner R, Fink GR, Drzezga A, Schmidt M, Langen K-J. Diagnosis of pseudoprogression in patients with glioblastoma using O-(2-[18F]fluoroethyl)-l-tyrosine PET. Eur J Nucl Med Mol Imaging. 2015;42:685-95; DOI:10.1007/s00259-014-2959-4.
263. Puranik AD, Boon M, Purandare N, Rangarajan V, Gupta T, Moiyadi A, Shetty P, Sridhar E, Agrawal A, Dev I, Shah S. Utility of FET-PET in detecting high-grade gliomas presenting with equivocal MR imaging features. World J Nucl Med. 2019;18:266-72; DOI:10.4103/wjnm.WJNM_89_18.
264. Kranz M, Bergmann R, Kniess T, Belter B, Neuber C, Cai Z, Deng G, Fischer S, Zhou J, Huang Y, Brust P, Deuther-Conrad W, Pietzsch J. Bridging from brain to tumor imaging: (S)-(−)- and (R)-(+)-[18F]fluspidine for investigation of sigma-1 receptors in tumor-bearing mice. Molecules. 2018;23:702; DOI:10.3390/molecules23030702.
265. Drake LR, Hillmer AT, Cai Z. Approaches to PET imaging of glioblastoma. Molecules. 2020;25(3):568; DOI:10.3390/molecules25030568.
266. Chitneni SK, Reitman ZJ, Spicehandler R, Gooden DM, Yan H, Zalutsky MR. Synthesis and evaluation of radiolabeled AGI-5198 analogues as candidate radiotracers for imaging mutant IDH1 expression in tumors. Bioorg Med Chem Lett. 2018;28:694-9; DOI:10.1016/j.bmcl.2018.01.015.
267. Roberts JW, Powlovich L, Sheybani N, LeBlang S. Focused ultrasound for the treatment of glioblastoma. J Neurooncol. 2022;157:237-47; DOI:10.1007/s11060-022-03974-0.
268. Bunevicius A, McDannold NJ, Golby AJ. Focused ultrasound strategies for brain tumor therapy. Oper Neurosurg. 2020;19:9-18; DOI:10.1093/ons/opz374.
269. Meng Y, Pople CB, Suppiah S, Llinas M, Huang Y, Sahgal A, Perry J, Keith J, Davidson B, Hamani C, Amemiya Y, Seth A, Leong H, Heyn CC, Aubert I, Hynynen K, Lipsman N. MR-guided focused ultrasound liquid biopsy enriches circulating biomarkers in patients with brain tumors. Neuro Oncol. 2021;23:1789-97; DOI:10.1093/neuonc/noab057.
270. Nance E, Timbie K, Miller GW, Song J, Louttit C, Klibanov AL, Shih T-Y, Swaminathan G, Tamargo RJ, Woodworth GF, Hanes J, Price RJ. Non-invasive delivery of stealth, brain-penetrating nanoparticles across the blood – brain barrier using MRI-guided focused ultrasound. J Control Release. 2014;189:123-32; DOI:10.1016/j.jconrel.2014.06.031.
271. Hersh AM, Bhimreddy M, Weber-Levine C, Jiang K, Alomari S, Theodore N, Manbachi A, Tyler BM. Applications of focused ultrasound for the treatment of glioblastoma: a new frontier. Cancers (Basel). 2022;14:4920; DOI:10.3390/cancers14194920.
272. Bunevicius A, Pikis S, Padilla F, Prada F, Sheehan J. Sonodynamic therapy for gliomas. J Neurooncol. 2022;156:1-10; DOI:10.1007/s11060-021-03807-6.
273. Iturrioz-Rodríguez N, Bertorelli R, Ciofani G. Lipid-based nanocarriers for the treatment of glioblastoma. Adv Nanobiomed Res. 2021;1(2):2000054; DOI:10.1002/anbr.202000054.
274. Lin Q, Mao K-L, Tian F-R, Yang J-J, Chen P-P, Xu J, Fan Z-L, Zhao Y-P, Li W-F, Zheng L, Zhao Y-Z, Lu C-T. Brain tumor-targeted delivery and therapy by focused ultrasound introduced doxorubicin-loaded cationic liposomes. Cancer Chemother Pharmacol. 2016;77:269-80; DOI:10.1007/s00280-015-2926-1.
275. Seo S, Kim EH, Chang W-S, Lee W-S, Kim K-H, Kim J-K. Enhanced proton treatment with a LDLR-ligand peptide-conjugated gold nanoparticles targeting the tumor microenvironment in an infiltrative brain tumor model. Am J Cancer Res. 2022;12(1):198-209.
276. Ismail M, Yang W, Li Y, Chai T, Zhang D, Du Q, Muhammad P, Hanif S, Zheng M, Shi B. Targeted liposomes for combined delivery of artesunate and temozolomide to resistant glioblastoma. Biomaterials. 2022;287:121608; DOI:10.1016/j.biomaterials.2022.121608.
277. Kim EL, Sorokin M, Kantelhardt SR, Kalasauskas D, Sprang B, Fauss J, Ringel F, Garazha A, Albert E, Gaifullin N, Hartmann C, Naumann N, Bikar S-E, Giese A, Buzdin A. Intratumoral heterogeneity and longitudinal changes in gene expression predict differential drug sensitivity in newly diagnosed and recurrent glioblastoma. Cancers (Basel). 2020;12:520; DOI:10.3390/cancers12020520.
278. Ladbury C, Amini A, Schwer A, Liu A, Williams T, Lee P. Clinical Applications of magnetic resonance-guided radiotherapy: a narrative review. Cancers (Basel). 2023;15:2916; DOI:10.3390/cancers15112916.
279. Maziero D, Straza MW, Ford JC, Bovi JA, Diwanji T, Stoyanova R, Paulson ES, Mellon EA. MR-guided radiotherapy for brain and spine tumors. Front Oncol. 2021;11:626100; DOI:10.3389/fonc.2021.626100.
280. Ellingson BM, Malkin MG, Rand SD, Connelly JM, Quinsey C, LaViolette PS, Bedekar DP, Schmainda KM. Validation of functional diffusion maps (fDMs) as a biomarker for human glioma cellularity. J Magn Reson Imaging. 2010;31:538-48; DOI:10.1002/jmri.22068.
281. Hattingen E, Müller A, Jurcoane A, Mädler B, Ditter P, Schild H, Herrlinger U, Glas M, Kebir S. Value of quantitative magnetic resonance imaging T1-relaxometry in predicting contrast-enhancement in glioblastoma patients. Oncotarget. 2017;8:53542-51; DOI:10.18632/oncotarget.18612.
282. Trejo-Solís C, Serrano-Garcia N, Escamilla-Ramírez Á, Castillo-Rodríguez R, Jimenez-Farfan D, Palencia G, Calvillo M, Alvarez-Lemus M, Flores-Nájera A, Cruz-Salgado A, Sotelo J. Autophagic and apoptotic pathways as targets for chemotherapy in glioblastoma. Int J Mol Sci. 2018;19:3773; DOI:10.3390/ijms19123773.
283. Han W, Yu F, Cao J, Dong B, Guan W, Shi J. Valproic acid enhanced apoptosis by promoting autophagy via Akt/mTOR signaling in glioma. Cell Transplant. 2020;29:096368972098187; DOI:10.1177/0963689720981878.
284. Yuan Y, Xiang W, Qing M, Yanhui L, Jiewen L, Yunhe M. Survival analysis for valproic acid use in adult glioblastoma multiforme: a meta-analysis of individual patient data and a systematic review. Seizure. 2014;23:830-5; DOI:10.1016/j.seizure.2014.06.015.
285. Osuka S, Takano S, Watanabe S, Ishikawa E, Yamamoto T, Matsumura A. Valproic acid inhibits angiogenesis in vitro and glioma angiogenesis in vivo in the brain. Neurol Med Chir (Tokyo). 2012;52(4):186-93; DOI:10.2176/nmc.52.186.
286. Shaw P, Kumar N, Privat-Maldonado A, Smits E, Bogaerts A. Cold atmospheric plasma increases temozolomide sensitivity of three-dimensional glioblastoma spheroids via oxidative stress-mediated DNA damage. Cancers (Basel). 2021;13:1780; DOI:10.3390/cancers13081780.
287. Pinheiro Lopes B, O'Neill L, Bourke P, Boehm D. Combined effect of plasma-activated water and topotecan in glioblastoma cells. Cancers (Basel). 2023;15:4858; DOI:10.3390/cancers15194858.
288. Gjika E, Pal-Ghosh S, Kirschner ME, Lin L, Sherman JH, Stepp MA, Keidar M. Combination therapy of cold atmospheric plasma (CAP) with temozolomide in the treatment of U87MG glioblastoma cells. Sci Rep. 2020;10:16495; DOI:10.1038/s41598-020-73457-7.