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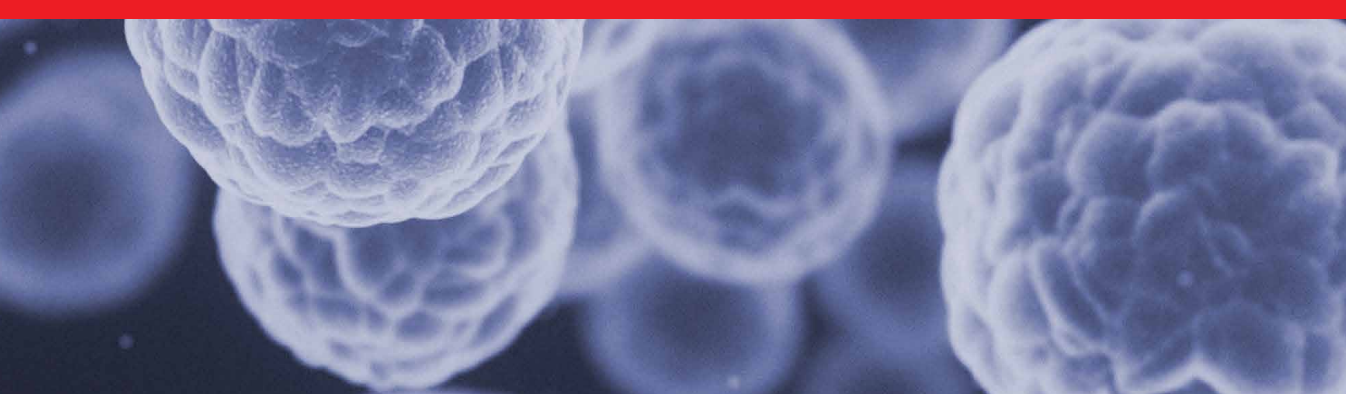
Glioblastoma

New Solutions for Brain Cancerogenesis

Edited by Jerzy Trojan



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Volume 7

Aims and Scope of the Series

The field of oncology has undergone extraordinary change and progress over the past several decades. Today, information is evolving at a rapid rate, with standards of management far different than standards of care applied during the training of most practicing oncologists. Oncology practitioners in all disciplines must remain current to optimize patient care. Basic and translational science remain critical in developing process improvements for patient care. The closer we understand the mechanism, the more we can improve targeted therapies and apply them to patient care. The pace of information is moving faster than at any previous time in history, and all oncology disciplines must remain current to provide excellent service to patients. The modern oncologist must be fluent in using big data and the volume of information generated in clinical trials. As we move closer to personalized patient care based on genomics and molecular biomarkers, the modern oncologist has to be nimble in assessing all available information and how this would be applied to each patient, balanced by the clinical status and medical co-morbidities of each patient. Targeted therapies can bring new and different sequelae, and oncology teams need to remain fluent in managing the consequences of therapy and primary management. In this book series, we will present how modern care has progressed in multiple disease areas and how modern oncology teams need to adapt in order to manage the cancer patients of today successfully. Surgery, radiation therapy, and medical oncology are practiced today with the support of exceptional modern technology, and in this series, we will review how these improvements are applied to each disease site to maintain excellence in patient care.

Meet the Series Editor

Dr. FitzGerald is the professor and chair of the Department of Radiation Oncology at UMass Chan Medical School in the USA. He serves as one of the principal investigators of the Imaging and Radiation Oncology Core (IROC) service for the National Cancer Institute clinical trials program and is directly involved in the quality assurance of clinical trials in the National Clinical Trials Network (NCTN). Dr. Fitzgerald manages NCTN clinical trials with a real-time pre-therapy review of imaging and radiation therapy treatment objects to ensure the care plan complies with study objectives and the patient stage has been assigned to the correct study. His basic science interest is in hematopoietic stem cell biology and cellular adhesion molecules as they pertain to therapeutic resistance and mitigation of injury from therapy.

Meet the Volume Editor

Jerzy Trojan completed his Doctorat ès Sciences at the Sorbonne – Pierre & Marie Curie University. He has conducted scientific and clinical research at institutions including the French National Institute of Health (Paris), Case Western Reserve University (Cleveland), and the Colombian National Institute of Health (Bogotá), among others. His research, focused on the development of neoplastic brain diseases and their translational application in cancer therapies, has been documented in nearly 380 publications. He was elected a member of the International Academy of Medicine (ANM) in Paris. He is internationally recognized for his novel contributions to biomedical science, notably in demonstrating the convergence between neuro-ontogenesis and neuro-oncogenesis through the use of AFP and IGF-I as innovative biomarkers for brain development and tumorigenesis [Developmental Neuroscience, 1984; PNAS USA, 1994; AMOR, 2016]. He also pioneered the field of cancer gene therapy, specifically immuno-gene therapy for glioblastoma, helping establish this approach as a distinct and promising domain within oncology [Science, 1993; Neuroscience, 2007; Current Medicinal Chemistry, 2024].

Contents

Preface

Glioblastoma multiforme (GBM) is the most common malignant brain tumour, with a minimum incidence of 4 cases per 100,000 people. Generally diagnosed from the age of 60, the average life expectancy of patients is typically one year [1–3]. In the pathogenesis of GBM, the scientific literature acknowledges the development of tumours through various genetic pathways [4, 5]. Other studies have demonstrated the important role played by the abnormal "deregulation" of growth factors, especially IGF-I, VEGF, EGF, and PDGF [1, 2, 6, 7]. The GBM is accompanied by corresponding neurological symptoms [5] at the site of the tumour [1, 8]. Despite ongoing diagnostic and therapeutic efforts, GBM remains the most challenging brain pathology to treat [6, 9]. In GBM recurrence, maximal tumour resection remains the first-line treatment [10]. To increase life expectancy, a combined therapy of surgery and radiation therapy must include immunotherapy [11, 12]. The current therapeutic strategy of GBM is associated with the use of glucocorticoids (GCs) [3]. Patients receiving exclusively excess doses of GCs had elevated expression of CD8-T cells [13] and activated B cells, the two lymphocyte populations together playing a role in the antitumor response [13, 14]. Regarding postoperative radiation therapy, an improved survival rate is observed [1, 6]. Concomitant with radiotherapy, chemotherapy with temozolomide (TMZ) is used with caution [15]: approximately 55% of patients with GBM are resistant to temozolomide due to their DNA repair system, specifically methylguanine methyltransferase (MGMT) [9]. On the other hand, vaccines targeting tumour growth factors, such as VEGF, eGFR, and PDGFR, have limitations, including low immunogenicity [1, 16]. Bevacizumab, which binds to the VEGF factor, is recommended in combination with irinotecan, carmustine, lomustin, carboplatin, or temozolomide chemotherapy [17]. The GBM therapies linked to IGF-I and TGF have resulted in vaccines with an immunogenic character [18, 19].

The development of new therapeutic approaches, especially gene therapy, by Anderson et al. in 1990 [20] and the "creation" of cancer gene therapy or cancer gene immunotherapy by Trojan et al. in 1992/3 [21], have been widely followed [22, 23]. Cancer gene therapy targeting IGF-I (the main factor of oncogenesis [7, 21]) using IGF-I antisense and IGF-I triple helix approaches has been successfully introduced in clinical trials in the USA, Europe, China, and Latin America [7]. At the same time, cancer immunotherapy "created" by Townsend et al. in 1993 [24] and Guo et al. in 1994 [14] was implemented on the concept of immunotherapy [25]. Since 2015, immunotherapy has been launched in the United States as part of the "Cancer Moonshot" program [26]. Therapeutic immunotherapy strategies involve different approaches. Viral immunotherapy includes viral vector vaccines that contain DNA-antigen-producing immune responses [1, 27]. In the ACT strategy of adoptive T cells (CAR-T therapies), allogeneic T cells are modified by recognizing a unique antigen associated with a tumour [28]. Recently, intraventricular injection of CAR-T cells in GBM patients has been shown to improve their efficiency [29]. Regarding therapies based on immune checkpoint inhibitors, such as PD-1, PD-L1, and CTLA-4, they have also promoted the immune activation of T cells [30]. The immunotherapy strategies, particularly anti-IGF-I

immuno-gene therapy, have given very encouraging results: the increase in survival has reached two years and, in some cases, three years [7, 15]. Although we are far from victory, the permanent clinical progress related to the development of new techniques in diagnostics and therapies described in this book is already promising.

The presented first chapter (Asem A. Muhsen et al. "Glioblastoma's Infrequent Locations and Synchronous Tumors") is a source of knowledge about glioblastomas located outside of the cerebral hemispheres, arising in less common regions of the central nervous system.

The second chapter (Weichi Wu et al. "Identifying Glioma Margins Rapidly during Microsurgery via Microendoscope Systems: History and Prospects") constitutes a considerable advancement in glioblastoma surgical technology for identifying tumour boundaries. Moreover, microsurgery should maximize tumour removal while preserving neurological functions and ensuring patient safety. Additionally, microsurgery is often obligatory for any type of gene therapy or immunotherapy, which are post-surgery approaches. The third chapter (Hulya Torun, Ihsan Solaroglu. "Innovative Diagnostic Approaches in Glioblastoma: The Role of Raman Spectroscopy and Liquid Biopsy") presents the essential up-to-date techniques of GBM diagnostic - Raman spectroscopy and liquid biopsy, having the potential to significantly improve survival rates in GBM patients by enabling earlier detection, more precise monitoring, and personalized treatment strategies. That has an impact on survival, considering that early diagnosis facilitated by Raman spectroscopy's ability to detect tumour biomarkers, such as IGF-I, in biofluids and its potential for tumour margin detection (distinguishing between healthy and malignant tissues with high accuracy). The combination of liquid biopsy and Raman spectroscopy offers a powerful diagnostic approach that captures both circulating and tissue-specific biomarkers. The fourth chapter ("Astrocytes in Glioblastoma Therapy: A Novel Approach to Targeting Tumor Microenvironment" by Ravindri Jayasinghe, Nadun Danushka) is a real source of knowledge as well as astrocytes physiology and pathology, as well as the GBM tumour microenvironment, the latter constituting the potential therapy target. The fifth chapter (Zuowen Zhang, Mingchang Li, Jie Huang, "Dendritic Cell-Based Glioblastoma Vaccines: Advances and Challenges") provides information on various types of current immunotherapies and related immune mechanisms targeting GBM. In this context, the authors propose the new GBM vaccines, whose preparation is based on the use of dendritic cells. The sixth book chapter (Xiaohui Ren, Ling Qin, "Glioblastoma Management in the Post-COVID-19 Era: Challenges, Strategies, and Adaptations") is an essential description of managing GBM during the global health crisis caused by the COVID-19 pandemic.

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Section 1

Location and Surgery of Tumour

Chapter 1

Glioblastoma's Infrequent Locations and Synchronous Tumors

Asem A. Muhsen and Bahaeddin A. Muhsen

Abstract

Glioblastoma (GBM) is an exceedingly aggressive Grade IV astrocytoma and the most prevalent primary malignant brain tumor in adults, representing 45–50% of all gliomas. In our chapter, we have highlighted the rare locations of GBM, the occurrence of multiple GBM, and its coexistence, alongside other cerebral neoplasms such as meningioma. While mostly situated in the cerebral hemispheres, glioblastomas can also arise in atypical locations such as the brainstem, thalamus, basal ganglia, corpus callosum, cerebellum, and spinal cord. Multiple GBMs were identified in 11.7% of cases. Meningiomas are common benign brain tumors that can coexist with GBMs as collision tumors. This link may just be a coincidence or a sign of a related pathogenesis. Further molecular and biological studies are necessary to confirm the existence of shared molecular mechanisms and genetic determinants.

Keywords: collision tumors, synchronous tumors, multiple GBM, meningioma, glioblastoma

1. Introduction

Glioblastoma (GBM) is an aggressive and highly malignant form of primary brain tumor classified as a Grade IV astrocytoma by the World Health Organization (WHO) [1]. It is the most common and lethal form of primary brain cancer in adults, accounting for approximately 45–50% of all gliomas.

While glioblastomas most often develop in the cerebral hemispheres, particularly the frontal, temporal, parietal, and occipital lobes, they can occasionally arise in less common regions of the central nervous system (CNS) [2]. These infrequent locations include the brainstem, thalamus, basal ganglia, corpus callosum, cerebellum, and spinal cord. Tumors in these regions pose unique diagnostic and therapeutic challenges due to the critical nature of surrounding brain structures [3]. For example, glioblastomas in the brainstem are often inoperable due to the dense concentration of essential motor and sensory pathways.

The etiology of glioblastomas in these rare sites is not entirely understood, but they may be associated with underlying genetic predispositions or cellular differences

in neural progenitor cell populations [4]. Their clinical presentation may also differ from glioblastomas in more common regions, as symptoms are often site-specific, such as ataxia for cerebellar tumors or motor impairments for brainstem tumors.

2. Main body

2.1 Brainstem

Glioblastomas are part of the most malignant brain tumors and are most often localized in the cerebral hemispheres. However, GBMs found in the brainstem are rare compared with other glioblastoma cases and thus are diagnosed infrequently [1]. Their incidence is higher among children than adults. The reasons for the higher incidence of the phenomenon in children are still unclear; however, certain inherited tendencies or perhaps favorable molecular profile of the tumor in children might influence that. They found that pediatric brainstem GBMs may have different clinical and molecular features than adult brainstem GBMs, which could be a reason for the difference in incidence between the two populations [2]. However, as noted earlier, brainstem gliomas are more frequent during childhood, but they are still rare; this fact makes the diagnostic and therapeutic approach challenging.

In adults, there are far fewer cases of brainstem glioblastomas; these tumors are usually diagnosed at an advanced stage because they cause mild and nonspecific signs and symptoms that progress slowly [3]. Incidentally, the brainstem, which controls vital autonomic functions and is less common in primary brain tumors, is infiltrated more frequently by other forms of gliomas, such as pontine gliomas and diffuse intrinsic pontine gliomas (DIPG) that, while related, pathologically, to GBMs, are a distinct entity in the pediatric population [4]. This points to the need for further research on the epidemiological factors and pathogenesis of brainstem glioblastomas, as well as the molecular features of the tumor.

Both traditional and sophisticated MR imaging offer non-invasive methods that reveal the required molecular regional adaptations of the various GBM lesions and mirror the morphologic changes that have been characterized. The various m-GBM lesions may show diverse sizes, growth rates, and composite intensities with distinct degrees and patterns of contrast enhancement on contrast-enhanced T1-weighted imaging (T1-WI) **Figure 1A** [5]. This reflects the underlying heterogeneity in the tumor bulk, texture, and angiogenesis. The non-enhancing tumor component with hazy borders and vasogenic edema is represented by the increased water content of the heterogeneous hyperintense signal abnormality on T2 Fluid-Attenuated Inversion Recovery (FLAIR) that is linked to each enhancing lesion (**Figure 2**).

There are no distinguishable imaging characteristics that allow the two components to be separated. The T2 FLAIR sequence is thought to be an infiltrative non-enhancing GBM component that joins two or more different enhancing lesions in multifocal GBM. The presence of T2 FLAIR hyperintense foci in a specific brain region may indicate an early emergence of m-GBM, even if contrast enhancement is necessary for the diagnosis of both multifocal and multicentric GBM [6]. The apparent diffusion coefficient, which is obtained from diffusion imaging, can be used to quantitatively evaluate the variation in cellular density among the various GBM lesions, or it can manifest as variations in diffusion restriction. Vascular growth, permeability, and blood flow within the GBM foci may be more precisely reflected by qualitative and quantitative assessments of several perfusion measures.

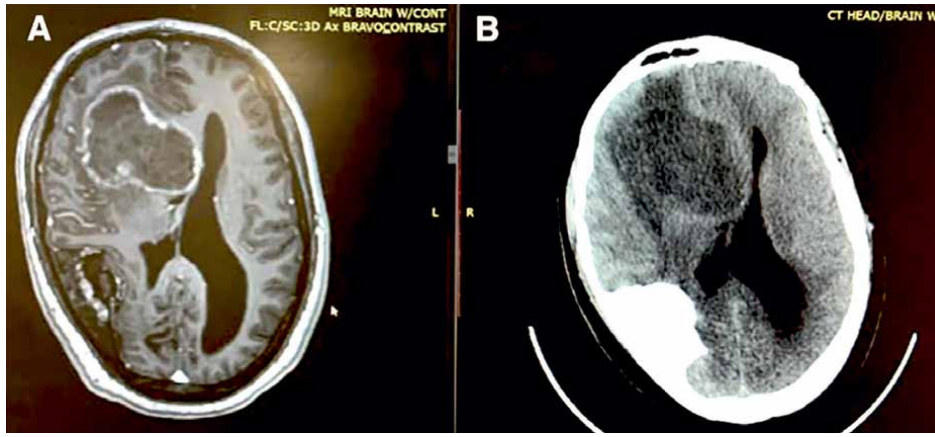

Figure 1.
A: Axial brain MRI with contrast demonstrating a left frontal lesion extending to the corpus callosum, exhibiting rim enhancement consistent with glioblastoma multiforme (GBM), confirmed by biopsy. B: Axial brain CT scan showing a calcified convexity meningioma and left frontal GBM (collision tumors).

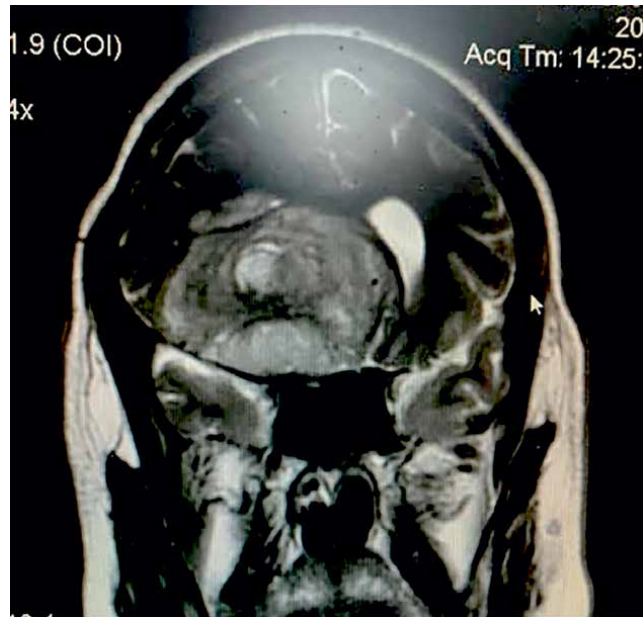

Figure 2.
T2-weighted FLAIR (Fluid-Attenuated Inversion Recovery) sequences exhibit hyperintense signals in glioblastoma multiforme (GBM). This manifestation usually aligns with areas of vasogenic edema, infiltrative neoplasm, and, at times, necrosis.

The clinical presentations of the brainstem glioblastomas are usually suggestive of the functions of the brainstem in regulating fundamental physiological processes. Being a part of the brainstem, many basic life-supporting and controlling functions like respiration, regulation of heartbeat, and swallowing are damaged [5]. A unique feature of brainstem GBMs is that the patients often present with cranial nerve palsies in terms of visual dysfunction due to the involvement of the optic nerve, weakness of the face due to impaired facial nerve, and dysphagia with impaired pathways of

swallowing and speech [6]. A second vivid sign is an ataxia, which is uncoordinated movement and even loss of balance due to injury in the cerebellum or the coordination centers of the brainstem [7]. Ataxia could be disabling, and a patient might experience vertigo and dizziness, making their motor problem worse. Patients may develop other posterior fossa signs as the size of the tumor increases, such as headaches and vomiting due to raised intracranial pressure (ICP) associated with the mass effect [8].

Neurological changes are frequent and can be progressive, with symptoms worsening rapidly as the tumor invades cerebral structures. Other complications include papilledema and swelling of the optic disc [9, 10].

The brainstem is a small but very important part of the superior location, which is a crucial complex for many automatic functions, including respiration and circulation, swallowing, and speech. Because of this, any tumor that may develop in this area also poses many diagnostic and therapeutic problems [11]. Therefore, the removal of the tumor requires the delving approach, which is still associated with a stern neurological deficit or even lethal outcomes due to seizing the important cranial nerve nuclei and centers [12]. For a variety of reasons, radiation therapy used to be viewed as the primary approach to treating brainstem glioblastomas, given the complexity of the brainstem structure [13]. However, a dangerous side effect, which is toxicity, is associated with radiation and becomes alarming when such regions are targeted. Accidents and radiation may affect combinatorial healthy cells, increasing symptoms, and detrimental neurological consequences [14]. Further, due to the blood-brain barrier, the delivery of chemotherapeutic agents to the brain is relatively restricted. Although temozolomide (TMZ) is used routinely in the treatment of GBMs at other anatomic sites in the brain, the use of TMZ for brainstem-located tumors is relatively ineffective due to poor penetration of the drug into this region [15].

Moreover, the brainstem has vital functions, so the glioblastomas progress extremely fast [16]. Locally invasive GBMs within the brainstem are considered difficult to cure despite surgery, radiation, and chemotherapy, and patients' overall life expectancy might be significantly reduced [17]. In addition to the diagnostic enigma, the volume and aggressiveness of cancer, along with the numerous and often highly technical treatment modalities, exert the clinical and patient burden on both stakeholders [18]. Further research for new treatments, including targeted therapies, immunotherapy, as well as gene therapy [19], brings hope for better results, but brainstem glioblastomas remain one of the most difficult in the field of neuro-oncology [3, 17].

2.2 Cerebellum

GBMs can be considered the most malignant type of primary brain tumor; they are localized mainly in the cerebral hemispheres. Still, GBMs occurring in the cerebellum constitute only about 1% of all glioblastomas [4]. GBMs are rare in children, and even in adults, the majority of the tumors are found in the cerebral hemispheres. This low incidence rate could explain the low level of research and documentation data and rare clinical cases of cerebellar GBMs, which complicates their diagnosis and treatment [19]. Despite the fact that cerebellar GBMs are very rare when diagnosed, they will be somewhat different from the patients with GBMs located in other parts of the brain. Motor deficits and a rapid decline in the quality of life for patients are rational when a cerebellar tumor arises due to the importance of this region in movement and balance [20].

Along with the neurological complaints consistent with the location of a cerebellar glioblastoma, patients may develop a variety of neurological symptoms depending on the size, location, and growth kinetics of the tumor [21]. Ataxia ensues out of disturbances of the cerebellum in that this body is solitary and in charge of refining the motor movements [22]. This can be presented in terms of poor mobility, involuntary movements, and uncoordinated movements of the limbs. When the tumor advances, one might experience dysmetria, which is a kind of ataxia for which the patient fails to estimate the distances in reaching for an object.

Headaches are relatively rare with GBMs, but when they occur, they are primarily associated with a cerebellar location that is often observed in GBMs, like vertigo [23]. Vertigo is a pathological condition that entails a spinning or whirling feeling due to an impairing ability of the brain to interpret spatial data. To the degree that balance and equilibrium are governed in the cerebellum, lesions in this area may affect these two straightaway, the reason for vertigo or imbalance, which is worse in case the patient tries to stand or move around [24].

Some headache patients who have a brain tumor develop increased ICP when the tumor expands and exerts pressure on adjacent tissues. High intracranial pressure is acquired from the growing mass of the tumor within the limited compartment of the skull, headaches, fatigue, and vomiting [25]. In further progression, if the cancer grows, it may lead to hydrocephalus, which means increased accumulation of cerebrospinal fluid within the brain parenchyma, causing an increase in ICP and thus, worsening the signs with visual complaints possibly accompanied by changes in mental status [26].

Common approaches to the surgical treatment of glioblastomas are challenging to apply due to the localization of the lesion and the peculiarities of cerebellar functions. Differential growth from other cancers in the head and brain again is in contrast to different locations in which there is often extra space for surgery and resection since cerebellar tumors arise from one of the most structurally sensitive areas in the head [27]. This complicates both diagnostic propositions as well as treatment strategies.

Cerebellar GBMs are challenging to remove through surgery because of their closeness to very sensitive structures when they are affected, for instance, the fourth ventricle and the brain stem, which controls some of the essential integrity of our bodies, like breathing and heartbeat [28]. In patients who undergo partial resection, complications may arise in the form of even a worsened motor function or cerebellar ataxia due to the highly sensitive nature of the cerebellar tissue and the possibility of damage during surgery and injury to other nearby tissues [29].

Another concern is radiation treatment for the cerebellar GBMs because of myelinization and subsequent damage to neurons. Although radiation allows for the limitation of tumor growth, the cerebellum has excellent sensitivity to radiation, and patients have permanent complications [30]. These tumors often have an aggressive course, and even if diagnosed early and treated with surgery, radiation, and chemotherapy, the outcome is still poor, and most patients live for only a short time after the diagnosis is made [31]. Thus, glioblastomas (GBMs) located in the region of the cerebellum can be considered a rare and complex type of brain tumor that can create specific diagnostic and therapeutic problems [32]. Treatment is rendered challenging by the fact that the cerebellum is a highly specialized region of the brain, and the key goal is to eliminate the tumor without causing significant neurological deficits [33]. Even a highly aggressive tumor has a poorer prognosis, as is generally witnessed.

2.3 Spinal cord

Superior mediastinal glioblastomas are considered rare, making up less than 1% of all glioblastomas. Although glioblastomas affect the brain predominantly in the cerebral hemispheres, spinal cord glioblastomas are rare, accounting for less than 1% of primary spinal tumors [34]. Spinal GBMs are rare, adding to the lack of accessible data concerning epidemiology, clinical manifestation, and optimal management plans.

Although spinal cord gliomas, a more extensive category of spinal malignancies, can happen at any age, spinal GBMs are highly uncommon in children and increasingly experienced in the fifth to the sixth decade of life [35]. Spinal GBMs again have symptoms that correlate with the position of the tumor, its size, and the extent to which it has infiltrated the spinal columns [36]. Another of the defining characteristics of spinal GBMs is myelopathy, which also translates as spinal cord dysfunction caused by direct infiltration of the cord and compression. This dysfunction may lead to neurological signs such as weakness, numbness, and ataxia, which is the inability to coordinate movements correctly [37]. Myelopathy occurs first in the lower extremities to result in difficulty in walking, and the case may progress to paraplegia or quadriplegia if the tumor infiltrates the spinal cord further.

A second reasonably frequent symptomatology of spinal GBMs is radicular pain due to the lesion of peripheral nerves in the neighborhood of the spinal hump that is created by the tumor [38]. These deficits can be weakness, paralysis, or loss of reflexes and are usually found in the limbs supplied by the part of the spinal cord in question. As the tumor advances and puts even more pressure on the spinal cord, the parts of the autonomic nervous system that control bowel and bladder function become affected [39]. Consequently, the early diagnosis of spinal GBMs is minimal, and most patients are diagnosed when their neurologic manifestations have progressed to an advanced stage [40].

MRI remains the imaging method of choice for spinal tumors, but even with the use of advanced MRI techniques, differentiating spinal GBMs from other types of spinal gliomas or tumors is a challenge [41]. This often makes them seem different and heterogeneous, as well as allows them to penetrate deep and thus obscure their numbers. Also, the dreadful intramedullary spinal tumors do not appear as clearly on the X-ray as the extramedullary spinal tumors. Thus, early diagnosis of the cancer can be challenging [42].

Conditions in treating spinal GBMs are even more complicated. The spinal cord is also very sensitive, and during the operation, it is hazardous to touch, remove, or injure any part of it because it leads to paralysis or other kinds of neurological disability [43]. Surgical removal of the tumor is also complicated by the fact that spinal nerve roots are in the vicinity; it becomes hard to excise the whole mass without causing damage to the motor and sensory function of the patient.

In addition, radiation therapy can be applied in conjunction with surgery where, for various reasons, total excision is difficult. Nevertheless, spinal GBMs treated with radiation therapy are associated with risks such as radiation-induced myelopathy and chronic neurological decline [44]. The blood-spinal cord barrier also restricts universal medications such as chemotherapy, hence making the treatment of spinal GBMs very challenging. Spinal GBMs are particularly aggressive, and because of the inherent impossibility of obtaining complete resection of these lesions [45], the prognosis for such patients is comparatively dismal, with the majority of patients experiencing rapid neurological decline.

2.4 Corpus callosum

Glioblastomas in the corpus callosum are rare tumors but are essential to the clinician due to their characteristics. The corpus callosum is the most prominent structure in the white matter, and it links the cerebral half spheres and guides the wires between them [46]. As much as GBMs involve the cerebral hemispheres, CC is a small percentage of all glioblastomas. It is almost impossible to determine the actual incidence of corpus callosum GBMs separately due to confusion in differential diagnosis or attribution into other gliomas or brain tumors involving the midline structures of the brain. Nonetheless, it is appreciated that such tumors are relatively rare, and it is estimated that less than 5% of all glioblastomas arise within the corpus callosum [47].

Tumors in this specific brain area are so rare that they present a unique diagnostic and treatment problem. CC glioblastomas appear distinct from glioblastomas in other brain areas in how they grow and clinically manifest themselves. Because the corpus callosum is involved in IHC connection, its' tumors must affect cognitive and motor processes differently from tumors in other areas, which is why they are essential for investigating the integrity of brain networks and neurofunction [48].

Disconnection syndromes usually accompany the manifestation of GBMs in the corpus callosum due to the disruption of communication pathways between the two halves of the brain. According to the disconnection hypothesis, the central role of the corpus callosum is to facilitate the interaction between the two hemispheres. Consequently, the lesions in the area can lead to meaningful and latent cognitive and neurological deficits [49].

Studies show that the significant symptoms of this type of GBM include, but are not limited to, memory impairment [50]. This characteristically presents as short-term memory disturbances, in which patients cannot absorb new information or recall events that have occurred. Ideally, long-term memory can also be compromised based on the position and size of this tumor. Neuropsychological deficits include deficits in higher-order executive functions such as planning, problem-solving abilities, decision-making, and attention span. These functions are mainly seated in the frontal lobes [51]. However, due to the integral role of the corpus callosum in integrating the function of the two frontal lobes, any damage to this area leads to a lack of synergy of the cognitive jobs.

Other such signs may include visual-organizational impairments that stem from the breakdown of the lateral connections between the vertical halves of the brain. These may consist of spatial judgment, how one interacts with an environment, navigation, and interpreting details of sight. Disorientation, poor judging of distances, and difficulties in copying objects or recognizing spatial arrangements may be expected. In severe manifestations, there may be hemispatial neglect, which is the inability of the patient to detect or acknowledge objects or events occurring in one half of their universe, typically the opposite side to the affected side of the brain [52]. These cognitive and visual-spatial impairments result from the damaged neural connections through which cortical areas of the brain relay information necessary for interhemispheric integration.

Besides cognitive and perceptual impairments, corpus callosum GBMs may bring about motor impairments not only if the tumor lesion areas are assigned with motoring coordination between the brain's two hemispheres [53]. Some of these signs could be weakness of arms or legs and other muscle formations, such as weakness because signals from the motor tracts in the brain may not be passing correctly. Seizures are also there because the tumor in the corpus callosum can

create electrical dysfunction in the brain and can result in focal or general colonic convulsions.

The glioblastomas in the corpus callosum are especially difficult to operate on because of the disease's butterfly shape and the ability to affect both sides of the brain [54]. Once GBM forms in the corpus callosum, it occupies a vast brain area, crossing over to the other side and forming the butterfly shape seen during scans. This pattern arises due to tumor growth across the midline from one hemisphere to another and is characteristic of corpus callosum glioblastomas. This bilateral spread makes the tumor peculiarly hard to manage as it transverses the hemispheres of the brain and is not singular to a given area [55].

Surgical resection is generally accepted as the mainstay of management of GBMs; however, complete excision of these tumors is especially difficult given the convoluted vasculature and delicate fiber connections in the region of the corpus callosum. The situation is such that even if there is a surgical attempt to remove the tumor, both hemispheres will be affected as well, and there will be potential for profound neurological deficits [56]. Surgical excision is typically incomplete because the cancer is infiltrative and situated in a densely populated region. Sometimes, only a part of the tumor can be removed; this puts a constraint on the efficiency of this operation in terms of patient prognosis.

Radiation is usually given after surgery to curtail cell division and tumor growth, but since the brain is so essential, potential damage to the latter in the process is an issue. It is known that neurocognitive decline in areas of radiation-related regions, such as the corpus callosum, is also a risk [57]. However, treatments such as chemotherapy with substances including temozolomide are likely to show only moderate effectiveness because many chemotherapeutic agents cannot cross the blood-brain barrier and penetrate the tumor mass [40]. Nevertheless, the chemotherapeutic agents can cross the blood-brain barrier more easily if nanotechnology is combined with chemotherapy [41]. These difficulties mean that optimal treatment outcomes for symptom and tumor control in corpus callosum GBM patients with the disease cannot be attained.

These are accompanied by other treatment difficulties of corpus callosum gliomas, including the functional decline patients experience during their treatment [57]. The lesioning effect of the tumor on cognitive functioning and interhemispheric exchange results in a severe decline in the quality of life. Despite early aggressive therapeutic regimens like surgery, radiation, and chemotherapy, the overall survival of patients with corpus callosum GBMs is generally considered poor [58]. It tends to be less favorable than GBMs at different anatomical sites. This is partly because this particular tumor is invasive and can impact critical cognitive and motor skills.

2.5 Optic pathways and hypothalamus

Optic pathway and hypothalamic glioblastomas are rare in adults and constitute less than 5% of all glioblastomas. Enlarging tumors usually occur in the midline brain structures—regions essential for physiological operations such as vision and hormonal control [59]. These tumors are not exclusive to patients with genetic syndromes but are more often encountered within the setting of Neurofibromatosis type 1 (NF 1). NF1 is a disease characterized by growing numerous body tumors, gliomas, and other changes, including café-au-lait spots, freckles, and optic gliomas. The optic pathways and hypothalamus are overgrown in patients with NF1, so glioblastomas in those individuals are constant [60].

In patients who do not have NF1, GBMs involving optic pathways and hypothalamic locations are extremely rare and are considered to have an adverse biomolecular profile and outcomes. These tumors are most often diagnosed in the late stage because they usually manifest very mild signs at the early stages and have the tendency to infiltrate the surrounding brain nervous tissue in critical areas, which makes detection and treatment more difficult [61].

The most frequent feature observed in patients with neoplastic processes of the optic pathways is visual pattern deterioration. Optic pathways encompass optic nerves, optic chiasm, and tracts, which act to relay optical information from the neural retina to the brain [62]. Another feature seen in these patients is endocrine dysfunction, of which the most frequently reported disturbance is diabetes insipidus [63]. This condition is due to injury to the hypothalamus or pituitary gland, which controls body water level and secretion of hormones. Diabetes insipidus means diabetes in Nigerians, which is the production of large volumes of dilute urine by the kidneys due to a deficiency of antidiuretic hormone (ADH) [64]. The hypothalamus plays a crucial role in regulating hormones within the organism; thus, the injuries sustained by this area may affect a number of hormones.

Another manifestation associated with hypothalamic disease is diencephalic syndrome. This syndrome is characterized by growth failure in stature, increased motor activity, and poor weight gain [65]. Surgical removal of GBMs in the optic pathways and hypothalamus is very challenging since the tumors are located in areas that contain critical neural elements [66]. The optic pathways and hypothalamus are placed sharply in the depth of the brain; certain other formations are nearby—ones that make it possible to see and regulate endocrine glands and feelings. Operations involving the removal of tumors in this area are hazardous because of the structures that may be damaged during surgery.

Another treatment method is radiation treatment; however, it is not very effective around the optic tracts and hypothalamus. They also pointed out that radiation also induces new neurological damage and worsens the condition further [67]. In addition, because of the invasive character of glioblastomas, these tumors are prone to recur after treatment. As they are almost impossible to eradicate and tend to invade the adjacent tissues, there is a tendency of recurrence, and patients' prognosis remains bleak when the tumor is located in such a region of the brain [68]. The impairing functional effects, such as hypothalamic and optic pathway glioblastomas, dramatically reduce the life quality of such patients, complicating treatment planning.

2.6 Ventricles

Supratentorial intraventricular glioblastomas are rare compared to the common infratentorial glioblastomas in the cerebral hemispheres or the brainstem [69]. These tumors are a rare occurrence in patients with GBMs and, according to some statistics, contribute to less than 1% of glioblastomas. However, intraventricular gliomas are detected more frequently in other types of tumors, e.g., ependymomas or choroid plexus tumors; invasive GBMs located in the ventricular system pose specific clinical and therapeutic considerations.

The lateral ventricles, the third ventricle, and the fourth ventricle are the parts of the ventricular system in the brain that are involved in producing the cerebrospinal fluid needed for the protection and cleaning of the brain [70]. For example, intraventricular GBMs tend to present considerable clinical signs and symptoms owing to their location and interference with CSF circulation. This results in constitutive

changes within the ventricle, with the formation of hydrocephalus, characterized by an influx of CSF-enhanced intracranial pressure (ICP) [71]. It may arise in any of the ventricular areas but is most dangerous when the tumor location is at the third ventricle and the fourth and when it blocks the passage between the lateral ventricles and the third ventricle.

Some of the distinguishing features of IVGGBM include the following: These headaches are generally due to hydrocephalus, which causes increased pressure in the cranial cavity. These headaches can be severe and are usually worse in the morning because CSF normally collects at night because of the patient's head's position, raising intracranial pressure [71]. Patients may also have nausea and vomiting, which are primarily related to elevated intracranial pressure and which can be aggravated by head positioning or vigorous movements [72]. Weakness or gait problems may arise if the tumor affects the motor tracts directly through infiltration or compression. In worse conditions, they may experience hydrocephalus or even an increased ICP that leads to a coma or death without treatment.

A further sign that may be attributed to intraventricular GBMs is seizures, which are caused by the disruption of the normal functioning of the brain by tumor tissue. Still, the presence of a tumor in an elementary or strategic part of the brain causes electrical dysfunction, which results in turmoil or seizures. It is worthwhile to note that intraventricular GBMs pose specific diagnostic and therapeutic problems because of their location and anatomy of the brain's ventricular system. These tumors are often not easily seen in the early stages by conventional imaging methods [73]. Other structures usually obscure them, are infiltrative, and may mimic others, such as ependymomas or choroid plexus papillomas.

MRI is the most used imaging technique for diagnosing intraventricular tumors because MR imaging offers good resolution of soft tissue and helps identify tumors located in the brain's ventricles. However, even with the help of high-resolution imaging, intraventricular GBMs can be challenging to diagnose in the early stages because they present heterogeneity diffusely in the tumor, which comprises solid malignant components and zones of necrosis/cystic change [74]. Moreover, these tumors may resemble the surrounding ependymal tissue, so differentiation from other pathological processes may be challenging, especially when the contrast enhancement is not very prominent.

The primary treatment of intraventricular GBMs is surgical resection, although it presents specific challenges due to the intricacy of the ventricular system. The ventricles are situated deep in the brain; hence, the tumors in this region exert pressure on and invade the neighboring neural tissue, making it difficult to surgically remove without risking destruction of the cerebral aqueduct or the cranial nerves, or any other important part of the brainstem [75]. Radiation therapy is often applied alongside surgery because complete removal of the tumor is impossible in many cases. However, since the ventricles are near the brainstem and the optic pathways, the radiation toxicity results in long-term effects such as decreased neurocognitive function, vision impairment, and endocrine disturbances [76]; radiation therapy may not even be curative since GBMs are often infiltrative and aggressive tumors.

Temozolomide and other chemotherapy drugs have been combined with surgery or given after surgery in an attempt to increase their effectiveness. However, they face the same limitation as radiation in treating intraventricular GBMs in that they cannot penetrate the blood-brain barrier to deliver high concentrations of the drug

to the tumor site. Issues related to providing sufficient amounts of chemotherapy to the tumor site, as well as the general aggressiveness of glioblastomas, make sustaining treatment of these tumors particularly problematic.

2.7 Pineal region

Pineal region glioblastomas are rare, comprising only a tiny fraction of all glioblastomas; thus, they are an uncommon and challenging type of brain tumor [77]. It is a small endocrine gland in the middle of the brain between the two halves or between the cerebral hemispheres and very close to the midbrain, thalamus, and cerebellum. Due to such a location, it may manifest unique symptoms from those seen in glioblastomas developing in more typical places such as the frontal or temporal lobes. Pineal GBMs are seldom diagnosed because these kinds of tumors rarely occur within this area of the brain and because they are frequently confused with other forms of tumors like those of the pineal gland or germ cell tumors [78].

Because the pineal gland is located adjacent to the third ventricle and other crucial structures in the brain, tumors in this area can severely impair typical brain functionality and cause individual neurological manifestations [79]. However, these types of tumors are pretty rare, and consequently, the diagnostic and therapeutic approaches are quite problematic due to the location of the growth and its increased anaplasticity. This results in the accumulation of CSF, thus hydrocephalus, and may lead to ICP [80]. Headache, vomiting, nausea, significantly increased intracranial pressure, and papilledema, established by ophthalmoscopy, may point toward the diagnosis. The involvement of the midbrain can alter autonomic functions, and the patient can experience irregular heart rate, breathing difficulties, and fluctuating blood pressure. Further, since the pineal gland is part of circadian rhythm regulation, people with tumors in this region may have sleep problems or disruptions in their biological clock, causing sleeplessness or oversleeping during the day. Because many of the tumors are located near spinal pathways, a patient may experience motor deficits, including ataxia or paraplegia [81].

The management of pineal GBMs is incredibly daunting given the site of tumor involvement in the midline of the posterior third ventricle and close to structures such as the midbrain, thalamus, and upper brainstem. Surgical removal remains the mainstay of therapy for glioblastomas. However, the surgery of pineal region tumors is very hazardous. The pineal gland is vulnerable; it is located within the brain core and surrounded by essential neural elements for respiratory, cardiac, and motor functions [82]. Lesions in this region are often infiltrative, and although we can attempt to remove as much of the tumor as possible, a safe margin may be impossible without compromising these structures. However, the overwhelming feature of this tumor reflects grave difficulties in early diagnosis since the lesion is deep within the breast parenchymal tissue, and the enhancement pattern might be heterogeneous [83].

2.8 Possible mechanisms and etiologies

One potential explanation is independent tumorigenesis, where both tumors (meningioma and GBM) develop as separate and unrelated events due to distinct pathophysiological processes. Factors such as age-related genetic instability or environmental influences, like prior radiation exposure, may independently trigger the development of both tumor types [75, 76].

Another possibility is the presence of common etiological factors that contribute to the formation of both tumors. Patients with a history of cranial irradiation are known to have a higher risk of developing multiple primary brain tumors. Additionally, certain genetic syndromes, such as Li-Fraumeni syndrome or Neurofibromatosis Type 2 (NF2), predispose individuals to multiple brain tumors, including GBM and meningiomas [78].

A third proposed mechanism involves paracrine or local interactions, where the presence of one tumor alters the local microenvironment in a way that promotes the development of another tumor nearby. This hypothesis suggests that cellular signaling pathways or changes in the extracellular matrix might play a role in the coexistence of the two tumors [80].

Finally, the possibility of chance coexistence cannot be ruled out. Meningiomas are relatively common brain tumors, and their co-occurrence with GBMs, Collision Tumor, may be coincidental rather than indicative of a shared causal mechanism. Given the high prevalence of meningiomas, it is possible that the simultaneous appearance of a meningioma and GBM in a patient is a matter of statistical probability [81].

By considering these potential mechanisms—independent tumorigenesis, common etiological factors, paracrine or local interactions, and chance coexistence—clinicians and researchers can better understand the underlying factors that contribute to the development of multiple primary brain tumors. This understanding is essential for accurate diagnosis, prognosis, and the development of effective therapeutic approaches [82].

3. Summary

The likelihood of developing multifocal GBM should therefore be even lower. Ultrasonography and MRI scans are widely in use today, and more surgeons are employing surgical excision even on multiple tumors, which has led to the increase in reporting of these lesions in the modern world. Multiple GBMs were seen in 11.7% of patients with GBMs in our study. Recurrent gliomas are those that are disseminated or growing by a defined pathway such as commissural fibers, cerebral spinal fluid, or local metastasis. On the contrary, multicentric gliomas are situated far from each other in different lobes or hemispheres, and the presence of two tumors in the same cannot be caused by the mentioned above mechanisms. The practical application of this distinction between multiple GBMs as either multifocal or multicentric is gradually becoming irrelevant. Different research indicates that the distinction between the two groups offers no clear pattern of clinical usefulness. Multifocality and multicentricity of the tumors did not influence the pathology of the GBMs in our context. For this reason, we have not divided GBMs into these groups and rather have put them together into multiple GBMs.

Unfortunately, the detailed pathogenetic processes of multifocal/centric gliomas have not been fully understood. Nevertheless, previous and current research has significantly expanded what is already known about the etiology of these types of lesions. According to the hypothesis proposed by Willis, there was an attempt to advocate a two-stage model of tumorigenesis resulting in multiple gliomas. In the first stage, a large area, or perhaps the whole brain, underwent some transformation, which was labeled as initiation. This step was considered something that made the barrier of the brain very susceptible to frank malignant changes. In the second step, because of different types of stimulation (mechanical, viral, or biochemical), there

was excessive cell division in various regions of the program, giving rise to gliomas in different places. This is also described as "promotion" delineated by metachronous development of these tumors.

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Chapter 2

Identifying Glioma Margins Rapidly during Microsurgery via Microendoscope Systems: History and Prospects

Weichi Wu, Po Zhang, Baoshu Xie and Kejun He

Abstract

The standard protocol of treatment for glioma currently involves maximal surgical resection while maintaining neurological function. Thus, accurately and promptly delineating the glioma's margins during microsurgery is crucial. This is to ensure minimal residual tumor tissue and avoid excessive removal, which can result in neurological impairments. The conventional and widely practiced method in neurosurgery is the use of frozen pathology. However, due to the significant time and effort this process demands, quick identification is not feasible, especially when there is a large remaining cavity requiring multiple site samplings. In the past few decades, there has been considerable advancement in surgical technology for identifying tumor boundaries. Systems like DiveScope, such as endoscopic Raman spectroscopy, multiphoton endomicroscopy, and confocal endomicroscopy, have shown significant progress. In this chapter, we aim to review the historical development and future potential of handheld pathology microscopes in neurosurgical microsurgery.

Keywords: glioma, surgical margins, Raman spectroscopy, confocal endomicroscopy, DiveScope, histopathology

1. Introduction

Gliomas are the predominant malignant tumors of the central nervous system (CNS) [1], with high-grade gliomas being especially lethal, carrying the highest mortality rates [2]. Epidemiological studies indicate that the incidence rate of glioblastoma multiforme (GBM) is approximately 5.26 per 100,000 individuals, with close to 17,000 new diagnoses annually [3–5]. GBM is associated with both gender and age [6], exhibiting a male-to-female ratio of around 1.632:1 [7]. The age distribution shows a single peak, predominantly affecting those aged 60–80 years, who make up about 63% of cases [7]. The most important prognostic factor for glioma patients is the residual tumor burden [8]. Research by Petrecca K and colleagues has

demonstrated that 90% of glioblastoma cases will recur at the original site within 2 years, even if gross total resection (GTR) is performed [9]. Currently, the standard treatment for gliomas is maximal surgical resection while preserving neurological function [10–12]. The extent of tumor resection is increasing the survival. In numerous cases of recurrence of GBM, maximalist tumor resection remains the first-line treatment followed often by a three-year survival in patients who had such resection [13]. Therefore, it is essential to accurately and swiftly delineate the boundaries of the glioma during microsurgery. This precision is necessary to minimize residual tumor tissue and prevent excessive removal, which can lead to neurological damage [14].

In the field of neurosurgery, frozen pathology has long been recognized as a traditional and well-established method [15]. Despite its effectiveness, this technique is both labor-intensive and time-consuming, making it challenging to achieve rapid identification, particularly when dealing with a large residual cavity and multiple sampling sites [16]. Over the past few decades, there have been significant advancements in microsurgical techniques for the delineation of brain lesions [14]. Currently, various intraoperative marginal exploration methods have been documented. These methods can be categorized into radiography techniques, fluorescein-guided surgery (FGS) techniques, and optical techniques. Microendoscope systems such as the DiveScope, endoscopic Raman spectroscopy, multiphoton endomicroscopy, and confocal endomicroscopy have been developed [14]. The radiography technique, however, only provides macroscopic visibility, which does not align with the principle of maximal resection [16]. By the way, as long as laboratory diagnosis techniques are considered, MRI and CT exams being used in emergency situations, other techniques such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT) facilitate early detection and treatment monitoring [17]. In this context, we aim to review the historical development and prospects of handheld pathology microscopes in neurosurgical microsurgery.

2. Fluorescence-guided surgery

Fluorescence-guided surgery (FGS) is a medical imaging technique that assists neurosurgeons in enhancing the visualization of specific tissues during microsurgeries [14]. It utilizes special dyes or near-infrared emitting lights that bind to target tissues like tumors. When hit with a specific light, these dyes glow, and the fluorescence can be detected by special cameras. This makes it easier for surgeons to spot and remove diseased areas while keeping the healthy tissues intact. FGS helps make surgeries more accurate and cuts down on complications.

FGS has revolutionized intraoperative visualization for neurosurgeons tackling glioblastomas. Fluorophores like indocyanine green (ICG), 5-aminolevulinic acid (5-ALA), and fluorescein have been extensively examined in the context of glioblastomas (**Table 1**). The light emitted by these fluorophores aids in distinguishing the tumor's core from its invasive margins, usually within the visible and near-infrared spectrums. Here, we mainly introduce three imaging agents: indocyanine green, 5-aminolevulinic acid, and fluorescein.

2.1 Indocyanine green

Indocyanine green (ICG) is a fluorescent dye that works in the first near infrared window (NIR-I, 700–900 nm) with an excitation wavelength of 740–800 nm and

Fluorescent dye	Number of patients	Pathology result	Golden standard	Sensitivity (%)	Specificity (%)
5-ALA [18–20]	82	Glioblastoma/HGG and metastasis	Traditional fluorescence imaging/pathology	88.67	87
Fluorescein [21–25]	111	Glioblastoma/HGG	Pathology	83.16	89.9
Indocyanine green [26–28]	74	Gliomas/HGG	Pathology/ Traditional neural network	95	60.95

Table 1.
A summary of studies exploring diagnostic accuracy of FGS in brain tumors.

emission wavelength in the 800–860 nm [29]. Similar to fluorescein, after intravenous injection, ICG can penetrate areas where blood-brain barrier (BBB) is incomplete. In addition to its traditional application as a fluorescent dye, second-window-ICG (SWIG) has recently shown promise in identifying tumor tissue. For this application, ICG is intravenously injected in high doses to patients 1 day before surgery, allowing it to accumulate in the extracellular matrix of the tumor microenvironment and surrounding edema [30, 31].

The pilot study involving SWIG in 15 glioma patients in 2016 showed a sensitivity of 98% and a specificity of 45% for marking tumor tissue, compared to 84% sensitivity and 80% specificity in bright field [26]. Subsequent research by the same research group demonstrated that SWIG achieved a 97% sensitivity and 56% specificity for border specimens in contrast-enhancing tumors, compared to 78% sensitivity and 100% specificity in bright field [27].

Nowadays artificial intelligence is getting involved in a large amount of research fields [28]. More recently, researchers have developed a technique combining fluorescent imaging with artificial intelligence, specifically deep learning, to identify between tumor and normal tissues during microsurgery. This method achieved a sensitivity of 93.8% and a specificity of 82.2%. NIR fluorescence generally offers higher sensitivity but lower specificity in comparison with other fluorescent dyes. However, when combined with deep learning techniques, both sensitivity and specificity improved [28].

Additionally, NIR-II fluorophores (operating between 1000 and 1700 nm) offer more precise detection, more outstanding contrast, and smaller absorption in comparison to NIR-I and the visible spectrum. Although the exciting results above have primarily been observed in animal experiments for now [32], recent clinical trials involving seven patients tested the efficacy of NIR-II fluorophores. Utilizing a multispectral fluorescence imaging equipment and an image fusion strategy, researchers were able to precisely block the vessels surrounding the tumor, significantly reducing blood loss during microsurgery [33]. Future clinical studies are needed to further validate the increased sensitivity and specificity of these fluorophores for distinguishing between normal and tumor tissue in human gliomas.

2.2 Fluorescein

Fluorescein sodium salt, known for its yellow-green fluorescence in bright fields (with a maximum absorbance at 494 nm and an emission peak at 521 nm),

was applied to identify brain tumors like glioblastomas during open biopsies since 1948 [34]. As an intravascular dye, fluorescein is administered intravenously and can pass brain regions with a disrupted BBB [21]. However, the fluorescence emitted by fluorescein is not specific to glioblastoma cells. Numerous research has assessed the possibility of application of fluorescein on guided brain tumor resection [21–24], reporting median values of sensitivity and specificity of 82.2 and 90.9%, respectively.

In a phase II trial involving 57 high-grade glioma (HGG) patients, the utilization of a specialized fluorescence filter in the microsurgical scope was tested for its efficacy in HGG [35]. In this study, 82.6% of patients completed GTR, with the fluorescent tissue at the tumor border being distinguished with a sensitivity of 80.8% and specificity of 79.1%. Another research group also examined the use of fluorescein for distinguishing biopsies from the tumor border through quantitative fluorescence measurements, finding decreased sensitivity (69.4%) and specificity (66.7%) [22].

The association between fluorescence intensity at the tumor border and the presence of residual tumors on postoperative MRI was also explored [36]. Researchers reported a sensitivity of 66.7% and a specificity of 75%, which aligned with the findings of Acerbi et al. and Neira et al., despite differences in fluorescent agent injection timing and kinetics in both studies [22, 35].

In conclusion, further assessment of the application of FGS is necessary, incorporating postoperative CT/MRI and quantitative calculation of fluorescence levels in resected tumors during microsurgeries. For now, this method is primarily used in vascular neurosurgery, as fluorescein, being an intravascular fluorescent agent, can blur the surgical area during microsurgeries when blood obscures the operative site.

2.3 5-Aminolevulinic acid

In the heme biosynthesis pathway, 5-ALA acts as a precursor to the fluorescent compound protoporphyrin IX (PpIX). PpIX is able to accumulate within malignant cells of both epithelial and mesenchymal origin, emitting bright red fluorescence under violet-blue light, whereas normal cells stay non-fluorescent [37]. The endogenous PpIX captures light at 405 nm and emits it with a peak at 634 nm [37, 38]. A research group investigated the application of 5-ALA in initially diagnosed glioblastomas [39] and discovered a notable increase in the complete GTR rate of glioblastomas (65% for the group with 5-ALA compared to 36% for the regular group). Furthermore, patients treated with 5-ALA exhibited longer progression-free intervals and median survival times. Subsequent research has consistently demonstrated the high sensitivity and positive predictive value of 5-ALA in both initially diagnosed and recurrent HGGs [18, 37, 40].

Two comprehensive analyses compiling sensitivity and specificity data for 5-ALA in application on HGGs resection microsurgeries reported sensitivity in 81–83%, and specificity ranging in 89–90% [41, 42]. Nonetheless, on a microscopic level, this result is still inadequate for microsurgery resection guidance at tumor edges, primarily because PpIX levels generally fall below the detection threshold of traditional microsurgical microscopes. Within the invasive edges of diffuse HGG, the intensity of PpIX fluorescence reduces, complicating differentiation from nearby non-fluorescent normal tissue. Recent research indicated that “dark” tissue retains a median residual cell density of 13% [43]. In contrast, for low-grade gliomas, there is limited utility of 5-ALA, with detectable fluorescence in only 10–20% of patients applying fluorescence microscopy [44, 45]. Additionally, assessing fluorescence intensity largely relies on the neurosurgeon’s experience, making quantification challenging.

Therefore, cutting-edge technologies like confocal microscopy and spectroscopy are being applied to more precisely measure absolute PpIX fluorescence concentrations [19, 46]. Despite this, 5-ALA remains the gold standard for malignant glioma resections due to its well-established efficacy and safety.

2.3.1 False-negative fluorescence

The invisibility of glioblastoma under 5-ALA-induced fluorescence can be attributed to certain conditions. First, glioblastoma have a diffusely infiltrative growth feature, meaning tumor cells can be present at lower densities, even several centimeters away from the main contrast-enhancing tumor mass. 5-ALA FGS has become essential in many neurosurgies. However, its negative predictive value varies between 22 and 91% [18, 47, 48], in areas with low-density cancer cell invasion. Furthermore, false-negative 5-ALA fluorescence can result from structural barriers that hinder the fluorescence of tumor cells. The fluorescent glioblastoma cells may be obscured by photobleaching, blood, or overhanging brain tissue, which blocks the visualization of the resection chamber. Additionally, the timing of 5-ALA injection is crucial and may explain the absence of tumor fluorescence in some instances.

2.3.2 False-positive fluorescence

Instances of 5-ALA-emitted fluorescence in tissues not related to glioblastoma have occasionally been observed. Multiple research teams have reported fluorescence in areas around the tumor resection cavity near viable cancerous cells, but not in healthy brain tissues far from the main tumor mass [48–50]. Additionally, this phenomenon has been observed in patients with recurrent glioblastomas who have undergone previous adjuvant therapies [51]. Reactive astrocytes might contribute to false-positive fluorescence in these patients [20, 51]. Another uncommon cause for false-positive fluorescence could be the autofluorescence of healthy tissue at the microscopic level [20]. In a study involving 313 patients who underwent surgery for suspected recurrent glioblastomas according to medical imaging, approximately 3% were found to have pathology consistent with radiation necrosis in tissues exhibiting fluorescence [52].

Lastly, it has been observed that necrotic regions of glioblastoma show either no tumor fluorescence or significantly reduced fluorescence [53]. Likewise, anaplastic oligodendrogliomas have been noted to exhibit diminished fluorescence following the injection of 5-ALA.

3. Optical technique

Confocal laser microscopes and Raman spectrometers are two principal categories in the field of optics [54]. The ZEISS CONVIVO® System is an example of confocal laser microscopes, using histomorphology to distinguish tumor cells. Although the handheld use of the probe is more convenient, it must be mounted on the retractor arm to produce higher-quality images, as handheld operation results in lower image quality [55].

3.1 Confocal laser microscopes

First commercialized in the 1980s, confocal microscopy is widely employed for imaging of thick tissues on molecular level within the biomedical research. The drive

to reduce the size of confocal microscopy into an endoscope (LSCE) arose from the necessity to diagnose premalignant lesions in epithelial tumors in vivo [56]. LSCE has undergone wide evaluation for the identification of Barrett's esophagus, urothelial cancers, and cervical cancers, etc. [57].

LSCE allows for imaging in vivo in a noninvasive way through a technique known as optical sectioning [58, 59]. This method enables the microscope to specifically focus on a selective depth by filtering out most out-of-focus photons above and below the focal plane using a pinhole [60]. This results in higher contrast imaging of thick tissues versus traditional wide-field microscopy, which often produces background light and scatter [61]. Additionally, endomicroscopy facilitates 3D image reconstruction and enhanced imaging parameters [61]. Lastly, this technology allows for histomorphological analysis in real time with improved diagnostic yield, eliminating the need for conventional processes of excision, fixation, and staining.

Although neurosurgeons use macroscopic FGS, distinguishing between normal and tumor cells remains challenging during microsurgeries. Hence, LSCE could offer new cellular insights into glioblastoma architecture. Research has shown that confocal endomicroscopy with fluorescent agents is effective for precise visualization and resection of glioblastoma in vivo in both animal experiments and, to some degree, clinical trials [46, 62, 63]. These findings highlight the in vivo imaging of glioblastoma with fluorophores. Selectively, 5-ALA and LSCE systems can aid in intraoperative diagnosis and real-time, fluorescence-guided glioblastoma resections. Further clinical experiments are necessary for nonspecific fluorescent dyes like ICG and fluorescein, which stay in the extracellular space.

3.2 Raman spectroscopy

Raman spectroscopy is a visual technique that can deliver comprehensive quantitative chemical data regarding a specimen [64]. It is especially suitable for in vivo assessments since the energy levels and excitation wavelengths employed do not harm the tissue and can penetrate relatively deeply.

Raman spectroscopy has been instrumental in the precise in vivo detection of various tumors, such as breast, skin, colon, gastric, and esophageal tumors [65–69]. This technique utilizes the interaction of light with molecular binding to determine the chemical components present in a tissue.

Despite the promising results of Raman spectroscopy in accurately identifying tumor tissues, its use for delineating tumor edges is still restricted. The rarity of Raman scattering events requires quite long acquisition times (around 1 second per acquisition) to obtain a sufficient signal [70]. This makes pixel-by-pixel measurement of a microsurgical field of view (FOV) impractical, as it can take several hours to image even a tissue smaller than 1 mm^2. Although efforts have been made to shorten imaging times for tumor edges identification, such as integrating autofluorescence guidance with specific Raman spectroscopic sampling, these methods still need a minimum of 12 minutes for the processing.

In practical application, in vivo use of Raman spectroscopy has typically depended on gathering point spectra using handheld fiber-optic probes. This method provides diagnostic data at specific points instead of offering a wide-area diagnostic image, as fluorescence imaging does. While this approach can yield extremely precise diagnoses at individual points, identifying tumor edges is impractical unless multiple points can be simultaneously visualized and documented. Despite these limitations, it delivers extensive biomedical data that complements the macroscopic morphological data obtained from

widefield imaging. Consequently, building a system that integrates in-space co-registered spectroscopic diagnostic data with widefield imaging is able to significantly enhance the application of Raman spectroscopic probes for tumor edge identification.

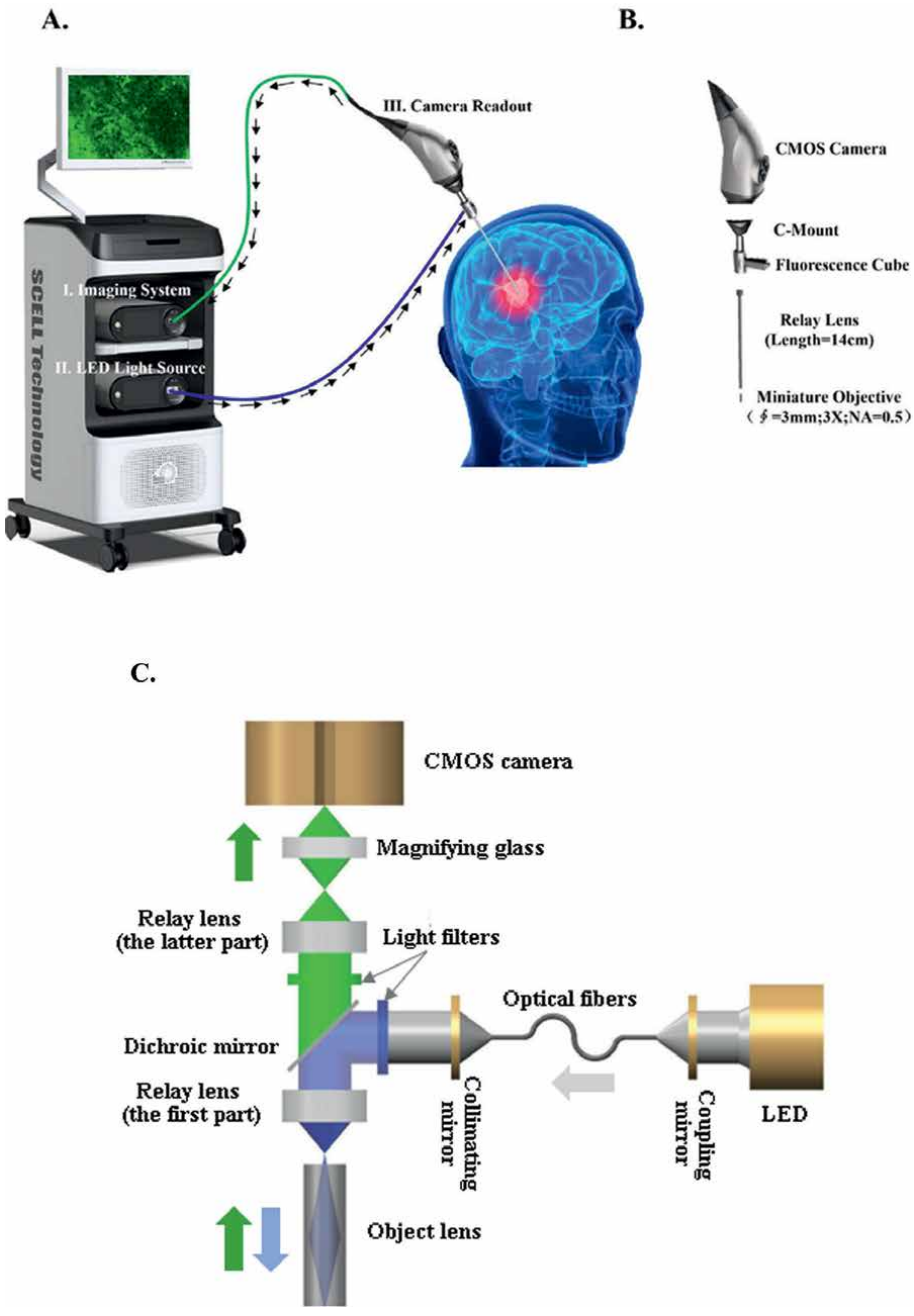

Figure 1.
The structure of DiveScope, the intraoperative histopathological microscope. (A) The system schematics of DiveScope, which is composed of the mainframe of imaging system, LED light source, a camera readout, a monitor, a distal hood, and a power cord (the power cord is not drawn in the figure). (B) The optical components of the camera readout include a CMOS camera, a C-Mount, a fluorescence cube, relay lens, and the miniature objective. This figure is original.

3.3 DiveScope

Systems akin to the DiveScope microendoscope include endoscopic Raman spectroscopy, multiphoton endomicroscopy, and confocal endomicroscopy. With a penetration depth of 50 μm, the DiveScope endomicroscope is well-suited for visualizing cell histomorphology.

One of DiveScope's primary benefits is its OIS anti-shaking feature, which prevents image distortions due to pulsatile arteries and involuntary movements (**Figure 1**). This design supports real-time tissue morphology feedback with a video output of 60 frames per second [54]. The DiveScope can provide the capture of tissue and cellular sub-structures including nucleoli, at an extreme-high resolution of up to 50 μm [54]. Glioblastomas are distinguished by upregulated cell density, irregular borders, larger mass, and deeply stained nuclei. On the other hand, reactive gliosis presents an increased density of glial cells but lacks atypical cells. Conversely, normal neurons in brain are sparse and uniformly distributed [71]. In conclusion, it is essential for neurosurgeons to employ techniques like the DiveScope for intraoperative identification of glioblastoma edges.

In human in vitro experiments, researchers confirmed that the diagnostic accuracy of DiveScope surpassed that of pathologists' intraoperative frozen sections, despite the lack of statistical significance. This discrepancy can be attributed to the fact that intraoperative frozen sections are easily destroyed by ice crystals and tissue compression during resection [72], extensively impacting frozen section diagnostic value. Compared to conventional frozen sections, DiveScope can circumvent these limitations with a non-invasive probe in real-time. Concerning time effectiveness, the time of DiveScope consumption is much less than pathologists' reports which may avoid the adverse effects of extending operation time.

4. Future prospect

Although FGS and Optical techniques are still immature and imperfect in many aspects, people have not given up the pursuit of rapid intraoperative identification of glioma margins. Next, we will provide a preliminary discussion from several aspects to illustrate the current research directions of researchers, in order to inspire readers to think about the future development direction of intraoperative tumor margin identification technology.

4.1 Molecular targeted near-infrared fluorescence imaging

As previously mentioned in the context of FGS, many fluorescent agents in application at present still face challenges such as non-specific fluorescence and adverse effects. However, recent years have seen the advances of new approaches in this field, including the exploration of new fluorescent dyes and probes. One innovative method targets ligand specifically on tumor cells, such as epidermal growth factor receptor (EGFR). These fluorophores can aggregate in areas of the tumor with high expression, allowing for intra-operative visualization of the tumor [73, 74]. The protein is abundantly expressed in glioblastomas, making it a great target in the future. Currently not only EGFR, but also a principal neoplastic differentiation biomarker, IGF-I, present in glial neoplastic cells, but not in neuronal neoplastic cells, constitutes the target in cancer gene therapies approaches, especially in glioblastoma treatment [75, 76].

The first clinical trial with a combination of cetuximab, an EGFR inhibitor, and IRDye 800 CW, a near-infrared dye that emits at 805 nm, was conducted. The researchers also studied two patients (one given a low dose and the other a high dose) and were able to differentiate tumor from healthy tissue with a sensitivity of 73% and specificity of 66.3% in the low-dose patient, and a sensitivity of 98.2% and specificity of 69.8% in the high-dose patient [77]. In the future, studies should focus on various fluorophores, targets, and potential combinations with other fluorescent dyes to decide the optimal injection schedule and dosage [78]. For now, many fluorescent probes are currently in the early stages of clinical and preclinical trials.

4.2 Confocal reflectance microscopy

By employing light scattering technique, confocal reflectance microscopy (CRM) serves as a high-resolution imaging method that creates thin tissue sections images without invasion [79, 80]. CRM produces contrast by leveraging the inherent refractive differences of the focused tissue. Consequently, images feature lower refractive structures that emit fewer signals to the final output [80]. Because CRM does not depend on a Stoke's shift, it introduces significantly less energy into samples versus fluorescence imaging techniques [80]. This method enables the analysis of tissue cellularity and cytoarchitecture in real time with minimal generation of reactive oxygen species (ROS) [79, 80]. However, CRM technology is restricted by its shallow imaging depth (200–300 μm) and reduced clarity in visualizing subcellular images. Although upregulating wavelengths can enhance imaging depth, this comes at the expense of resolution and contrast [79].

CRM was explored for visualizing the structure of cellular and subcellular tissues. It has been used for noninvasive detection of skin conditions, intraoperative identification of tumor tissues and their edges, and evaluation of cellular and subcellular characteristics in both tumor and healthy liver tissues [79–83]. Recently, CRM has been employed to assess the cellular characteristics of glioma specimens in vitro [84]. The effective utility of this imaging technology in glioma microsurgery could increase the speed of sampling from the surgical area and improve diagnostic outcomes.

4.3 Two-photon and second harmonic production

The imaging depth of confocal and other linear fluorescence microscopy techniques may be restricted, but two-photon microscopy offers a unique strength in imaging deeper cell architectures [85]. Utilizing concentrated photons from a femtosecond laser, two photons, each carrying about half the energy needed to excite a fluorophore, simultaneously engage with the same molecule [86]. The intensity of those pulsed photons diminishes circumferentially around the targeted area, causing fluorescence being emitted from a small volume directly at the targeted point. This in-space limitation of fluorescence to the targeted point decreases scattering. Furthermore, two-photon microscopy employs infrared and far-red excitation wavelengths, which penetrate tissues more effectively than the visible wavelength light used in superficial imaging [86].

A different nonlinear imaging technique is second harmonic generation (SHG). This method produces contrast by measuring the extent of photon scattering when incident photons engage with heterogeneous tissues. In SHG, though two photons with half of the anticipated energy also converge at the targeted point, they interact

after being scattered by the identical molecule instead of being absorbed. This results in an "emitting" photon that effectively has double the energy, which is then received to form a picture with potential applications for in-vivo surgery. Nonetheless, further advancement of these techniques is necessary, particularly in enhancing image processing speed and addressing spatial recognition challenges [87].

5. Conclusion

The primary aim of glioblastoma microsurgery is to maximize tumor removal while keeping neurological functions and ensuring patient safety. Over time, various methods have been explored and assessed for detecting and distinguishing tumor tissue, each demonstrating varying degrees of diagnostic efficacy for various types and grades of gliomas. Consequently, it is crucial to recognize that resection in critical brain regions cannot rely solely on fluorescence imaging. Brain tumor microsurgery should always be dominated by the surgeon's experience and the ability to correlate surgical anatomy with supplementary techniques. This article summarizes the strengths and weaknesses of both traditional and experimental methods for glioblastoma microsurgery, with particular focus on the invasive edges of tumors and the intra-operative, real-time identification between tumor and healthy tissue.

In conclusion, all the approaches developed had their own strategical and practical constraints, leading us to advocate for a combined utility of intraoperative imaging approaches. This integrated approach often resulted in better efficacy concerning the degree of tumor removal and patients' progression-free survival. With the application of more and more techniques into the operating room, surgeons can choose the most suitable technology according to the tumor's features and their familiarity with various approaches. We sincerely hope that, in the future, after large-scale RCTs with improved and mature technology, intraoperative tumor identification will be a routine but crucial component of glioblastoma microsurgery.

Conflict of interest

The authors declare no conflict of interest.

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Section 2

Diagnostic of Tumour

Chapter 3

Innovative Diagnostic Approaches in Glioblastoma: The Role of Raman Spectroscopy and Liquid Biopsy

Hulya Torun and Ihsan Solaroglu

Abstract

Glioblastoma (GBM), the most aggressive and fatal type of adult brain cancer, poses significant diagnosis, treatment, and patient survival. Accurate diagnosis typically relies on invasive procedures such as tissue biopsy or surgical resection, sophisticated and time-intensive molecular testing, and histopathological evaluation. Despite significant advancements in understanding the molecular and genetic features of GBM, the overall prognosis remains dismal, with current treatment approaches offering only limited improvements in survival. Moreover, the majority of GBM patients experience tumor recurrence within 2 years of initial treatment, highlighting the inadequacies of existing diagnostic and monitoring approaches. While standard imaging techniques are substantial, they often lack the sensitivity and specificity to detect residual disease, evaluate recurrence, or effectively monitor therapeutic efficacy. With 14 months of average survival, there is a pressing need for innovative diagnostic approaches that are rapid, accurate, cost-effective, and minimally invasive. Liquid biopsy has emerged as a promising tool for GBM, enabling the analysis of circulating tumor materials and providing real-time insights into tumor dynamics. Surface-enhanced Raman spectroscopy (SERS) combined with artificial intelligence (AI) is a promising diagnostic paradigm for brain tumor detection, leveraging the sensitivity of SERS for molecular fingerprinting and the predictive power of AI. Together, these approaches offer significant potential to advance early diagnosis, improve postsurgical follow-up, and improve treatment assessment in GBM. By addressing current diagnostic limitations, these methods may pave the way for improved clinical outcomes and personalized treatment strategies.

Keywords: glioblastoma, liquid biopsy, Raman spectroscopy, blood biomarkers, artificial intelligence

1. Introduction

Glioblastoma (GBM) is the most common malignant central nervous system tumor, presenting significant challenges in prognosis, diagnosis, and therapy monitoring due to its aggressive nature, marked genetic heterogeneity, and unpredictable clinical behavior [1, 2]. In clinical practice, standard diagnostic techniques involve magnetic resonance imaging (MRI) to detect tumors and histopathology and molecular evaluation of tumor tissue for accurate diagnosis. Although these methods provide critical information, they have limitations [3, 4]. In clinical diagnostics, imaging techniques such as MRI and CT are primarily utilized in acute or emergency settings, while advanced imaging modalities like positron emission tomography (PET) and single photon emission computed tomography (SPECT) serve as critical laboratory tools for comprehensive evaluation. Additionally, molecular biomarkers including P53, S100, stem cell markers such as CD44, and particularly IGF-I, play vital roles in supporting the accurate diagnosis and characterization of tumors [5–7]. MRI, though noninvasive, often lacks the sensitivity to detect early molecular changes and can miss microscopic signs of tumor progression. Moreover, imaging may sometimes lack the specificity and sensitivity necessary to assess recurrence after treatment [4, 7] accurately. Histopathology, although specific, requires invasive surgical biopsies, which carry risks, particularly in brain tissues, and is not ideal for monitoring GBM's rapid and dynamic progression or diagnosing treatment-related radiological changes [8].

The *2021 WHO Classification of Tumors of the Central Nervous System* introduced key molecular parameters, fundamentally transforming the diagnosis and classification of GBM [1]. Under this updated framework, GBM is classified as an IDH-wildtype, CNS WHO grade-4 tumor, integrating both histopathological and molecular features into a layered reporting format to enhance diagnostic accuracy and prognostic stratification [9–11]. As established by Stupp et al., standard treatment includes radiation therapy combined with concomitant and adjuvant temozolomide, which offers modest survival benefits [12]. Despite these advances, the prognosis remains poor, with a median survival of only 14 months [12, 13].

Recent genomic studies have revealed the somatic landscape of GBM, highlighting alterations in key genes such as EGF and its receptor (EGFR), TERT, and PTEN [1, 9–11], as well as DNA methylation status [10, 14, 15]. From a pathogenic perspective, the development of the disease arises from disruptions in genetic pathways and growth factor signaling pathways, particularly those involving epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), PDGF, TGF-β, and most notably, IGF-I [1, 11, 16–20]. These findings have informed the development of molecularly defined subtypes (e.g., proneural and mesenchymal), each with distinct clinical behaviors and implications for therapy [10, 14, 15]. Comprehensive reviews of GBM biology emphasize the significant heterogeneity of the tumor, complicating therapeutic strategies [15, 21]. Although advances in treatment modalities such as surgery, chemotherapy, and radiotherapy continue to improve outcomes, significant challenges remain in tackling this aggressive disease [13, 22]. Fortunately, recent advancements in immunotherapy have yielded promising outcomes. Following the pioneering work on cancer gene therapy (immunogene therapy) by Trojan et al. in 1993 and the foundational developments in cancer immunotherapy by Townsend et al. in the same year, numerous other studies have emerged. Since 2015, immunotherapy has gained further recognition through initiatives such as the "Cancer Moonshot" program, and various immunotherapeutic strategies have increasingly been implemented in the treatment of solid tumors, including GBM [23–27].

Managing GBM remains a significant challenge due to the lack of reliable methods for early diagnosis and monitoring recurrence during treatment and/or differential diagnosis of posttreatment [13, 21]. Detection often occurs at advanced stages, when the tumor has already infiltrated surrounding brain tissue, making intervention less effective [22, 28–30]. Furthermore, GBMs are highly heterogeneous, comprising diverse cellular populations with distinct genetic, proteomic, and metabolic profiles [22, 31, 32]. This complexity hinders accurate diagnosis and tailored treatment, as conventional diagnostic tools struggle to capture the full molecular landscape of these tumors [22, 33, 34]. Addressing these limitations requires innovative diagnostic approaches that can provide early detection, evaluate treatment response, and provide better insights into the tumor's molecular diversity.

Distinguishing recurring GBM from therapy-related tissue changes, such as pseudoprogression or radiation necrosis, is a significant challenge in clinical practice. Current management of gliomas primarily relies on diagnostic imaging, which often lacks the sensitivity and specificity required to reliably assess recurrence after primary treatment [3, 35, 36]. Differentiating pseudoprogression from true progression is critical for clinicians to avoid unnecessary surgeries and ineffective treatments [35]. Traditionally, GBM response is evaluated using contrast-enhanced (gadolinium) T1-weighted MRI (**Figure 1**) [37]. However, this imaging method primarily reflects blood-brain barrier disruption and does not specifically measure tumor activity, leading to potential misinterpretations.

True progression reflects actual tumor regrowth and invasion despite treatment, whereas pseudoprogression, a temporary condition following radiotherapy or chemotherapy, mimics tumor growth on MRI but lacks active tumor proliferation [35, 38–40]. Pseudoprogression occurs in approximately 20–30% of GBM patients [3, 35] and can induce clinical symptoms, further complicating its diagnosis [36, 38]. Additionally, pseudoresponse [3], characterized by reduced contrast enhancement in patients treated with antiangiogenic agents like bevacizumab, can appear similar to therapeutic success but does not represent true tumor control [35, 38].

Given the limitations of contrast-enhanced MRI in differentiating these conditions, advanced imaging modalities, such as perfusion-weighted imaging, diffusion-weighted imaging, amide proton transfer, magnetic resonance spectroscopy, and

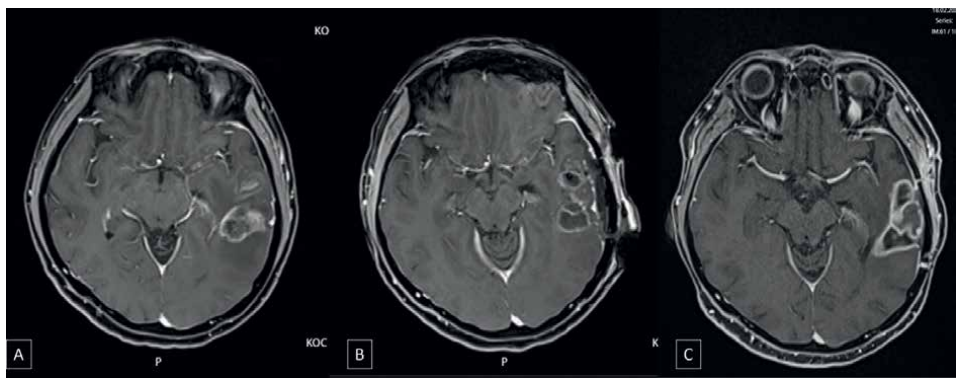

Figure 1.
Axial T1-weighted postcontrast MR images of a left temporal GBM. (A) Preoperative MRI showing left temporal GBM. (B) Image obtained 24 hours after surgical excision demonstrating total resection of the tumor. (C) Image obtained 3 months after completion of radiation therapy showing a rim-enhancing necrotic mass with a suspected recurrent tumor.

positron emission tomography (PET), are increasingly used to improve diagnostic accuracy [3, 37, 39, 41, 42]. These methods provide additional physiological and metabolic information, aiding in distinguishing true tumor progression from treatment-related changes [41–43].

Advanced imaging techniques, such as Dynamic Susceptibility Contrast MRI (DSC-MRI) and amino acid PET imaging, have demonstrated utility in distinguishing true tumor progression from treatment-related changes [41, 43]. DSC-MRI evaluates cerebral blood flow and volume, offering a more accurate assessment of tumor activity compared to conventional anatomical MRI [38, 43]. While advanced MRI techniques provide greater diagnostic accuracy for GBM patients, recommendations for posttreatment imaging vary in terms of frequency and timing [38, 42]. Currently, no reliable biomarkers or clinical features exist to differentiate true glioma progression from pseudoprogression detection [38]. Liquid biopsy techniques hold promise in addressing these challenges by providing noninvasive diagnostic tools [44–50]. Moreover, growing insights into the glioma tumor microenvironment and advances in immunotherapy emphasize the need to evaluate bodily fluid biomarkers for improved therapeutic planning and clinical management [44–48, 50].

Surgical resection or biopsy of a brain tumor poses significant postoperative complication risks to patients, including brain swelling, infection, hemorrhage, hematoma, and potential neurological impairment [51–53]. Furthermore, the location of some tumors may render them inaccessible for safe biopsy [54–56]. Diffuse intrinsic pontine tumors (DIPG), which account for 10–15% of all childhood brain tumors, are among the most challenging cases in pediatric neuro-oncology [56–58]. Diagnostic biopsies in DIPGs remain controversial due to the high sensitivity of the brainstem and the limited direct therapeutic benefits for the patient [59]. However, liquid biopsy techniques could facilitate the molecular profiling of DIPGs, help identify novel therapeutic targets, and enable the monitoring of treatment responses [59, 60].

Accurate delineation of tumor margins is critical during surgical interventions to achieve maximal tumor removal, minimize residual disease, and reduce the likelihood of recurrence. However, precise identification of tumor boundaries can be challenging due to the infiltrative growth patterns of gliomas and their indistinct borders, which often blend into adjacent healthy brain tissues [54, 61]. Conventional surgical navigation techniques based on preoperative imaging may not accurately represent real-time tumor margins, leading to incomplete resections or unintended damage to normal brain structures (**Figure 2**) [62]. To address this limitation, fluorescent agents

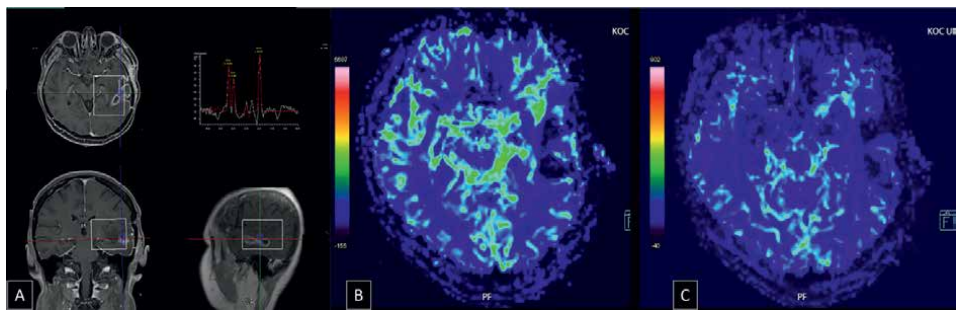

Figure 2.
(A) Three-dimensional proton MR spectroscopy of the lesion shows a decrease in NAA/choline ratio, (B) decreased cerebral blood volume (CBV), and (C) decreased cerebral blood flow (CBF), which are suggestive of pseudoprogression rather than recurrent tumor.

such as 5-aminolevulinic acid (5-ALA) have been employed to improve intraoperative visualization of tumor tissues. The use of 5-ALA has shown significant promise, with radiologically confirmed complete resection rates reaching up to 83% [54, 61, 63]. Nonetheless, this technique has its own limitations, including variable fluorescence intensity and limited specificity for low-grade or diffusely infiltrative tumors [64]. Thus, there remains a critical need for additional techniques that can reliably distinguish tumor margins at a microscopic or molecular level to further enhance surgical precision, reduce morbidity, and improve patient outcomes. Emerging techniques, such as intraoperative Raman spectroscopy and advanced molecular imaging modalities, hold the potential to address these shortcomings and further refine the accuracy of tumor margin delineation [65–79].

Moreover, traditional tissue biopsies often fail to capture the tumor's heterogeneity or represent real-time tumor activity [80, 81]. Liquid biopsies offer a promising

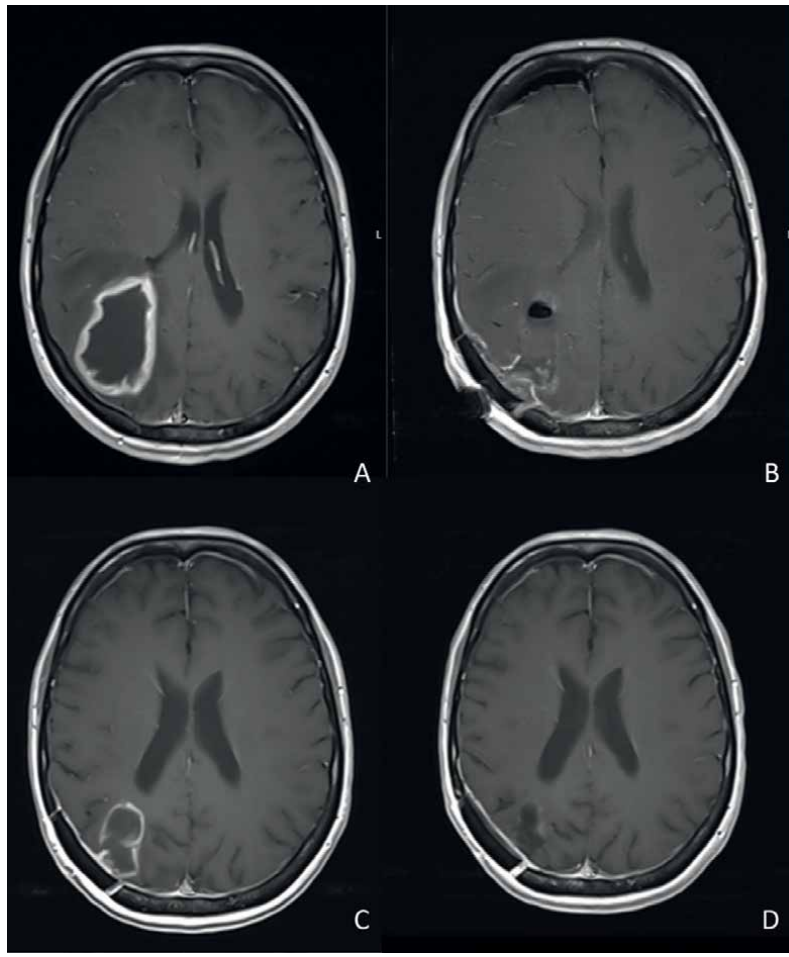

Figure 3.
Axial T1-weighted postcontrast MR images of a right parieto-occipital GBM. (A) Preoperative MRI showing right parieto-occipital GBM. (B) Image obtained 24 hours after surgical excision demonstrating total resection of the tumor. (C) Image obtained 2 months after completion of radiation therapy showing rim-enhancing necrotic borders of the resection cavity. (D) Image obtained 6 months after completion of radiation therapy showing resection cavity without recurrence.

alternative for gliomas, enabling early diagnosis, detection of minimal residual disease after surgery, posttreatment disease monitoring, identification of treatment resistance mechanisms, and outcome prediction (**Figure 3**) [82, 83].

To address the limitations of current diagnostic approaches, there is growing interest in noninvasive or minimally invasive techniques capable of detecting GBM at earlier stages and enabling continuous monitoring over time [29, 84]. Among these innovative methods, liquid biopsy and Raman spectroscopy have demonstrated significant potential [47, 85]. Liquid biopsy offers a minimally invasive approach to analyzing circulating tumor cells (CTCs), cell-free DNA (cfDNA), and extracellular vesicles (EVs) derived from blood samples, providing real-time insights into tumor biology and progression without the need for invasive procedures [44–50, 83, 85].

Similarly, Raman spectroscopy, a label-free and noninvasive optical technique, allows for the detailed molecular fingerprinting of biological samples. By detecting vibrational shifts in molecular bonds, this technique identifies specific biochemical signatures, enabling rapid and precise analysis. Applied to GBM, Raman spectroscopy has the potential to detect and characterize tumor-specific biomarkers without requiring extensive sample preparation or destructive processes [65–79].

This chapter investigates the potential of combining liquid biopsy and Raman spectroscopy to advance GBM diagnostics [47, 85, 86]. By emphasizing the role of cancer growth factors, particularly insulin-like growth factor I (IGF-I), it examines how these techniques could facilitate earlier detection, real-time monitoring, and personalized treatment strategies for GBM [87, 88]. This integrated approach seeks to enhance diagnostic accuracy while addressing the tumor heterogeneity that poses significant challenges in GBM care [47, 80, 85, 86].

2. Overview of emerging diagnostic approaches

Traditional diagnostic methods, such as MRI and histopathological analysis, are indispensable for GBM diagnosis but remain insufficient for capturing the intricate and evolving molecular landscape of the disease [84, 89]. These conventional approaches often fail to reflect the tumor's real-time heterogeneity, progression, and response to treatment, limiting their utility in guiding therapeutic decisions [29, 89]. To address these challenges, emerging noninvasive and minimally invasive technologies, including liquid biopsy and Raman spectroscopy, have demonstrated significant promise as complementary diagnostic tools for advancing GBM care [47, 85, 86]. These innovative techniques offer deeper insights into tumor biology, enabling earlier detection and continuous monitoring-key factors for optimizing therapeutic strategies [71, 90].

Liquid biopsy offers a transformative approach to GBM diagnostics by enabling the analysis of tumor-associated materials, including circulating tumor cells (CTCs), cell-free DNA (cfDNA), EVs, and tumor-educated platelets (TEPs). By examining these biomarkers in bodily fluids such as blood and cerebrospinal fluid (CSF), liquid biopsy provides dynamic, real-time insights into the genetic and molecular landscape of GBM. This approach is particularly valuable for tracking tumor progression, detecting therapy resistance, and monitoring clonal evolution-factors that are challenging to capture using traditional methods. For instance, cfDNA reflects the tumor's genetic profile, revealing mutations, copy number variations, and epigenetic changes. Similarly, EVs and TEPs offer critical information on tumor-derived proteins and RNA, broadening the diagnostic scope. The minimally invasive nature of liquid

biopsy allows for serial sampling, enabling longitudinal studies of tumor dynamics without subjecting patients to repeated invasive procedures [44–50, 90, 91].

Raman spectroscopy, a label-free, noninvasive optical technique, provides precise molecular fingerprinting of biological samples. By detecting vibrational shifts in molecular bonds, it identifies tumor-specific biochemical signatures with minimal sample preparation. When applied to GBM, Raman spectroscopy has proven to be a powerful tool for characterizing tumor heterogeneity and monitoring molecular changes in real time. Its ability to detect subtle biochemical alterations in tissues and fluids complements the genomic and proteomic insights provided, making it a versatile addition to the diagnostic arsenal. Furthermore, Raman spectroscopy enables spatially resolved analysis, which is particularly useful for localizing and characterizing specific tumor regions [65–79].

The integration of liquid biopsy and Raman spectroscopy represents a synergistic approach to overcoming the diagnostic challenges associated with GBM. While liquid biopsy excels in capturing dynamic, systemic tumor changes, Raman spectroscopy adds a layer of spatial and molecular precision, especially for localized tumor characterization [47, 85, 86]. Together, these technologies address the complexities of tumor heterogeneity, focusing on critical cancer biomarkers such as insulin-like growth factor I (IGF-I). Their combined application can potentially improve early detection, enable real-time monitoring of disease progression, and facilitate personalized treatment strategies [87, 88, 92].

This chapter explores the synergistic potential of these emerging technologies in advancing GBM diagnostics and care. By addressing the limitations of traditional diagnostic methods and leveraging the strengths of liquid biopsy and Raman spectroscopy, this integrated approach aims to enhance diagnostic precision, provide insights into tumor heterogeneity, and improve clinical outcomes [91]. As the field continues to evolve, combining these techniques could revolutionize the standard of care for GBM, paving the way for more effective and personalized interventions [93–96].

3. Biomarkers in glioblastoma detection

Emerging biomarker technologies focus on a diverse set of circulating tumor components in GBM, each offering unique insights into the disease's progression, therapeutic resistance, and response. The following subsections outline these biomarkers and their relevance in clinical practice.

3.1 Circulating tumor cells (CTCs)

Circulating tumor cells (CTCs) are tumor-derived nucleated cells shed into the bloodstream or CSF from primary or metastatic tumors, reflecting the molecular and genetic characteristics of the original tumor [45, 97, 98]. In GBM, the detection of CTCs is of immense diagnostic and prognostic value but presents unique challenges due to their scarcity in circulation, estimated at approximately one CTC per billion blood cells [45, 98, 99]. Furthermore, unlike epithelial cancers, GBM lacks EpCAM and other epithelial markers, limiting the effectiveness of traditional CTC detection platforms such as CellSearch, the only FDA-approved CTC isolation platform [98]. Instead, GBM-derived CTCs often express mesenchymal markers, such as SERPINE1 and TGFB1, reflecting the tumor's mesenchymal phenotype [98, 100, 101].

3.1.1 Detection technologies

The rarity and heterogeneity of GBM-derived CTCs have driven the development of advanced detection platforms, including label-free techniques, microfluidic devices, and immunoaffinity-based separation methods. Microfluidic technologies, such as the CTC-iChip, have demonstrated high sensitivity by isolating CTCs based on size, deformability, and surface markers [45, 102]. Another approach involves telomerase-based probes, which exploit the elevated telomerase activity in GBM cells to selectively capture and detect CTCs [98, 101]. Additionally, recent advances in fluorescence in situ hybridization (FISH), reverse transcription polymerase chain reaction (RT-PCR), and single-cell RNA sequencing have enabled molecular profiling of CTCs, revealing mutations in EGFR, PTEN, and other GBM-specific genes [98, 101].

3.1.2 Applications in glioblastoma

CTC enumeration and molecular profiling provide critical insights into GBM biology. Studies have shown that elevated CTC levels correlate with tumor burden, progression, and resistance to therapy [98, 101]. For instance, GBM-derived CTCs expressing mesenchymal markers are associated with aggressive phenotypes and poor prognosis [45, 99]. Furthermore, CTCs offer a noninvasive means of monitoring clonal evolution and therapy-induced mutations, enabling personalized treatment strategies [45, 102]. By distinguishing true tumor progression from pseudoprogression, CTC analysis aids in treatment decision-making and disease monitoring.

3.1.3 Emerging techniques

Single-cell sequencing of CTCs has revolutionized GBM research by providing unprecedented insights into intratumoral heterogeneity. Emerging technologies, such as RNA-seq and proteomics, have identified novel biomarkers, including extracellular matrix proteins and transcription factors, in GBM-derived CTCs [98, 101]. Additionally, integration with artificial intelligence (AI) has enabled automated analysis of CTC images and gene expression data, significantly improving detection accuracy and predictive capabilities [103, 104].

3.1.4 Challenges and future directions

Despite significant advancements, several challenges remain in the detection and analysis of GBM-derived CTCs. Their extreme rarity, coupled with the lack of universal markers, necessitates the development of marker-independent detection platforms. Moreover, the integration of multi-omics data from CTCs with clinical and imaging data holds promise for improving diagnostic precision and therapeutic outcomes in GBM [45, 101]. Future efforts should focus on enhancing sensitivity, reducing false positives, and validating CTC-based biomarkers in large-scale clinical studies.

3.2 Circulating nucleic acids (CNAs)

Circulating nucleic acids (CNAs), including cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA), are fragments of nucleic acids shed by tumor cells into the bloodstream or CSF. These fragments provide a molecular snapshot of the tumor's genetic and epigenetic landscape [101, 105]. In GBM, CNAs hold immense potential

as noninvasive biomarkers for early diagnosis, disease monitoring, and treatment evaluation [98, 101].

3.2.1 cfDNA and ctDNA in GBM

cfDNA refers to extracellular DNA fragments released by normal and tumor cells through apoptosis, necrosis, or active secretion. A subset of cfDNA, ctDNA, is derived explicitly from tumor cells and carries genetic alterations reflective of the primary tumor [98, 101]. In GBM, ctDNA analysis can detect hallmark mutations, such as IDH1/2, EGFR, and TERT promoter mutations, which are crucial for diagnosis and prognosis [106, 107]. Additionally, ctDNA provides insights into copy number variations, DNA methylation patterns, and tumor heterogeneity [107, 108].

3.2.2 Technological advancements in detection

The detection of ctDNA in GBM presents unique challenges due to its low abundance in circulation, particularly in early-stage tumors with an intact blood-brain barrier (BBB) [98]. Advanced methods, such as digital droplet PCR (ddPCR) and next-generation sequencing (NGS), have significantly improved the sensitivity and specificity of ctDNA detection [109]. ddPCR enables the precise quantification of rare mutations, including single-nucleotide variations (SNVs), with unparalleled accuracy [109]. NGS, on the other hand, provides comprehensive genomic profiling, allowing for the simultaneous detection of mutations, fusions, and structural rearrangements [110].

Epigenetic biomarkers, such as DNA methylation and histone modifications, are emerging as powerful tools for GBM diagnostics. Recent studies have shown that methylation profiles of ctDNA can distinguish GBM from other brain tumors with high sensitivity [107, 108]. For example, MGMT promoter methylation status in ctDNA correlates with patient response to temozolomide, aiding in personalized treatment planning [105, 107, 108].

3.2.3 Applications in clinical practice

The clinical utility of ctDNA in GBM extends beyond diagnosis. ctDNA levels correlate with tumor burden, grade, and progression, enabling real-time monitoring of disease dynamics. During treatment, ctDNA analysis can identify therapy-resistant clones and emerging mutations, guiding adjustments to therapeutic strategies. Additionally, ctDNA-based liquid biopsy is particularly valuable for patients with inaccessible tumors, where traditional tissue biopsies are not feasible [98, 105, 111, 112].

3.2.4 Emerging directions and challenges

Despite the promise of CNAs, several limitations remain. The low yield of ctDNA in GBM samples, coupled with the complexity of distinguishing tumor-derived DNA from normal cfDNA, necessitates highly sensitive techniques [98, 101]. Combining ctDNA analysis with other biomarkers, such as circulating tumor cells (CTCs) and EVs, could provide a more comprehensive view of tumor biology [98, 107, 108]. Future research should focus on standardizing ctDNA isolation and analysis protocols, as well as validating ctDNA biomarkers in large, multicenter clinical trials [98, 105].

3.3 Extracellular vesicles (EVs)

Extracellular vesicles (EVs) are nano-sized particles with lipid bilayer secreted by cells into the extracellular environment, mediating intercellular communication. These vesicles include exosomes, microvesicles, apoptotic bodies, oncosomes, exomeres, ectosomes, and other subtypes. EVs are enriched with biomolecules such as nucleic acids, proteins, lipids, metabolites, and glycans reflective of their cell of origin [113–116]. EVs are particularly significant in GBM research because they can cross the blood-brain barrier (BBB) and serve as accessible biomarkers through liquid biopsy for diagnosis, therapy monitoring, and even therapeutic delivery [98, 113, 117–119]. The International Society for Extracellular Vesicles (ISEV) provides guidelines to standardize EV isolation, characterization, and functional validation, ensuring reproducibility and rigor in EV studies [118, 119].

3.3.1 Types and characteristics of EVs

EVs are categorized based on their size, biogenesis, and molecular content. Subtypes relevant to GBM include:

- *Exosomes:* Small vesicles (30–150 nm) derived from the endosomal pathway. Exosomes carry tumor-specific molecules such as EGFRvIII, miR-21, and miR-222, which are critical for GBM diagnostics and therapy [114, 116, 120].

- *Microvesicles (MVs):* Medium-sized vesicles (100–1000 nm) that bud directly from the plasma membrane. MVs transport diverse RNA, proteins, and lipids that play roles in tumor microenvironment remodeling [120, 121].

- *Apoptotic bodies:* Large vesicles (500–2000 nm) released during programmed cell death. These vesicles contain genomic DNA, histones, and cellular debris, reflecting immune responses and tumor microenvironment characteristics [120, 122].

- *Oncosomes:* Large tumor-derived vesicles (1–10 μm) secreted by aggressive cancer cells. Oncosomes are enriched with oncogenic proteins, mutated RNA, and growth factors, promoting tumor invasion, angiogenesis, and immune evasion [121, 123, 124].

- *Exomeres:* Nano-sized particles (~35 nm) distinct from exosomes and microvesicles. Exomeres contribute to metabolism, angiogenesis, and extracellular matrix remodeling [123, 125].

- *Ectosomes:* Vesicles (100–500 nm) shed directly from the plasma membrane, carrying tumor-specific molecules involved in immune modulation and tumor-stroma interactions [126, 127].

- *Vault particles:* Ribonucleoprotein complexes involved in RNA transport and drug resistance. Vault particles contribute to therapy resistance in GBM [128].

- *Prostasomes:* Vesicles derived from prostate tissues but now identified in GBM-related extracellular environments, where they mediate intercellular signaling [114].

3.3.2 Molecular cargo in EVs

The molecular cargo of EVs reflects their cell of origin and provides insights into GBM biology, progression, and therapy resistance:

- *DNA:*
  - Tumor-derived genomic DNA (gDNA) fragments carry mutations such as TERT promoter mutations and EGFR amplifications, which are key diagnostic and prognostic markers [118, 119].
  - Mitochondrial DNA (mtDNA) reflects metabolic dysregulation, often linked to altered oxidative phosphorylation in GBM [129].
- *RNA:*
  - Messenger RNAs (mRNAs) provide insights into transcriptional activity, including oncogene overexpression and tumor suppressor silencing [114].
  - MicroRNAs (miRNAs) such as miR-21 and miR-222 regulate gene expression, promoting tumor growth, angiogenesis, and resistance to therapies [114].
  - Long noncoding RNAs (lncRNAs), such as MALAT1, play roles in epigenetic regulation, invasion, and immune modulation [114].
  - Circular RNAs (circRNAs) are emerging as regulators of gene expression, linked to GBM progression and therapeutic resistance [114].
- *Proteins:*
  - Tumor-specific proteins such as EGFRvIII, PD-L1, and integrins enhance immune evasion, invasion, and metastasis [98].
  - Heat shock proteins (HSP70, HSP90) reflect cellular stress, promoting tumor survival and immune modulation [130].
  - Matrix metalloproteinases (MMPs) carried by EVs degrade the extracellular matrix, facilitating invasion and angiogenesis [131].
- *Lipids:*
  - Elevated levels of sphingomyelins, ceramides, and phosphatidylserines in EVs reflect metabolic adaptations in GBM [132].
- *Metabolites:*
  - Small molecules such as lactate and citrate indicate shifts in tumor metabolism and hypoxia [133, 134].
- *Glycans:*
  - Glycosylated proteins, including galectins and glypicans, mediate adhesion, signaling, and immune evasion in GBM [135].

3.3.3 Emerging technologies and characterization of EVs

Advanced technologies and established methods are transforming the characterization of extracellular vesicles (EVs). Accurate EV characterization is critical for ensuring reproducibility and functional validation while emerging technologies are broadening the scope of EV analysis and enabling high-throughput applications [118, 119].

3.3.3.1 Established characterization techniques for EVs

A variety of well-established methods are used to characterize the physical, biochemical, and molecular properties of EVs:

- *Transmission electron microscopy (TEM):* TEM is the gold standard for visualizing EV morphology and size with high-resolution imaging. It is widely used to confirm the structural integrity of isolated EVs [118, 119].

- *Cryo-electron microscopy (Cryo-EM):* Cryo-EM preserves the native structure of EVs under near-physiological conditions, providing detailed morphological information and resolving sub-vesicular structures [119].

- *Nanoparticle tracking analysis (NTA):* NTA enables real-time measurement of EV size distribution, concentration, and Brownian motion. It is frequently used to quantify EV populations in biological samples [119].

- *Dynamic light scattering (DLS):* DLS measures the hydrodynamic diameter of EVs, providing insights into their stability and aggregation properties in solution [118, 119].

- *Raman spectroscopy:* This label-free analytical technique captures the molecular fingerprints of EVs, offering detailed insights into their biochemical composition. Raman spectroscopy is particularly valuable for detecting tumor-specific signatures in GBM [118, 119].

- *Interferometric scattering microscopy (iSCAT):* A highly sensitive technique that detects single EV particles without the need for fluorescent labeling, enabling quantitative studies of EV populations [118, 119].

- *Flow cytometry:* Flow cytometry detects EV surface markers such as CD63, CD81, and CD9. Fluorescently labeled antibodies improve the specificity of EV subtype identification [118, 119].

- *Western blotting:* This method validates the presence of EV-specific proteins (e.g., TSG101, ALIX) and ensures the absence of non-EV contaminants such as calnexin. Western blotting is a standard quality control step for EV characterization [118, 119].

3.3.3.2 Emerging technologies for EV characterization

Recent technological advancements have introduced innovative approaches to EV isolation, detection, and molecular profiling:

- *Single particle interferometric reflectance imaging sensor (SP-IRIS):* This label-free platform enables single-particle EV detection with high throughput and sensitivity. SP-IRIS is particularly effective for studying heterogeneous EV populations and detecting specific biomarkers [136].

- *Microfluidics platforms:* Microfluidics systems streamline EV isolation, purification, and molecular analysis from limited sample volumes. These platforms are ideal for clinical applications, offering enhanced specificity and efficiency [137].

- *Super-resolution microscopy:* Techniques such as stimulated emission depletion (STED) and photoactivated localization microscopy (PALM) microscopy enable nanoscale imaging of EVs, providing detailed insights into their biogenesis, dynamic release patterns, and molecular composition [138].

- *Izon technology (qNano):* A tunable resistive pulse sensing platform that allows EV quantification and size measurement. This method addresses challenges in detecting smaller vesicles [139].

3.3.4 Applications and emerging technologies in GBM management

EVs offer significant promise in advancing GBM research, diagnostics, and therapy. Their diverse roles are summarized below:

- *Noninvasive diagnostics:* Exosomal RNA (e.g., miR-21) and protein markers (e.g., EGFRvIII) serve as diagnostic tools, providing insights into tumor heterogeneity and progression through liquid biopsy approaches [140].

- *Therapy monitoring:* Longitudinal analysis of EV cargo enables real-time monitoring of tumor progression and therapeutic response, offering noninvasive methods to track treatment efficacy [140].

- *Biomarker discovery:* EVs act as reservoirs of tumor-specific biomarkers for identifying GBM, providing molecular insights that can inform personalized treatment strategies [140].

- *Drug delivery vehicles:* Engineered EVs deliver chemotherapeutics, siRNAs, and immunomodulators with high specificity and reduced off-target effects. They offer a promising alternative for targeted drug delivery in GBM [141].

- *Immune regulation:* EVs influence immune cells, including tumor-associated macrophages (TAMs), by modulating cytokine expression and immune checkpoint pathways, contributing to immune evasion and tumor progression [142].

- *CRISPR-Cas9 delivery:* Emerging research demonstrates the potential of EVs to deliver CRISPR-Cas9 gene-editing tools, enabling precise genomic modifications for targeted tumor suppression [143].

- *Drug resistance mechanisms:* EVs mediate the transfer of resistance-associated genes and proteins, facilitating adaptive mechanisms that contribute to treatment failure in GBM [114, 144].

3.3.5 Challenges in EV research and translation

Despite advancements, EV research faces critical challenges:

- *Heterogeneity of EV populations:* Differentiating EV subtypes remains difficult due to overlapping size and content profiles [118, 119].
- *Quantitative challenges:* Accurate quantification is hindered by the presence of non-EV particles, requiring refined isolation techniques [118, 119].
- *Regulatory issues:* Lack of standardized clinical-grade EV protocols complicates translational research [118, 119].
- *Emerging solutions:* AI-driven EV profiling and high-throughput platforms hold promise for addressing these limitations [145].

3.4 Tumor-educated platelets (TEPs)

Tumor-educated platelets (TEPs) are circulating blood platelets that undergo molecular and functional changes when exposed to tumor-derived biomolecules, such as proteins, RNA, metabolites, and lipids. These alterations reflect the molecular biology of the tumor, enabling TEPs to serve as a dynamic, real-time mirror of tumor activity. This unique property makes TEPs an invaluable tool for cancer diagnostics, monitoring, and personalized treatment strategies, particularly in GBM [146, 147].

3.4.1 TEPs as diagnostic biomarkers

Platforms such as ThromboSeq, a sequencing-based technology, have demonstrated exceptional accuracy in distinguishing GBM from other neurological disorders. ThromboSeq analyzes TEP RNA profiles, which include alterations in long noncoding RNAs (lncRNAs), microRNAs (miRNAs), and mRNA splicing variants. These profiles provide critical insights into tumor progression, therapeutic resistance, and overall disease dynamics [146–148]. For example, elevated levels of tumor-specific mutations, such as EGFRvIII, can be detected in TEPs. EGFRvIII is a hallmark of GBM and serves as a biomarker for distinguishing true tumor progression from pseudoprogression during treatment monitoring [89]. Moreover, the ability to longitudinally monitor TEP RNA signatures provides clinicians with a noninvasive, real-time tool for tracking therapeutic efficacy and disease evolution [149].

3.4.2 Functional contributions of TEPs in tumor biology

In addition to their diagnostic potential, TEPs play active roles in tumor progression by interacting with other tumor microenvironment components. TEPs can interact with circulating tumor cells (CTCs), immune cells, and endothelial cells, facilitating metastasis and immune evasion. These interactions involve the secretion of proinflammatory cytokines (e.g., IL-6, IL-8), angiogenic factors (e.g., VEGF), and growth factors, which collectively create a tumor-supportive niche [146–149]. For instance:

- *Cytokine secretion:* TEPs release cytokines that enhance tumor growth and promote immune evasion by inhibiting the activity of natural killer (NK) cells and cytotoxic T lymphocytes [150].

- *Angiogenesis promotion:* TEPs secrete VEGF, contributing to neovascularization and supplying tumors with nutrients necessary for their growth [151].

- *Tumor metastasis:* By interacting with CTCs, TEPs facilitate the adhesion and extravasation of tumor cells, promoting metastatic dissemination [152].

These functional roles underscore the ability of TEPs to actively shape the tumor microenvironment, making them not only passive biomarkers but also active participants in GBM progression.

3.4.3 TEPs in therapy resistance and clonal evolution

TEPs provide dynamic insights into therapy resistance and clonal evolution in GBM. By analyzing RNA profiles, clinicians can detect early signs of resistance, such as upregulated pathways related to efflux pumps or apoptosis inhibition. For example:

- *Emergence of resistant clones:* Changes in TEP RNA profiles, such as alterations in drug-metabolizing enzymes or cell cycle regulators, can indicate the emergence of therapy-resistant clones [150, 153].

- *Real-time monitoring of tumor evolution:* Longitudinal tracking of TEP RNA profiles provides a minimally invasive means to study tumor adaptation to treatments, such as chemotherapy, radiotherapy, or targeted therapies [153, 154].

This ability to dynamically track tumor evolution and resistance mechanisms makes TEPs an invaluable resource for guiding personalized treatment strategies. TEPs complement other liquid biopsy approaches, such as circulating tumor DNA (ctDNA) and extracellular vesicle (EV) profiling, providing a multidimensional view of tumor biology [154].

3.4.4 Future directions and clinical applications

The use of TEPs in GBM diagnostics and monitoring is rapidly evolving. Advances in sequencing technologies, such as digital droplet PCR (ddPCR) and next-generation sequencing (NGS), are enabling increasingly sensitive and specific analyses of TEP-derived biomarkers. Additionally, artificial intelligence (AI) and machine learning algorithms are being integrated into TEP data analysis to improve the predictive power of these biomarkers for early detection, prognosis, and therapeutic decision-making [146, 155, 156].

Future research aims to:

- *Expand the TEP biomarker repertoire:* Identify additional tumor-specific RNA signatures and proteins in TEPs to enhance diagnostic accuracy and specificity [146–148].

- *Integrate multi-omic data:* Combine TEP data with other omics, such as ctDNA and proteomics, to provide a comprehensive molecular snapshot of tumor dynamics [148, 153, 157, 158].

- *Develop clinical protocols:* Translate TEP-based diagnostics into standardized clinical workflows for real-time monitoring of GBM patients [150, 154].

As a minimally invasive tool, TEP profiling has the potential to revolutionize GBM care by enabling earlier detection, improving patient stratification, and tailoring treatments to individual tumor profiles.

3.5 Circulating miRNAs

Circulating microRNAs (miRNAs) are small noncoding RNA molecules, approximately 18–24 nucleotides in length, that regulate gene expression by binding to complementary sequences on messenger RNAs (mRNAs), leading to their degradation or translational repression. These molecules play a central role in regulating key biological processes, including cell proliferation, apoptosis, differentiation, and stress response, all of which are critically altered in GBM [116, 159–161].

Circulating miRNAs have emerged as pivotal biomarkers in GBM due to their high stability in blood, cerebrospinal fluid (CSF), and other biofluids, where they are protected by their association with extracellular vesicles (EVs), lipoproteins, or RNA-binding proteins. This stability allows them to persist under harsh conditions, making them highly suitable for liquid biopsy applications. Profiling of circulating miRNAs in GBM patients has revealed distinct expression patterns that can be exploited for diagnostics, prognostics, and therapy monitoring [116, 161].

Among the numerous miRNAs identified, miR-21 is consistently overexpressed in GBM and is associated with poor prognosis. MiR-21 promotes oncogenesis by targeting tumor suppressor genes such as PTEN, PDCD4, and TPM1, which regulate apoptosis, cell cycle arrest, and tumor invasion. Elevated levels of miR-21 in the plasma and CSF of GBM patients correlate with aggressive disease and therapeutic resistance, making it a key biomarker for monitoring tumor dynamics [116, 161].

Beyond miR-21, other circulating miRNAs, including miR-15b, miR-23a, and miR-197, have demonstrated high sensitivity and specificity in distinguishing GBM from other brain tumors, enhancing their potential for diagnostic applications. Panels of miRNAs, such as combinations of miR-124, miR-125b, and miR-222, have been identified as markers for therapeutic resistance and disease progression, providing additional tools for patient stratification and personalized treatment. For example, downregulation of miR-124 has been linked to resistance to radiotherapy, while overexpression of miR-125b contributes to chemoresistance by modulating apoptotic pathways [116, 161–163].

Recent technological advancements, including next-generation sequencing (NGS), digital droplet PCR (ddPCR), and qRT-PCR, have enabled the precise and high-throughput quantification of circulating miRNAs. These tools provide dynamic insights into tumor heterogeneity, progression, and response to therapy, supporting the growing importance of circulating miRNAs in GBM diagnostics and therapeutic monitoring [116, 161–163]. Future research is focusing on integrating miRNA profiling with artificial intelligence (AI) models to enhance the predictive

power of these biomarkers, enabling earlier detection and more accurate prognostication [153, 159, 164].

3.6 Cancer growth factors

Cancer growth factors are signaling molecules that regulate critical cellular processes, including cell proliferation, differentiation, survival, angiogenesis, and immune modulation. These factors play a pivotal role in the development and progression of GBM by activating oncogenic pathways, contributing to therapeutic resistance, and supporting tumor invasiveness. Notably, elevated levels of specific growth factors have been implicated not only in tumor progression but also in signaling precancerous conditions, providing opportunities for early detection and intervention [165–169].

3.6.1 The role of IGF-I in pre-cancer and GBM detection

Insulin-like growth factor I (IGF-I), initially identified by Daughaday in the 1970s, is a critical growth factor involved in tumor growth and progression, particularly playing a pivotal role in glioblastoma (GBM). Overexpression of IGF-I primarily results from the activation of the IGF-I receptor. Targeting this receptor, notably through gene therapy approaches aimed at inhibiting IGF-I, has shown promise in enhancing tumor immunogenicity by upregulating major histocompatibility complex class I (MHC-I) and co-stimulatory molecule B7 expression. These changes are closely associated with the activation of signal transduction pathways, such as PI3K, MAPK, and PKC. Similar mechanisms, albeit to a lesser extent, are also implicated in the roles of other growth factors, including vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and particularly transforming growth factor-beta (TGF-β), which will be further discussed in the subsequent section (3.6.2) [19, 20, 170–172]. IGF-I is among the most studied cancer growth factors, recognized for its dual role in precancer and advanced tumor stages. IGF-I is a peptide hormone produced primarily in the liver, but its expression is modulated by both systemic and local factors, including tumor microenvironment dynamics. By binding to the insulin-like growth factor 1 receptor (IGF-1R), a tyrosine kinase receptor, IGF-I activates critical downstream pathways such as PI3K/AKT and MAPK. These pathways drive cell proliferation, survival, and angiogenesis while inhibiting apoptosis, creating a fertile environment for tumor initiation and progression [166, 168].

Recent studies have demonstrated that elevated serum levels of IGF-I may signal precancerous cellular changes, particularly in highly metabolic tissues such as the brain. IGF-I induces early molecular alterations, including the dysregulation of cell cycle checkpoints, enhanced growth signaling, and increased resistance to apoptosis—all of which contribute to the transformation of normal glial cells into malignant phenotypes. These early alterations provide a window for precancer detection, particularly in high-risk populations or individuals with genetic predispositions [166, 168, 173, 174].

3.6.2 Other key growth factors in GBM and precancer detection

Among novel therapeutic strategies targeting growth factors and their downstream signaling components, particular emphasis has been placed on interventions

involving VEGF, IGF-I, and EGFR pathways, frequently employing advanced nanotechnology-based delivery systems [175–178]. While IGF-I plays a central role [166, 168, 173, 174], other growth factors contribute significantly to both precancer signaling and GBM pathophysiology:

- *Vascular endothelial growth factor (VEGF):* VEGF is a critical driver of angiogenesis in GBM. In precancer conditions, VEGF overexpression often signals the transition from a benign state to aggressive vascular proliferation, creating a nutrient-rich microenvironment for tumor growth [17, 179–183].

- *Epidermal growth factor (EGF):* EGF and its receptor (EGFR) are frequently overexpressed in GBM, particularly in precancerous conditions where dysregulated EGF signaling contributes to abnormal cell proliferation and early oncogenic mutations [17, 184–187].

- *Transforming growth factor-beta (TGF-β):* TGF-β plays a dual role, acting as a tumor suppressor in normal cells but switching to an oncogenic driver during tumorigenesis. Elevated TGF-β levels in precancer stages are associated with immune evasion and enhanced epithelial-to-mesenchymal transition (EMT), facilitating tumor invasion [17, 169, 188].

3.6.3 Advancements in growth factor detection for precancer symptoms

Technological advancements have enabled the noninvasive detection and quantification of growth factors, offering significant potential for identifying precancer symptoms [17]. Liquid biopsy techniques are emerging as powerful tools for monitoring circulating growth factors in blood and cerebrospinal fluid (CSF), enabling early detection of dysregulated signaling pathways [189, 190]. Growth factors can be detected in serum and CSF [191] using highly sensitive assays such as:

- *Digital droplet PCR (ddPCR):* This method provides precise quantification of growth factor-related transcripts, such as IGF-I mRNA, even at low concentrations [192].

- *Raman spectroscopy:* A label-free optical technique capable of detecting IGF-I and other growth factors in biofluids with high specificity. Raman spectroscopy captures molecular fingerprints of tumor-derived biomolecules, making it particularly valuable for precancer diagnostics [85, 193].

- *Enzyme-linked immunosorbent assay (ELISA):* A gold standard for quantifying protein levels, ELISA is routinely used to measure circulating IGF-I levels in research and clinical settings [194].

3.6.4 Clinical implications and future directions

The ability to detect precancer symptoms signaled by growth factors, particularly IGF-I, represents a paradigm shift in oncology. By identifying molecular changes associated with early tumorigenesis, clinicians can intervene before the onset of

invasive disease, improving outcomes for high-risk populations. In GBM, integrating growth factor profiling into routine screening protocols could enable earlier detection, personalized risk assessment, and stratification of patients for preventive therapies [17, 166, 169].

Future research is focusing on combining growth factor detection with AI-driven algorithms to enhance predictive accuracy and integrate multi-omic data. Additionally, novel therapeutic approaches targeting growth factor pathways, such as IGF-1R inhibitors and anti-VEGF therapies, are being developed to suppress precancer signaling and prevent disease progression [17, 179, 180, 194].

4. Liquid biopsy: A breakthrough in GBM diagnostics

Liquid biopsy represents a transformative approach in cancer diagnostics, providing a minimally invasive method to assess tumor dynamics by analyzing biofluids such as blood, cerebrospinal fluid (CSF), and urine. Unlike traditional tissue biopsies, which are invasive and limited in capturing tumor heterogeneity, liquid biopsy enables real-time monitoring of tumor characteristics, addressing the dynamic and multifocal nature of GBM [116, 161]. Given the infiltrative growth patterns and critical location of GBM within the brain, liquid biopsy holds immense promise as a safer and more comprehensive alternative to conventional biopsy techniques [98].

4.1 The critical need for liquid biopsy in GBM

GBM presents unique diagnostic challenges that underscore the dire need for innovative, minimally invasive diagnostic tools like liquid biopsy:

- *Limited accessibility of tumor tissue:* Surgical biopsies are often infeasible due to the proximity of the tumor to critical brain regions, posing risks such as neurological damage, hemorrhage, and infection. This is particularly problematic for recurrent GBM, where tissue access becomes increasingly difficult [45, 98].

- *Tumor heterogeneity:* GBM exhibits substantial spatial and temporal heterogeneity, with genetic and molecular variations across regions of the tumor. A single tissue biopsy cannot capture this complexity, leading to incomplete diagnostics and less effective treatment plans [45, 98].

- *Challenges in therapy monitoring:* Imaging methods such as MRI often struggle to differentiate between true tumor progression, radiation necrosis, and pseudoprogression, leading to diagnostic uncertainty and potential delays in care. Liquid biopsy provides molecular-level insights to address these challenges [45, 98].

- *Early detection of recurrence:* Biomarkers detectable through liquid biopsy, such as ctDNA, miR-21, and IGF-I, allow clinicians to identify tumor recurrence earlier than imaging, enabling timely intervention and improving patient outcomes [45, 98].

4.2 How liquid biopsy improves patient care

Liquid biopsy offers multiple benefits that directly impact GBM patient care and survival:

- *Real-time monitoring:* By analyzing biofluids at regular intervals, liquid biopsy enables the dynamic tracking of tumor evolution, therapeutic efficacy, and emerging resistance mechanisms [45, 98, 195].

- *Guiding personalized therapy:* Molecular profiling through liquid biopsy helps identify patient-specific tumor characteristics, allowing for tailored treatments that target actionable mutations or pathways [45, 98, 195].

- *Reducing patient burden:* Noninvasive sampling eliminates the need for repeat surgical biopsies, minimizing physical risks and improving patient comfort [45, 98, 195].

- *Clarifying treatment effects:* Liquid biopsy aids in distinguishing pseudoprogression and radiation necrosis from true tumor regrowth, reducing unnecessary interventions and ensuring timely, appropriate care [3, 36, 45].

4.3 Bridging liquid biopsy and advanced analytical techniques

The success of liquid biopsy in GBM depends on integrating advanced analytical technologies that enhance its sensitivity, specificity, and clinical utility. Among these, Raman spectroscopy and artificial intelligence (AI) are emerging as transformative tools:

- *Raman spectroscopy:* Raman spectroscopy is a label-free, noninvasive optical technique that detects molecular fingerprints of tumor-derived components in biofluids. Its ability to identify biomarkers such as IGF-I, miR-21, and EV cargo enhances the diagnostic power of liquid biopsy, particularly in low-abundance samples [85, 196–198].

- *AI-driven analysis:* AI models analyze complex biomarker datasets, identify diagnostic patterns, and predict clinical outcomes with high accuracy. For example, machine learning applied to Raman spectra can classify GBM-specific molecular changes with improved sensitivity and reproducibility [86, 164, 199].

The integration of these technologies into liquid biopsy workflows not only improves diagnostic accuracy but also facilitates real-time, scalable diagnostics, paving the way for standardized clinical protocols [45, 86, 91].

4.4 Transforming GBM diagnostics through liquid biopsy

The combination of liquid biopsy, Raman spectroscopy, and AI offers a paradigm shift in GBM diagnostics and care. By addressing limitations such as the blood-brain barrier and tumor heterogeneity, these approaches provide a comprehensive view of tumor biology that complements traditional methods [44, 45, 86, 89, 140, 200, 201]. Key clinical applications include:

- *Early recurrence detection:* Liquid biopsy biomarkers can signal recurrence before imaging detects structural changes, enabling earlier and more effective interventions [44, 45, 89, 98, 101, 198, 201].

- *Therapeutic monitoring and adaptation:* Regular sampling allows clinicians to track biomarker dynamics in real time, optimizing treatment strategies based on evolving tumor profiles [45, 48, 91, 98, 101].

- *Personalized medicine:* Multimodal integration of liquid biopsy with Raman spectroscopy and AI enhances the specificity of molecular profiling, enabling highly individualized treatment plans [86, 104, 198, 199].

4.5 Clinical outlook and transition to Raman spectroscopy

The adaptability and versatility of liquid biopsy, particularly when paired with Raman spectroscopy and AI, are reshaping the landscape of GBM care. These technologies enable clinicians to overcome traditional limitations, such as distinguishing pseudoprogression from true tumor recurrence, monitoring treatment efficacy, and detecting emerging resistance mechanisms in real time [69, 86, 197, 198, 201]. By providing minimally invasive and highly sensitive diagnostic solutions, liquid biopsy offers a dynamic approach to understanding tumor progression, while Raman spectroscopy enhances its precision through detailed molecular fingerprinting [69, 197, 201, 202].

The integration of these technologies has significant implications for addressing key clinical challenges in GBM. For instance, pseudoprogression and radiation necrosis often present with imaging characteristics indistinguishable from true tumor regrowth, leading to diagnostic uncertainty and potentially inappropriate treatment interventions [36, 39–41, 97]. Raman spectroscopy provides unique biochemical insights into biofluids and tissues, enabling the identification of tumor-specific signatures that can clarify these ambiguities. Additionally, the ability to monitor molecular changes in circulating biomarkers-such as IGF-I, miR-21, and ctDNA-through liquid biopsy allows clinicians to track therapeutic responses and adjust treatments promptly, improving patient outcomes [85, 98, 202].

The potential of Raman spectroscopy is further amplified by AI-driven analytical frameworks [86, 198, 199]. Machine learning algorithms process the high-dimensional spectral data generated by Raman spectroscopy, identifying patterns and correlations that may otherwise be missed [86, 198, 199, 202]. This enhances the specificity and sensitivity of the technique, enabling the identification of tumor progression markers, prediction of therapeutic responses, and differentiation of molecular subtypes within GBM [86, 97, 198, 199, 202]. AI integration also facilitates automated, real-time diagnostics, making Raman spectroscopy a scalable and practical tool for clinical workflows [86, 198, 199, 202, 203].

The synergy of liquid biopsy, Raman spectroscopy, and AI is particularly impactful in the context of personalized medicine. By providing a comprehensive molecular profile of the tumor, these technologies may enable tailored therapeutic strategies that address the unique characteristics of each patient's disease [198, 202–205]. This approach have potential in improving progression survival (PFS) and overall survival (OS) rates but may also reduce unnecessary interventions, enhancing the overall quality of care [203].

As the field evolves, Raman spectroscopy is positioned to expand the clinical applications of liquid biopsy beyond its current capabilities. Emerging evidence suggests that Raman spectroscopy could play a pivotal role in intraoperative

settings, guiding tumor resection by delineating tumor margins with unprecedented precision [97, 206]. Furthermore, its ability to analyze low-abundance biomarkers in blood and cerebrospinal fluid (CSF) positions it as a critical tool for detecting early molecular changes that precede radiological or symptomatic progression [97, 98].

The following section delves deeper into the transformative potential of Raman spectroscopy, exploring its applications in GBM diagnostics, tumor margin detection, and treatment monitoring. By integrating Raman spectroscopy into liquid biopsy workflows, clinicians and researchers can unlock new opportunities for improving GBM care and advancing the field of precision oncology [85, 201].

5. Raman spectroscopy in glioblastoma diagnosis

Raman spectroscopy is a noninvasive optical method capable of molecular profiling in GBM, offering the unique ability to identify biochemical changes at a cellular level. By detecting vibrational energy shifts in molecular bonds, Raman spectroscopy produces detailed molecular fingerprints of biological samples, making it particularly effective for identifying specific tumor biomarkers in tissues and biofluids [206]. Its ability to provide rapid, label-free analysis with minimal sample preparation enables real-time detection of molecular changes associated with tumor progression, treatment response, and tumor margins [70, 207]. These attributes make Raman spectroscopy a promising diagnostic tool for GBM, particularly when integrated with liquid biopsy and AI [198, 199].

This section explores how Raman spectroscopy can be applied to detect GBM biomarkers, including those associated with growth factors, and discusses its potential for supporting early diagnosis, monitoring treatment response, and improving survival outcomes.

5.1 Applications of Raman spectroscopy for tumor biomarker detection

Raman spectroscopy offers powerful capabilities for identifying tumor biomarkers associated with GBM, such as CTCs, extracellular vesicles (EVs), CNAs, and tumor-educated platelets (TEPs). These biomarkers are crucial for diagnosing GBM, monitoring therapy, and detecting recurrence.

- *Circulating tumor cells (CTCs):* Raman spectroscopy identifies unique biochemical signatures of CTCs by profiling their intracellular components, including proteins, lipids, and nucleic acids [208, 209]. It captures spectral differences between CTCs and normal blood cells, enabling label-free identification of these rare cells. For instance, glycolysis-related metabolic shifts can be detected as tumor-specific markers [209, 210].

- *Extracellular vesicles (EVs):* Raman spectroscopy profiles molecular cargo within EVs, such as RNA, DNA, proteins, and lipids, differentiating tumor-derived EVs from non-tumor EVs based on their unique biochemical signatures [202]. Tumor-specific exosomal markers, such as miR-21, can be identified with high

precision, providing insights into tumor progression and therapeutic response [113, 114, 117].

- *Circulating nucleic acids (CNAs):* Raman spectroscopy detects molecular vibrations in ctDNA and ctRNA, identifying tumor-specific mutations (e.g., EGFRvIII) and methylation patterns [211]. These insights enable label-free, nondestructive analysis of nucleic acids in biofluids [209, 212].

- *Tumor-educated platelets (TEPs):* Raman spectroscopy identifies molecular changes in TEPs, such as RNA cargo and protein content, which are influenced by tumor-secreted factors [148–150, 153]. These alterations can be used to distinguish tumor-educated platelets from normal ones, providing valuable diagnostic information [154].

5.2 Synergy of liquid biopsy and Raman spectroscopy for enhanced diagnostic precision

The combination of liquid biopsy and Raman spectroscopy offers a powerful diagnostic approach that captures both circulating and tissue-specific biomarkers. Liquid biopsy excels in detecting circulating tumor markers, such as ctDNA, EVs, and growth factors, while Raman spectroscopy provides precise molecular profiling of localized samples, including tumor tissues and isolated biofluid components [202].

By leveraging liquid biopsy's ability to analyze systemic tumor markers and Raman spectroscopy's precision in localized molecular analysis, these methods can significantly improve diagnostic accuracy. For example, analyzing growth factors, like IGF-I, through both approaches offers a comprehensive view of GBM progression, improving tracking of disease dynamics and enhancing treatment response evaluations [203].

Studies have shown that Raman spectroscopy enhances the sensitivity of liquid biopsy, detecting subtle molecular changes in tumor-derived biomarkers that are often missed by conventional methods [206]. This synergy supports the development of personalized treatment plans by providing a detailed molecular landscape of the tumor, enabling clinicians to monitor treatment efficacy in real time, detect resistance mechanisms, and adjust therapies accordingly [97, 202].

5.3 Applications in tumor margin detection

Raman spectroscopy has shown great promise in determining tumor margins, a critical factor in GBM surgery. Its ability to detect biochemical differences between healthy and tumor tissues provides surgeons with real-time, intraoperative guidance, reducing the risk of leaving residual tumor tissue behind [97, 206].

When combined with AI, Raman spectroscopy's potential for tumor margin detection is further amplified. Machine learning algorithms analyze the complex spectral data generated by Raman spectroscopy, distinguishing between healthy and malignant tissues with high accuracy [86, 198, 199, 202]. This integration allows for precise tumor boundary mapping, improving surgical outcomes and minimizing damage to healthy brain tissue [213].

5.4 Impact on survival outcomes

Innovative diagnostic approaches, such as Raman spectroscopy and liquid biopsy, have the potential to significantly improve survival rates in GBM patients by enabling earlier detection, more precise monitoring, and personalized treatment strategies. Early diagnosis facilitated by Raman spectroscopy's ability to detect tumor biomarkers such as IGF-I and ctDNA in biofluids provides clinicians with actionable information before radiological changes become apparent [204]. This early intervention can improve progression-free survival (PFS) and overall survival (OS), as evidenced by studies linking biomarker-driven diagnostics to better treatment outcomes [202].

Real-time monitoring of treatment response using Raman spectroscopy in combination with AI allows for dynamic adjustment of therapies, minimizing delays in addressing therapeutic resistance or recurrence. These capabilities reduce the likelihood of treatment failure and improve long-term patient outcomes [97].

5.5 Future directions and clinical integration

The combination of Raman spectroscopy, liquid biopsy, and AI represents a paradigm shift in GBM diagnostics. While Raman spectroscopy excels in molecular profiling and tumor margin detection, integrating it with liquid biopsy expands its utility for systemic biomarker analysis. AI further enhances these applications by automating data analysis, improving diagnostic accuracy, and enabling real-time decision-making [202].

Future research should focus on standardizing Raman spectroscopy protocols, validating AI-driven workflows, and integrating these tools into multimodal diagnostic frameworks. Large-scale clinical trials are needed to establish their efficacy and scalability for routine use in GBM care [203]. By addressing these challenges, Raman spectroscopy and its combination with liquid biopsy and AI could revolutionize GBM diagnostics, improving patient care and survival outcomes.

6. Future directions in glioblastoma diagnostics

Advances in diagnostic technologies are reshaping cancer care, and the potential applications in GBM are particularly exciting. Future research may focus on enhancing the sensitivity and accuracy of liquid biopsy and Raman spectroscopy to detect subtle molecular changes related to tumor initiation, progression, and therapeutic resistance [200, 214]. These innovations, combined with emerging technologies such as multi-omics integration, portable Raman devices, and AI-driven analytical frameworks, could revolutionize GBM diagnostics and improve patient care [215, 216].

One promising area of research lies in the development of multi-omics approaches that combine genomic, transcriptomic, proteomic, and metabolomic data to provide a comprehensive molecular profile of GBM [217, 218]. By integrating data from multiple layers of biological regulation, researchers can uncover new biomarkers that better reflect the tumor's spatial and temporal heterogeneity. These multi-omics

approaches can also identify actionable therapeutic targets, enabling more precise and personalized treatment strategies [203, 219].

Technological advancements in Raman spectroscopy are expected to address current challenges, such as the detection of low-abundance biomarkers and real-time analysis of biofluids [207]. Portable and handheld Raman devices could enable point-of-care diagnostics, bringing this technology into clinical settings where rapid decision-making is critical [200, 215]. Additionally, combining Raman spectroscopy with advanced imaging modalities, such as PET or MRI, could provide a synergistic approach to tumor detection and monitoring, improving diagnostic precision [214].

AI and machine learning are poised to play an integral role in the future of GBM diagnostics. AI-driven tools can analyze large and complex datasets generated by liquid biopsy and Raman spectroscopy, identifying patterns and correlations that would be difficult to discern manually [207, 216]. These tools can improve the accuracy of biomarker detection, automate the classification of tumor subtypes, and predict patient outcomes based on molecular profiles [215, 218]. Furthermore, AI integration can reduce variability in diagnostic interpretations, paving the way for standardized and scalable diagnostic workflows [217, 219].

To fully realize the potential of these innovations, future efforts must focus on overcoming key challenges, including regulatory approval, cost-effectiveness, and accessibility in low-resource settings [200, 220]. Large-scale clinical trials are essential to validate these technologies and ensure their reliability and safety for routine use. By addressing these challenges, GBM diagnostics can evolve to provide earlier detection, more precise monitoring, and better therapeutic outcomes for patients facing this aggressive disease.

7. Conclusions

As GBM diagnostics evolve, innovative methods like liquid biopsy and Raman spectroscopy hold great promise for significantly improving patient outcomes [47, 85, 86]. These methods provide noninvasive, real-time molecular analysis that addresses critical limitations in traditional diagnostics, supporting more personalized treatment strategies [200, 219]. The integration of these technologies into clinical workflows has the potential to transform GBM care, enabling earlier detection, better monitoring of therapeutic responses, and identification of emerging resistance mechanisms [214, 218].

This chapter has highlighted the clinical potential of growth factors, particularly IGF-I, as early markers in GBM, underscoring their value in enhancing diagnostic accuracy and efficacy [219, 220]. By leveraging advanced techniques such as Raman spectroscopy and AI-driven liquid biopsy, clinicians can achieve a more comprehensive understanding of GBM's molecular landscape, leading to tailored treatment strategies that improve progression-free survival and overall survival rates [47, 85, 86, 207].

While challenges remain in translating these innovations into routine practice, ongoing advancements in diagnostic technologies, multi-omics research, and AI-driven analytics offer a promising path forward. With continued investment in research, validation, and implementation, these groundbreaking approaches could redefine GBM care and lead to better outcomes for patients facing this challenging disease [216, 221].

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Conflict of interest

The authors declare no conflict of interest.

Abbreviations

GBM	glioblastoma
AI	artificial intelligence
RS	Raman spectroscopy
SERS	surface-enhanced Raman spectroscopy
MRI	magnetic resonance imaging
CTC	circulating tumor cell
cfDNA	cell-free DNA
EV	extracellular vesicles
IGF	insulin-like growth factor I
CSF	cerebrospinal fluid
TEPs	tumor-educated platelets
ddPCR	digital droplet polymerase chain reaction
NGS	next-generation sequencing
FISH	fluorescence in situ hybridization
EpCAM	epithelial cell adhesion molecule
BBB	blood-brain barrier
PET	positron emission tomography
DSC-MRI	dynamic susceptibility contrast magnetic resonance imaging
miRNA	microRNA
EGFR	epidermal growth factor receptor
IDH1/2	isocitrate dehydrogenase 1/2
5-ALA	5-aminolevulinic acid

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Section 3

Therapy of Tumour

Chapter 4

Astrocytes in Glioblastoma Therapy: A Novel Approach to Targeting Tumor Microenvironment

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Abstract

Glioblastomas (GBM) are one of the most aggressive tumors of the brain with a devastating prognosis despite its standard treatment of chemoradiotherapy and surgery. Astrocytes play a vital role in the tumor microenvironment (TME) of GBM, significantly influencing tumor progression and therapeutic outcomes. This chapter explores the complex interplay between astrocytes and GBM cells, highlighting the potential of targeting astrocytes within the TME as a novel therapeutic approach. Astrocytes undergo astrogliosis in response to GBM, adopting a reactive phenotype that contributes to tumor growth and invasion. The heterogeneity of astrocytes within the GBM microenvironment has been revealed, with distinct subpopulations correlating with different GBM subtypes. Key signaling pathways, including NF-κB, Sonic hedgehog, p53, IL-6/JAK/STAT, and PI3K/Akt, mediate the crosstalk between astrocytes and GBM cells, promoting tumor proliferation, invasion, and angiogenesis. Reactive astrocytes secrete growth factors, cytokines, and extracellular vesicles that enhance GBM cell survival and invasiveness. Understanding these interactions provides insights into potential therapeutic targets for GBM treatment. This chapter aims to elucidate the molecular mechanisms underlying astrocyte-GBM interactions and explore promising avenues for developing astrocyte-targeted therapies to improve outcomes in this challenging malignancy.

Keywords: glioblastoma, astrocytes, tumor microenvironment, targeted therapy, GBM treatment

1. Introduction

Glioblastoma (GBM) is the most common primary malignant brain tumor in adults, with a median onset age of 55–60 years [1]. It is one of the most aggressive tumors of all, with a poor prognosis despite the standard treatment of surgery followed by chemoradiotherapy. They also display the highest recurrence rate and resistance to therapy. There is much focus drawn into exploring potential treatments through targeted therapy and molecular mechanisms; however, most patients have dismal outcomes with a survival rate of up to 18–20 months, and in rare cases, 2 years,

instead of previously 1-year survival following diagnosis [2–5]. This may be due to the hurdle of crossing the blood-brain barrier (BBB). Astrocytes play a major role in the BBB by selecting the exchange molecules through the barrier. Their oncogenic transformation in the progression of GBM would compromise the BBB and permit the entry of immune cells into blood-activating astrocytes [6].

Glioblastomas are in constant interaction with the surrounding tumor microenvironment (TME). The TME is a heterogeneous collection of cancerous and noncancerous cells including endothelial cells, immune cells, astrocytes, and glioblastoma stem cells within an extracellular matrix (ECM). The TME plays a critical role in tumor development. Extracellular vesicles (EV) in the TME establish bidirectional communication between tumor and TME [7, 8]. GBM communicates with host cells through cytokines and EVs, converting them into pro-tumor phenotypes promoting tumor evolution by the alteration of TME. Thus, recognizing this process and targeting the TME shows the potential to guide diagnostic and therapeutic strategies [9, 10]. Astrocytes and other myeloid-derived cells, including microglia and macrophages, are the first interactors of GBM. They have been found to aid tumor progression by promoting immune escape [9]. Astrocytes manipulate GBM behavior in diverse mechanisms and understanding the association between astrocytes and GBM would shed light on optimizing targeted therapy. Targeting astrocytes within the TME in inhibiting the progression of GBM shows much potential in guiding therapy.

Most literature focuses on targeting tumor cells to guide therapeutic targets for GBM. This chapter aims to depict the molecular interactions and understanding of the complex interplay between GBM cells and astrocytes. The chapter also focuses on the TME in identifying the promising therapeutic potential of manipulating astrocytes within the TME describing the current developments in astrocyte-targeted therapies for GBM and its future directions.

2. Tumor microenvironment in glioblastoma

The tumor microenvironment (TME) comprises extracellular matrix (ECM), noncancerous cells, and biomolecules. Astrocytes, glioblastoma stem cells (GSCs), vascular pericytes, fibroblasts, immune cells, microglia, and endothelial cells (ECs) constitute the noncancerous cells in the TME. Biomolecules are produced by noncancerous cells and include cytokines, chemokines, nitric oxide (NO), and hormones. TME regulates most functions of GBM and the tissue adjacent to it and this interaction plays a vital role in tumor development [11, 12]. The concept of the "Niche" was developed to describe the locations where the TME exerts the maximum influence and to describe the distribution of GSC in the tumor. The GSCs are localized into specific niches: the sub-ventricular zone (SVZ) and sub-granular zone (SGZ) [13].

Hypoxia is a notable character in GBM present in variable degrees within the tumor and the TME. They are characterized by extensive necrotic areas and thus a hallmark of GBM [13]. The hypoxia activates hypoxia-inducible factors (HIFs) which regulate the expression of oncogenes, transcription factors, and proangiogenic factors such as angiopoietins, vascular endothelial growth factor (VEGF), transforming growth factor β (TGF-β), which play a main role in GSC maintenance, self-renewal, invasion, and expansion. Furthermore, hypoxia contributes to the recruitment of macrophages and metabolic programming, which form the inflammatory niche. Macrophages facilitate the expansion of the GSCs by secreting IL-6 and TGF-β [14]. GSCs have been assumed to represent a subset of cells within GBM with the ability

to generate new tumor which contributes to the cellular heterogeneity of the tumor. Biomolecules including NO, cyclic guanosine monophosphate, and Fibroblast growth factor (FGF-2) were found to preserve the stemness of GSCs [15]. Glioma-associated microglia/macrophages (GAM) are known to contribute to tumor progression as they are related to immunological features of gliomas. They increase the invasiveness of the GBM by secreting factors such as TGF-β, which promotes the release of MMP2, MMP, versican, and the matrix metalloproteases that degrade collagen and elastin comprising the ECM [16]. Furthermore, TGF-β enhances the potential of GAMs to inhibit T cell proliferation and promote tumor progression.

Tumor angiogenesis is mainly determined by the ECs, pericytes, and GSCs specifically by the pericyte-EC interactions that are responsible for forming endothelial junctions and recruiting macrophages associated with tumor angiogenesis. Pericytes play a major role in the vascular maturation of the tumor, promoting tumor growth, and, together with the disruption of the BBB, they serve as a marker of neovascularization [17]. Interactions between ECM and GBM cells regulate the malignant nature of the tumor, facilitating invasiveness and infiltration of GBM.

3. Astrocyte function in glioblastoma

Astrocytes are glial cells that play a vital physiological role in the brain. They have numerous functions, which include synaptic transportation via calcium signaling, maintaining fluid homeostasis via aquaporin 4 channels, regulating ion homeostasis by the Na+/H+ exchanger, maintaining transmitter homeostasis at the synaptic interstitial fluid, and guiding the migration of neuroblasts and axons in development of neurons. They also serve as primary mediators of alterations in CNS blood flow [11]. Astrocytes play a pivotal role in synaptic activity and plasticity by regulating the balance between excitatory and inhibitory transmission, contributing to normal brain function. Furthermore, they are an important component of the BBB, which is formed by ECs forming tight junctions surrounded by basal lamina, pericytes, and astrocyte end-feet controlling the transport of metabolites from blood to the brain parenchyma and transport of waste products from the brain to blood using multiple substrate-specific transport systems, thus serving as a key homeostatic point between the systemic circulation and the CNS [11].

3.1 Astrogliosis

Astrogliosis, a phenomenon observed in various CNS pathologies including GBM, represents a complex cellular response characterized by the transformation of astrocytes into reactive states. This process is marked by significant morphological and functional alterations, notably cellular hypertrophy and enhanced proliferation at sites of injury. The reactive astrocytes exhibit upregulation of a diverse array of cellular components, encompassing intermediate filaments (e.g., nestin, vimentin), signaling receptors (e.g., c-MET), transcription factors (e.g., STAT-3), growth factors (e.g., brain-derived neurotrophic factor (BDNF), growth differentiation factor-15 (GDF-15)), cell adhesion molecules (e.g., CD44), and extracellular matrix constituents (e.g., collagens, versican). These molecular changes are integral to tissue repair mechanisms and the overall CNS response to pathological insults [18]. Concomitantly, reactive astrocytes secrete pro-inflammatory mediators, including cytokines such as Interleukin 6 (IL-6), and C-C motif chemokine ligand 2 (CCL2), as well as NO,

potentially exacerbating neuroinflammatory cascades [11]. In the context of acute brain injuries, astrogliosis would upregulate glial fibrillary acidic protein (GFAP), contributing to the formation of a "glial scar". This serves as a functional barrier, playing pivotal roles in modulating inflammation, isolation of the lesion, facilitating blood-brain barrier repair, enhancing synaptic plasticity, and initiating neuronal circuit reorganization, thereby potentially improving clinical outcomes and survival [11].

3.2 Astrocytes in tumor microenvironment

Recent advancements in astrocyte biology have unveiled the heterogeneous nature of these cells within the GBM microenvironment. Empirical studies have delineated five distinct astrocyte subpopulations, each characterized by unique morphological, molecular, and functional attributes across three regions in CNS [19]. The region-specific gene signatures of these astrocytes are correlated with gliomas harboring distinct genomic alterations, suggesting differential roles for astrocytes in their interactions within the GBM microenvironment. Moreover, investigations into tumor-associated astrocytes (TAAs) have revealed distinctive gene expression patterns, with certain stromal astrocytes in the tumor milieu expressing GBM-specific gene signatures that correlate with patient survival outcomes [20]. The intrinsic heterogeneity of astrocytes has been demonstrated to significantly influence glioma pathogenesis, with various astrocyte phenotypes showing correlations with different GBM subtypes [11].

3.3 Crosstalk and signaling pathways

The GBM microenvironment is critical for tumor progression, with adjacent parenchymal and non-malignant cells supporting its growth. Astrocytes, a key component of this environment, adopt a reactive phenotype upon interacting with tumor cells, releasing growth factors, chemokines, and cytokines that facilitate ongoing astrocyte activation and signaling with stroma and GBM cells. This interaction leads to increased expression of glial fibrillary acidic protein (GFAP) and connexin 43 (CX43), creating conditions conducive to glioma cell invasion [21]. Additionally, the TGF-β1 signaling pathway upregulates matrix metalloproteinase 2 (MMP2), enhancing the infiltrative capacity of GBM cells, while stromal cell-derived factor-1 (SDF1) secreted by reactive astrocytes promotes their uncontrolled proliferation. Thus, reactive astrocytes play a significant role in the TME by promoting malignancy through abnormal cell proliferation and malignant transformation. Understanding the pathways involved in these interactions is essential for developing targeted therapies [22].

3.3.1 NF-κB

Activated astrocytes increase significantly around invasive tumors expressing high levels of receptor activator of nuclear factor kappa-B ligand (RANKL). GBM-produced RANKL activates astrocytes via NF-κB signaling, leading to the secretion of factors regulating glioma invasion, particularly enhancing TGF-β signaling [23]. Fibulin-3 from GBM cells also activates NF-κB in peritumoral astrocytes, promoting tumor progression. Lipopolysaccharide (LPS), another NF-κB activator, can be inhibited by tetrandrine (TET), potentially preventing astrocyte reactivity. Additionally, astrocyte-derived C-C motif ligand 20 (CCL20) interacts with the C-C motif receptor (CCR6), stimulating NF-κB signaling and increasing HIF-1α under hypoxia, further enhancing GBM proliferation [24].

3.3.2 Sonic hedgehog (SHH)

Neuron-derived sonic hedgehog (SHH) plays a crucial role in regulating the molecular and functional profiles of astrocytes. In the perivascular niche of gliomas, two populations of SHH-producing stromal cells, astrocytes and endothelial cells, are highly concentrated. The SHH signaling pathway is activated when SHH, secreted by glioblastoma multiforme (GBM) cells, binds to the patched (PTCH) receptor on TAA, relieving the inhibition on the smoothened (SMO) receptor. This leads to the activation of GLI transcription factors, through TAA activation. Deregulation of SHH-Gli signaling has been associated with hyperproliferation of precursor cells and potential brain tumor initiation, while its suppression inhibits glioma cell migration and invasion. The SHH-Gli pathway facilitates astrocyte activation in the perivascular niche, promoting glioma invasion [25, 26].

3.3.3 p53

The tumor suppressor gene p53 plays a crucial role in the interaction between glioblastoma multiforme (GBM) and astrocytes, with mutations present in up to 87% of GBM cases [27]. While p53 normally initiates cell death or growth arrest under healthy conditions, this function is inhibited in p53-deficient cancer cells. p53 also modulates the expression of tumor cell-secreted proteins in the extracellular matrix (ECM) of GBM [28]. The ECM of p53+/− astrocytes exhibits higher levels of laminin and fibronectin, promoting epithelial-to-mesenchymal transition (EMT) and increasing GBM cell invasiveness promoting survival of GBM cells [29]. Additionally, GBM cells cultured in p53+/− astrocyte ECM show increased expression of N-cadherin and vimentin, markers associated with apoptosis resistance. This bidirectional relationship between GBM cells and the tumor microenvironment, where GBM hinders the expression of p53 in astrocytes, induces a lenient environment to tumor cells, with p53 reactivation emerging as a promising therapeutic approach [30].

3.3.4 IL-6/JAK/STAT

Recent studies have revealed the significant role of interleukin-6 (IL-6) in promoting glioblastoma multiforme (GBM) progression and malignancy. Tumor-associated astrocytes secrete IL-6, which activates the JAK/STAT signaling pathway in the TME. The activation of STAT3 protein is linked to aggressive clinical behavior in GBM. The activation of the IL6/STAT pathway leads to increased expression of matrix metalloproteinases (MMP2, MMP9, and MMP-14), enhancing GBM invasiveness through extracellular matrix degradation [31]. Furthermore, STAT3, activated by IL-6, directly upregulates vascular endothelial growth factor (VEGF) gene expression, promoting angiogenesis in GBM. STAT3 also regulates the expression of anti-apoptotic genes such as Bcl2l1, Bcl-2, and Mcl-1, contributing to GBM cell survival. These findings highlight the complex interplay between IL-6 signaling and GBM progression, suggesting potential therapeutic targets by inhibition of STAT3 in GBM cells [32].

3.3.5 PI3K/Akt

Reactive astrocytes in glioblastoma multiforme (GBM) secrete glial cell line-derived neurotrophic factor (GDNF), which activates the PI3K/Akt pathway through

RET/GFR1 receptor binding, promoting GBM migration. This pathway is often dysregulated in GBM due to phosphate and tensin homolog deleted on chromosome ten (PTEN) alterations. PTEN alterations by mutation, promotor methylation, and loss of heterozygosity are all proven to have significant tumor suppressor properties [33]. Activated Akt enhances GBM cell survival by regulating anti-apoptotic genes (Mcl-1, IAP), activating the NF-κB pathway, inhibiting p53-mediated apoptosis, and reducing caspase-9 activity. The PI3K/Akt pathway also increases epidermal growth factor receptor (EGFR) expression, including the constitutively active EGFRvIII variant associated with radioresistance in GBM [34]. Additionally, it regulates MMP-9 expression and AEG-1, enhancing GBM invasiveness and proliferation. High pAkt expression correlates with increased malignancy and poor prognosis in GBM patients, highlighting the pathway's significance in tumor progression [35]. Nevertheless, the works in cancerology on the signal transduction pathways related to growth factors have demonstrated that the overexpression of growth factors receptors signaling via TK/PI3K/AKT can induce immunogenicity in cancer cells. The last immunogenic cells increase the immune anti-tumor response [36–38].

3.4 Astrocyte function in GBM proliferation

Reactive astrocytes play a crucial role in glioblastoma multiforme (GBM) progression by secreting factors that enhance tumor growth and survival. In response to central nervous system injury, these astrocytes release TNF-α, TGF-β, IL-6, and insulin-like growth factor-1 (IGF-1), which increase the proliferation of primary brain tumors like GBM [39]. Additionally, extracellular vesicles (EVs) from GBM cells enrich the surrounding medium with fibroblast growth factor (FGF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), chemokines, and interleukins, further stimulating GBM cell proliferation.

Astrocytes upregulate connexin 43 (Cx43), which modulates apoptotic responses in GBM cells by inhibiting mitochondrial cytochrome C release, thereby preventing apoptosis. Protocadherin 7 (PCDH7) in GBM cells facilitates gap junction formation with astrocytes, allowing the transfer of cGAMP to TAAs. This activates the STING pathway, promoting TNF and interferon-alpha (IFN-α) production in the tumor microenvironment, which activates NF-κB and STAT-1 in GBM cells, supporting proliferation and invasion [40]. Astrocytes also meet the metabolic needs of GBM cells by synthesizing and secreting L-glutamine (Gln), essential for tumor growth, as GBM cells cannot produce enough themselves to satisfy their metabolic needs. GDF-15, overexpressed in reactive astrocytes, initially suppresses tumors by inducing Smad3 phosphorylation and apoptosis, inhibiting the division of tumor cells. However, as the tumor progresses, it enhances GBM cell viability by disrupting p53 function and promoting angiogenesis through increased HIF-1α and VEGF expression under hypoxic conditions [41]. Overall, reactive astrocytes create a supportive microenvironment that facilitates GBM proliferation and invasion, highlighting potential therapeutic targets to disrupt these interactions.

3.5 Astrocyte function in GBM invasion and migration

Glioblastoma multiforme (GBM) exhibits diffuse invasion, hindering complete surgical resection and effective chemoradiotherapy. GBM cells utilize extracellular migration routes similar to immature neurons and stem cells, often following blood vessels and exploiting the extracellular matrix (ECM) to support invasion [11].

These cells repurpose ion channels to adjust their volume for navigating narrow spaces and disrupting the blood-brain barrier. Astrocytes contribute significantly to GBM invasion by remodeling the ECM through the release of interleukin-6 (IL-6), which activates matrix metalloproteinases (MMPs). The interaction between GBM cells and astrocytes also activates MMP2 via the uPA-plasmin cascade [42]. Astrocytes further promote GBM invasion by upregulating fascin-1 expression and increasing VEGF production, supporting cell migration and angiogenesis.

Additionally, astrocytes facilitate communication with GBM cells through ion channels and neurotransmitters, participating in gliotransmission and exosome-mediated transport of miRNAs and proteins that enhance GBM progression and invasion. Pro-inflammatory cytokines secreted by glioma cells stimulate astrocyte reactivity, disrupt ion homeostasis, and regulate ion channel expression in glioma-associated stromal cells [43, 44]. These complex interactions between GBM cells, astrocytes, and the tumor microenvironment collectively contribute to the aggressive and invasive nature of GBM, presenting significant challenges for the treatment and management of the disease.

4. Therapeutic strategies targeting astrocytes

4.1 Immunotherapy-astrocyte-mediated immunosuppressive microenvironment

Despite significant advancements in understanding the immune regulation of glioblastoma multiforme (GBM) and the development of immunotherapies, therapeutic progress remains limited. The immune system initially activates natural killer (NK) cells and T lymphocytes to combat developing tumors, but cancer cells quickly develop resistance through immune evasion mechanisms. This evasion, coupled with T lymphocyte dysfunction, presents a major obstacle to effective immune responses, largely mediated by the immunosuppressive microenvironment in GBM patients [45].

Reactive astrocytes contribute significantly to this immunosuppressive environment through various mechanisms. They secrete factors such as tenascin-C, which impedes T cell migration, and IL-10, which inhibits pro-inflammatory mediators and antigen presentation [46]. The upregulation of STAT-3 in reactive astrocytes promotes angiogenesis, immunosuppression, and tumor invasion by recruiting regulatory T cells, expanding T-helper 17 cells, and promoting myeloid-derived suppressor cells (MDSCs). These processes collectively silence the anti-tumor Th1 response and inhibit CD4+ and CD8+ T cell activation [47].

Additionally, GDF-15, elevated in reactive astrocytes, impairs NK cell function, reduces immune cell infiltration, and disrupts dendritic cell function, further contributing to immune evasion. Astrocytes also upregulate PD-L1 expression, mediated by GBM-derived extracellular vesicles, which inhibits T cell function [48, 49].

A distinct reactive astrocytic subtype, characterized by JAK/STAT pathway activation and PD-L1 expression, contributes substantially to creating an immunologically "cold" tumor environment [22]. These findings highlight the complex role of reactive astrocytes in protecting GBM cells against anti-cancer immune responses, presenting significant challenges for developing effective immunotherapies and underscoring the need for targeted approaches to overcome this immunosuppressive microenvironment.

Considering JAK/STAT transduction pathway in relation to immunological mechanism (signaled in 3.4 Chapter), the expression of this pathway induces also immunogenicity playing a role in anti-tumor response; JAK/STAT is critical for MHC-1 expression on the cell surface [50–53].

4.2 Resistance to chemoradiotherapy

The current standard of care for glioblastoma multiforme (GBM) patients involves surgery followed by concomitant adjuvant temozolomide (TMZ) chemotherapy and radiotherapy, resulting in a median overall survival of 16 months [54]. Tumor treating field (TTF) therapy has shown promise, increasing median overall survival by 2.8 months. However, other novel treatments, including boron neutron capture therapy, antiangiogenic therapy, immunotherapy, epigenetic therapy, oncolytic virus therapy, and gene therapy, have yielded uncertain or disappointing clinical results despite promising preclinical data [55]. Fortunately, some recently applied immunotherapy and gene therapy approaches have given satisfactory clinical results in GBM treatments such as immune checkpoint inhibitors [56, 57] adoptive T cell therapy [58, 59], viral immunotherapy [60], and immuno-gene therapy [61]. The interaction between tumor cells and their microenvironment, particularly astrocytes, plays a crucial role in therapy resistance. Gap junction communication between glioma cells and astrocytes decreases tumor cell sensitivity to TMZ chemotherapy, while the gap junction protein Cx43 contributes to TMZ resistance [62].

Astrocytes also diminish glioma stem cell sensitivity to radiotherapy by influencing DNA double-strand break induction and repair. The JAK/STAT3 and PI3K/Akt pathways have been implicated in radioresistance, suggesting potential targets for enhancing treatment efficacy. In keeping with the above results, WP1066, a JAK/STAT pathway inhibitor, was known to enhance the radiosensitivity of GBM [63]. Given the significant role of astrocytes in GBM resistance to chemoradiotherapy, further research is needed to elucidate the underlying mechanisms and develop more effective treatment strategies.

4.3 Therapeutic challenge

GBM remains a formidable challenge in oncology due to its cellular heterogeneity and invasive nature. Despite decades of research and improvements in surgical techniques and chemotherapy protocols, the median survival for GBM patients is only 14.6 months, even with multimodal treatments including surgical resection, radiotherapy, and adjuvant chemotherapy with Temozolomide (TMZ) [64]. The rapid recurrence of GBM within 2–3 cm of the original tumor site, often with increased aggressiveness and resistance, underscores the limitations of current therapeutic approaches.

The complexity of GBM treatment is compounded by multiple factors, including the brain's limited cellular turnover, which restricts ablative therapies, the localization of tumors in functional brain areas that prevent complete surgical removal, and the blood-brain barrier's impediment of chemotherapeutic delivery. The genetic heterogeneity of GBM cells further complicates treatment, resulting in varied therapeutic responses. A critical factor in GBM's resistance is the presence of glioblastoma stem cells (GSCs), which are abundant in aggressive cancers and responsible for tumor maintenance and repopulation after treatment [65–67]. These GSCs possess an intrinsic resistance to current therapies, necessitating innovative strategies to

target this critical cancer cell subpopulation. Recent research has thus focused on understanding the role of GSCs in GBM, particularly their ability to survive therapy, with the ultimate goal of developing more effective treatment approaches to improve patient outcomes.

4.4 Therapeutic targets

Glioblastoma stem cells (GSCs) are believed to play a critical role in the resistance of GBM to chemoradiotherapy and subsequent tumor recurrence. These cells persist after standard treatments, such as surgery, radiation, and adjuvant chemotherapy with Temozolomide (TMZ), which is considered the most effective non-surgical therapy for GBM but remains largely palliative [68]. GSCs exhibit greater resistance to radiation compared to non-stem glioma cells, and while TMZ can delay tumor growth and extend survival by 6 months to a year, long-term survivors are rare due to the presence of TMZ-resistant cancer cells [69]. Although TMZ preferentially targets GSCs with high levels of the DNA repair protein MGMT, it does not inhibit the self-renewal of GSCs with normal MGMT levels. Furthermore, in vivo studies suggest that TMZ treatment may increase populations enriched in cancer stem cells, potentially favoring tumor recurrence. This highlights the urgent need for therapies specifically targeting the signaling pathways that maintain GSC functions. Traditional therapies primarily target proliferating non-tumorigenic cells, whereas GSCs remain mostly quiescent and resistant, serving as a reservoir for potential tumor recurrence. Despite advances in understanding GSC biology, further research is required to develop effective strategies targeting these cells to prevent GBM progression and improve patient outcomes [70].

4.4.1 Inducing differentiation

Bone Morphogenetic Proteins (BMPs) have emerged as promising agents for differentiation therapy targeting glioblastoma stem cells (GSCs). Recent studies have shown that the GBM tumor mass contains a central, necrotic core enriched with immature CD133 and Nestin-positive cells resistant to temozolomide (TMZ) treatment [71]. This highlights the need for novel therapeutic approaches combining conventional and innovative drugs. BMPs, members of the Transforming Growth Factor-β family, interact with type II (BMPR2) and type I (BMPR1A and BMPR1B) receptors [71]. During embryonic development, BMP2/4 initially promotes neuroepithelial proliferation via BMPR1A, later inducing neuronal and astrocytic differentiation of neural stem cells through BMPR1B. Several studies have demonstrated the potential of BMPs, particularly BMP2, BMP4, and BMP7, in promoting astroglial differentiation and reducing cell growth in GBM-derived cells. BMP7, released by neural precursor cells, has been shown to repress proliferation, self-renewal, and tumor initiation of GSCs. However, caution is warranted as approximately 20% of GBM tumors display epigenetic silencing of BMPR1B, potentially limiting the efficacy of BMP treatment in these cases. Further research is needed to determine if BMP-mediated inhibitory effects specifically target the cancer stem cell population and to identify alternative soluble factors for BMPR-silenced patients [72].

The Wnt/β-catenin signaling pathway plays a crucial role in glioblastoma multiforme (GBM) progression, with β-catenin, encoded by the CTNNB1 gene, being a key component of the canonical Wnt pathway. Studies have shown that β-catenin expression levels are elevated in high-grade gliomas compared to low-grade tumors

and normal brain tissue. Silencing β-catenin in U251 glioma cells induces apoptosis, inhibits growth, and arrests the cell cycle in G1 phase, accompanied by downregulation of cyclin D1, c-Myc, and c-jun expression. These findings are consistent with research demonstrating delayed tumor progression in vivo following Wnt2 and β-catenin silencing [73].

Furthermore, GBM samples exhibit reduced expression of three potent Wnt signaling inhibitors: DKK1, SFRPI, and WIFI, compared to normal brain tissues. Among these, restoring DKK1 expression alone resulted in growth reduction, suggesting its potential tumor suppressor function. Interestingly, DKK1 has also been reported to sensitize GBM cells to chemotherapy and exert growth-suppressive effects, partly through non-canonical Wnt signaling activation [74].

These findings highlight the complex role of Wnt/β-catenin signaling in GBM progression and suggest potential therapeutic targets for GBM treatment, particularly through modulation of Wnt pathway inhibitors and β-catenin expression [75].

4.4.2 GSC signaling pathways

The PI3K/Akt/mTOR signaling pathway plays a crucial role in regulating cell proliferation, differentiation, and survival in GBM. Dysregulation of this pathway, often due to PTEN mutations or deletions occurring in approximately 40% of GBM patients, is frequently observed and associated with reduced patient survival [76]. Recent studies have shown that loss of PTEN, in conjunction with p53 loss, maintains neural stem cells and glioblastoma stem cells (GSCs) in an undifferentiated state by promoting self-renewal and inhibiting differentiation via Myc [76].

Various PI3K inhibitors are currently in preclinical or clinical development, targeting different catalytic subunit isoforms of class IA PI3Ks. Notable examples include NVP-BKM120, a pan-class IA PI3K inhibitor that induces apoptosis and mitotic catastrophe in GBM cells, and PX-866, a synthetic wortmannin analog that inhibits PI3K/Akt/mTOR target genes and prolongs survival in experimental mouse models [77].

Akt inhibition has shown promise in targeting GSCs, with perifosine and A-443654 demonstrating potent effects. mTOR inhibitors, such as temsirolimus, everolimus, and sirolimus, have also been tested in clinical trials based on encouraging preclinical results. However, the use of PI3K/Akt/mTOR inhibitors as monotherapies has yielded unsatisfactory results in clinical studies, leading to the exploration of combination therapies and dual inhibitors targeting multiple components of the pathway [78].

Recent findings suggest that simultaneous inhibition of PI3K/Akt/mTOR and MEK/ERK pathways may more effectively suppress GSC tumorigenicity, with FoxO3a identified as a key molecular transducer integrating signals from these pathways [79]. This complex network of molecular pathways highlights the need for further research to develop more effective therapeutic strategies for GBM.

4.4.3 GSC microenvironment

Recent advances in targeting hypoxia-inducible factor-1 (HIF-1) have led to the development of numerous small molecule inhibitors, with some progressing toward preclinical and early clinical trials. Combining HIF-1 inhibitors with existing treatments or new targeted therapies shows potential for clinical application. The hypoxic niche and HIF-1α enhance cancer cell migration by promoting metalloproteinase expression and migration-associated receptors like CXCR4 [80].

The CXCR4/CXCL12 axis plays a crucial role in glioblastoma multiforme (GBM) migration and growth. CXCR4+ cells demonstrate stronger tumorigenic potential and increased resistance to temozolomide and radiotherapy. Blocking CXCR4 has shown a significant reduction in GBM xenograft growth, indicating its potential as a therapeutic target. Plerixafor (AMD3100), a CXCR4 antagonist, inhibits irradiation-induced vasculogenesis in vivo [81].

Targeting the vascular niche of glioblastoma stem cells (GSCs) is another approach to destabilize their function. Antiangiogenic drugs like Bevacizumab and Cediranib have shown some success in clinical therapy. Multi-tyrosine kinase inhibitors such as Sunitinib and Vandetanib have demonstrated potential in preclinical studies, although clinical trial results have been less promising [82].

In conclusion, targeting both the hypoxic and perivascular niches could be effective for GBM treatment. An optimal therapeutic strategy should combine therapies targeting GSC functions with those affecting microenvironmental factors that sustain them.

5. Clinical implications

The standard treatment for glioblastoma multiforme (GBM) includes surgery, radiation therapy (RT), and chemotherapy. The current standard of care combines concomitant adjuvant temozolomide (TMZ) chemotherapy with radiotherapy, resulting in a median overall survival (OS) of 14.6 to 16 months. Tumor treating fields (TTFields), approved by the FDA, can further increase median OS by 2.8 months. Despite promising preclinical results, other novel treatments such as boron neutron capture therapy (BNCT), antiangiogenic therapy, immunotherapy, epigenetic therapy, oncolytic virus therapy, and gene therapy have shown uncertain or discouraging clinical outcomes [54].

No monotherapy has proven sufficient to prevent GBM recurrence. The interaction between tumor cells and their microenvironment, particularly astrocytes, plays a crucial role in therapy resistance. Gap junction communication (GJC) between glioma cells and astrocytes decreases tumor cell sensitivity to TMZ chemotherapy. The gap junction protein Cx43, upregulated by astrocytes, contributes to TMZ resistance, while its knockdown increases TMZ-induced apoptosis of glioma cells [83].

Astrocytes also diminish the sensitivity of glioma stem cells (GSCs) to radiotherapy by influencing the induction and repair of DNA double-strand breaks (DSBs). Gene expression profiling of GSCs in astrocyte-mediated co-culture revealed potential targets for radiosensitization, including STAT3. The JAK/STAT3 inhibitor WP1066 has shown promise in enhancing GSC radiosensitivity [63]. Additionally, the PI3K/Akt pathway is involved in GBM radioresistance, with Akt targeting resulting in increased unrepaired DNA DSBs following irradiation.

Given the significant role of astrocytes in GBM resistance to chemoradiotherapy, further research is needed to elucidate the mechanisms underlying astrocyte-glioma cell interactions and develop more effective treatment strategies.

6. Conclusions

In conclusion, astrocytes play a pivotal role in the GBM tumor microenvironment (TME), significantly influencing tumor progression and therapeutic outcomes.

The complex interplay between astrocytes and GBM cells, mediated through various signaling pathways, presents both challenges and opportunities for targeted therapies. Astrocytes' heterogeneity and their region-specific gene signatures correlate with distinct GBM subtypes, highlighting the need for personalized treatment approaches. The reactive astrocytes' ability to promote GBM proliferation, invasion, and angiogenesis through the secretion of growth factors, cytokines, and extracellular vesicles underscores their potential as therapeutic targets. Future research should focus on developing strategies to modulate astrocyte function within the TME, potentially inhibiting their pro-tumor effects while enhancing their neuroprotective properties. By targeting the astrocyte-GBM cell interactions and the associated signaling pathways, novel therapies could be developed to improve GBM treatment outcomes. This approach represents a promising avenue in the ongoing efforts to combat this aggressive and challenging malignancy.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 5

Dendritic Cell-Based Glioblastoma Vaccines: Advances and Challenges

Zuowen Zhang, Mingchang Li and Jie Huang

Abstract

Glioblastoma (GBM) is the most aggressive malignancy of the central nervous system. Despite advances in standard treatments such as surgery, radiotherapy, and chemotherapy, patients have a very poor prognosis. Tumor vaccines based on dendritic cells (DCs) provide a promising new approach for GBM treatment. DCs, as the most effective antigen-presenting cells, initiate adaptive immune responses by activating tumor-specific T cells. However, the immunosuppressive microenvironment of GBM (characterized by regulatory T cells, myeloid suppressor cells, and immunosuppressive factors) and the physical barrier of the blood-brain barrier (BBB) greatly limit the efficacy of DC vaccines. This chapter explores the biological basis, preparation process, clinical progress, challenges, and future directions of DC-based GBM vaccines. Key aspects such as antigen selection, DC in vitro culture and activation, antigen loading, and delivery strategies are analyzed in detail. Early clinical trials have demonstrated the safety and potential efficacy of DC vaccines, while combination therapies and microenvironment reprogramming strategies are being used to overcome existing obstacles. Despite the challenges, the precision and personalization of DC vaccines highlight their potential as a focus of immunotherapy research. We believe that with the continuous advancement of technology and interdisciplinary collaboration, DC vaccines can significantly improve the survival rate and quality of life of GBM patients.

Keywords: glioblastoma, dendritic cell vaccine, antigen presentation, immunosuppressive microenvironment, blood-brain barrier, personalized immunotherapy

1. Introduction

Glioblastoma (GBM) is one of the most common and aggressive malignant tumors of the adult central nervous system, with high heterogeneity and invasiveness. Despite standard treatments including surgical resection, radiotherapy, and chemotherapy, the prognosis of GBM patients is still extremely poor, with a median overall survival (OS) of only 12–18 months [1]. The bottleneck of GBM treatment lies in its complex molecular biological characteristics and immunosuppressive microenvironment, which not only limit the effectiveness of traditional treatments but also pose a huge challenge to emerging immunotherapies [2].

In recent years, immunotherapy as a breakthrough in cancer treatment has provided new treatment strategies for a variety of malignant tumors, including GBM [3–6]. While technologies such as immune checkpoint inhibitors (such as anti-PD-1/PD-L1 and CTLA-4 antibodies), chimeric antigen receptor T cell (CAR-T) therapy, and oncolytic viruses have made progress, tumor vaccines based on dendritic cells (DCs) have also become an important part of the field of immunotherapy [7]. As the most powerful antigen-presenting cells (APC) in the immune system, DC plays a core role in activating tumor-specific T cells and initiating adaptive immune responses [8]. DC vaccines can effectively induce anti-tumor immune responses by loading tumor-associated antigens in vitro and activating DCs, providing a possibility for the treatment of "cold tumors" such as GBM [9]. However, the unique immunosuppressive microenvironment of GBM poses a significant challenge to the efficacy of DC vaccines. GBM inhibits the maturation and function of dendritic cells by secreting immunosuppressive factors (such as TGF-β, IL-10, IDO) and recruiting myeloid-derived suppressor cells (MDSC) and regulatory T cells (Treg) [10]. In addition, the blood-brain barrier (BBB) forms a physical restriction on the delivery of immune cells and vaccines, making it difficult for DC vaccines to exert their maximum efficacy in the central nervous system [11]. Therefore, although some clinical trials have confirmed the safety of DC vaccines and the immune response effect in some patients [12]. For example, DCVax®-L, a personalized DC vaccine based on the patient's own tumor antigens, has shown the potential to prolong survival in patients with recurrent and newly diagnosed GBM [13]. However, the low overall response rate and individual differences in efficacy suggest that the application of DC vaccines in the treatment of GBM still needs to be further optimized [14].

This chapter will comprehensively explore the application of dendritic cell vaccines in glioblastoma, focusing on its biological functions, preparation processes, clinical research progress, challenges, and future development directions. First, we will introduce the basic biological functions of DC and its mechanism of action in anti-tumor immunity; then, we will elaborate on the preparation technology of DC vaccines, including antigen selection, in vitro culture, antigen loading, and delivery strategies; then, we will review the latest progress in early clinical trials, efficacy evaluation, and combination therapy of DC vaccines in the treatment of GBM; finally, in response to the challenges of GBM immunosuppressive microenvironment, we will propose solutions for reprogramming microenvironment and combination therapy and look forward to future development directions (**Figure 1**).

2. Biological functions and mechanisms of dendritic cells

Dendritic cells (DC) are the most powerful antigen-presenting cells in the mammalian immune system. Their core function is to connect innate immunity with adaptive immunity [15]. DC originates from bone marrow progenitor cells and is distributed in peripheral tissues such as skin, mucosa, and organs in an immature state. They sense exogenous pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) through highly expressed pattern recognition receptors (PRRs) to capture external or endogenous antigens [16]. After these antigens are taken up, they are processed into small peptides in DC and presented through major histocompatibility complex (MHC) class I or class II molecules, laying the foundation for activating T cells [17]. Immature DCs have strong antigen

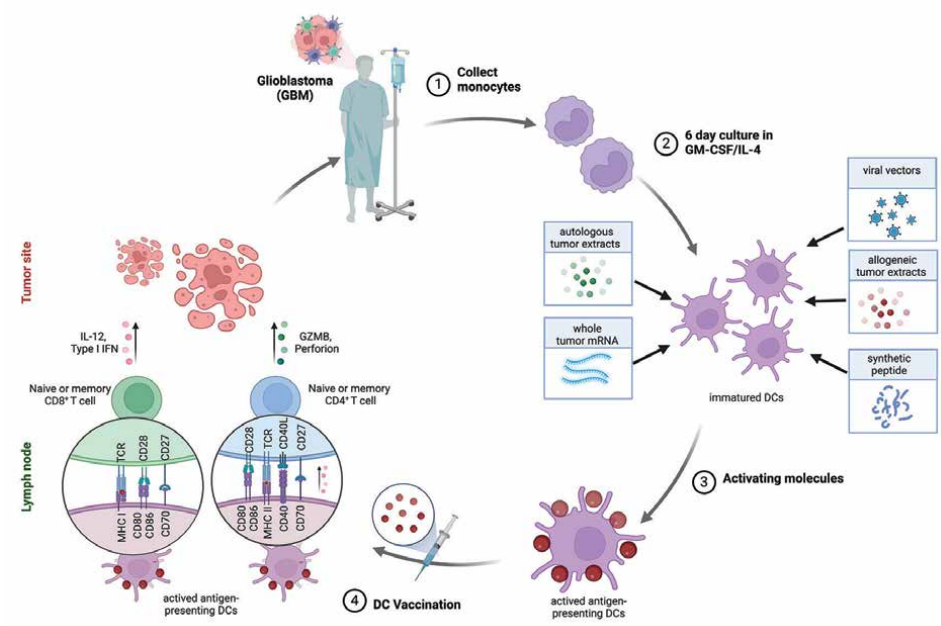

Figure 1.
Dendritic cell (DC) vaccine preparation process and its function in glioblastoma (GBM) immunotherapy. After mononuclear cells are collected from GBM patients, they are cultured in GM-CSF/IL-4 for six days to generate immature DCs. Tumor antigens are loaded onto DCs using autologous tumor extracts, mRNA, viral vectors, synthetic peptides, or allogeneic tumor extracts. The immature DCs are then activated using stimulatory molecules to generate mature antigen-presenting DCs. These activated DCs are infused back into the patient as a vaccine. In the lymph nodes, DCs present antigens to naive or memory CD4+ and CD8+ T cells, triggering an immune response against GBM cells at the tumor site.

uptake ability but weak antigen presentation ability; mature DCs can not only present antigens efficiently but also secrete immunomodulatory factors to comprehensively regulate immune responses [18].

Mature DCs activate specific T cells by migrating to lymph nodes, which is the core of their mechanism of action. The activation process of T cells depends on three signals provided by DCs: the binding of antigen-MHC complexes to T cell receptors (TCRs) provides specific recognition signals; the binding of costimulatory molecules (such as CD80/CD86) to CD28 provides a second activation signal; and the cytokines secreted by DCs (such as IL-12, IL-10, etc.) determine the functional differentiation direction of T cells, such as Th1 cell-mediated cytotoxic immunity or the immunosuppressive effect of Treg cells [19]. This multi-signal regulatory mechanism makes DCs the "commanders" of immune responses and plays an irreplaceable role in adaptive immunity [20]. In tumor immunity, the role of DCs is particularly important. By capturing and presenting tumor antigens, DCs can activate specific CD8+ cytotoxic T cells (CTLs) and eliminate tumor cells [21]. However, immunosuppressive factors in the tumor microenvironment (such as TGF-β, IDO) significantly weaken the function of DCs, resulting in a decrease in their presentation ability or inducing immune tolerance, which is also the main challenge facing DC-based immunotherapy [22]. Nevertheless, the use of DC vaccines to load tumor-specific antigens and reshape their functions has become an important strategy for cancer immunotherapy, especially in the treatment of highly invasive tumors such as glioblastoma [23].

3. Preparation and development of vaccines based on dendritic cells

3.1 Antigen source and selection

Antigen source and selection are the core links of dendritic cell (DC) vaccine preparation. Their precision and diversity directly determine the effect and specificity of the immune response. For DC vaccines targeting tumors, antigens are usually divided into tumor-associated antigens (TAAs) and tumor-specific neoantigens (Neoantigens) [24]. TAAs, such as EGFRvIII, MAGE family proteins, and Survivin, are highly expressed in tumor cells but relatively low in normal tissues and have certain specificity. However, since TAAs may be expressed in normal cells, there is a certain risk of immune tolerance [25]. In contrast, Neoantigens are produced by tumor mutations, have high specificity, and will not trigger immune responses in normal tissues, so they have been a research hotspot in recent years [26]. The sources of antigens also include tumor lysates and whole-cell antigens. This diverse source helps to enhance the breadth of immune response [27]. In addition, nucleic acid antigen (RNA or DNA) technology allows tumor antigens to be expressed in vivo, providing DC with persistent antigen stimulation [28]. Antigen selection needs to be combined with patient-specific tumor characteristics, and high-throughput sequencing and bioinformatics technologies are used to predict new antigens to develop personalized DC vaccines. This precise and personalized antigen strategy not only improves the immune efficacy of the vaccine but also significantly reduces off-target effects, providing a scientific basis for tumor immunotherapy [29].

3.2 In vitro culture and activation of dendritic cells

The in vitro culture and activation of dendritic cells (DC) is the core step in the preparation of DC vaccines. Its goal is to obtain functionally mature and efficient antigen-presenting cells to effectively stimulate the patient's immune response [30]. This process usually isolates monocytes from the patient's peripheral blood, differentiates them into immature DCs under specific culture conditions, and further activates them to enhance their antigen presentation ability [31]. First, monocyte isolation is a key starting step. Monocytes are isolated from peripheral blood mononuclear cells (PBMCs) by density gradient centrifugation or magnetic bead sorting technology [32]. These precursor cells can differentiate into immature DCs within 5–7 days after adding specific cytokines (such as GM-CSF and IL-4) to the in vitro culture medium [33]. Immature DCs have efficient antigen uptake but weak antigen presentation capabilities. Subsequently, maturation and activation are key steps. Immature DCs can be induced to mature after treatment with stimulatory factors (such as cytokine cocktails, including TNF-α, IL-1β, IL-6, and PGE2) or TLR (Toll-like receptor) agonists (such as LPS, Poly I) [34]. Mature DCs exhibit higher levels of MHC molecules and costimulatory molecules (such as CD80, CD86, and HLA-DR) and secrete cytokines such as IL-12 to enhance their antigen presentation function [35]. In addition, antigen loading and activation processes are often carried out simultaneously. Antigen loading methods include direct incubation of tumor lysates, synthetic peptides, or transfection of antigen-encoding RNA/DNA, which enable DCs to effectively process and present tumor-associated antigens [36]. To further enhance their immune function, gene editing technology or nanoparticle delivery can also be used to enhance the activity and stability of DCs [37]. Finally, quality control is an indispensable step in the in vitro culture and activation process. The maturation

state and functional activity of DCs can be evaluated by detecting DC surface markers (such as CD80, CD86, and HLA-DR) and the levels of cytokines secreted by DCs using flow cytometry [38]. High-quality DCs can more effectively activate patients' T cells, thus ensuring the clinical efficacy of vaccines [39].

3.3 Antigen loading technology

Antigen loading is a key step in the preparation of dendritic cell (DC) vaccines. Its purpose is to effectively deliver specific tumor-associated antigens to DCs to activate tumor-specific immune responses. Efficient antigen loading technology can enhance the antigen presentation ability and immune activation effect of DCs [40]. Commonly used antigen loading methods include direct incubation, nucleic acid transfection, electroporation, and fusion technology. Direct incubation is the simplest method, in which tumor lysates or synthetic peptides are co-incubated with mature DCs to allow them to ingest and process antigens. This method is suitable for broad-spectrum antigen presentation but may be limited by insufficient antigen concentration or low specificity [41]. Nucleic acid transfection introduces RNA or DNA encoding tumor antigens into DCs, causing them to express antigen proteins in vivo, thereby generating endogenous MHC class I presentation pathways and enhancing the activation effect on CD8+ cytotoxic T cells (CTLs) [42]. Electroporation increases cell membrane permeability through a short-term electric field, introducing nucleic acids or proteins into DCs, with high efficiency and rich antigen diversity [43]. Cell fusion technology fuses DC with tumor cells to form hybrid cells, thereby achieving comprehensive expression of tumor-associated antigens [44]. In addition, advanced delivery technologies such as nanoparticles and liposomes are also used for antigen loading, which further optimizes the antigen processing ability of DC by stabilizing antigens and improving uptake efficiency [45]. The strategy selection for antigen loading needs to comprehensively consider the type of antigen, loading efficiency, and clinical applicability to achieve a more efficient and specific anti-tumor immune response [46].

3.4 Vaccine delivery strategy

Vaccine delivery strategy is an important part of dendritic cell (DC) vaccine preparation. Its purpose is to accurately and efficiently deliver functionally mature DC to the immune-active area in the patient's body to activate anti-tumor immune response [47]. The choice of delivery method has a profound impact on the efficacy and safety of DC vaccines. Subcutaneous injection is the most commonly used delivery method. After injection, DC can migrate to the lymph nodes in the skin lymphatic tissue, contact T cells, and initiate an adaptive immune response. This method is simple to operate and has high safety, but its DC migration efficiency may be affected by individual differences in patients [48]. Intra-lymph node injection is to inject DC directly into the lymph nodes, which greatly increases the chances of DC contacting T cells, thereby enhancing the immune effect of the vaccine. However, this method requires precise positioning technology and may increase the complexity of the operation [49]. Intravenous injection is a feasible method for delivering DC vaccines, allowing them to be distributed throughout the body through the blood circulation. Although its ability to penetrate the blood-brain barrier (BBB) is limited, it remains a potential approach for treating central nervous system tumors such as glioblastoma [50]. Despite the challenges posed by the BBB, researchers are increasingly reconsidering its role as a major barrier to CNS immunotherapy given recent evidence of lymphocyte infiltration into the brain [51].

To enhance the delivery of dendritic cells (DCs) to tumor sites, direct intracerebral injection remains a viable strategy that can bypass the BBB and target glioblastoma or its surrounding areas [52]. Controlled release and targeted delivery technologies have also become a prominent research focus. For example, DCs can be encapsulated in bio-scaffolds, hydrogels, or nanoparticles to achieve slow release and prolonged action in vivo [53]. In addition, delivery vehicles modified with targeting molecules such as integrins or chemokine receptor ligands can precisely direct DCs to specific immune-active areas, further improving the precision and efficacy of treatment [54].

4. Advances in clinical research based on DC vaccines

4.1 Early trials

Early clinical trials based on dendritic cell (DC) vaccines laid the foundation for their application in tumor immunotherapy, especially in the treatment of recurrent and refractory tumors [55]. The earliest clinical studies focused on safety, immunogenicity, and preliminary efficacy evaluation, providing key data for subsequent studies. In 1996, a phase I clinical trial for melanoma patients verified the safety of DC vaccines for the first time. The results showed that patients tolerated the vaccine well, had no serious adverse reactions, and induced specific T cell immune responses in some patients [56]. Subsequently, early trials for glioblastoma (GBM) were also carried out. For example, in the phase I study of DCVax®-L vaccine, antigen-loaded DCs were prepared by collecting patient tumor tissues, and prolonged progression-free survival (PFS) and overall survival (OS) were observed in some patients after re-infusion [57]. Although the sample size was small, the trial results showed the potential benefits of DC vaccines in GBM. The effects of different antigen sources and delivery methods were also explored in early trials. For example, trials using tumor lysates as antigens showed a broader immune response, and the delivery strategy through intra-lymph node injection significantly enhanced the interaction between DC and T cells, further improving the immunogenicity of the vaccine [58]. These studies also revealed key challenges, including the limitation of the tumor immunosuppressive microenvironment on the efficacy of DC vaccines and the inconsistency of responses caused by individual differences among patients [59]. Although the early trials were limited in scale, their results provided strong support for the safety and preliminary efficacy of DC vaccines and pointed out the direction for optimizing the preparation process and treatment strategy [60]. These studies have laid a solid foundation for the further development of DC vaccines in glioblastoma and other tumors.

4.2 Efficacy evaluation

The efficacy evaluation of dendritic cell (DC) vaccines is a core part of clinical trials. Its main goal is to verify the effectiveness of vaccines in tumor treatment through multidimensional indicators. Common evaluation indicators include overall survival (OS), progression-free survival (PFS), immune response rate, and improvement in quality of life [61].

1. Overall survival (OS) is the gold standard for evaluating the clinical efficacy of DC vaccines. Early trials have shown that the overall survival of some glioblastoma (GBM) patients has been significantly prolonged after receiving DC vaccine

treatment. For example, in a Phase II clinical trial of DCVax®-L, the median OS of patients treated with the vaccine exceeded 23 months, which is significantly improved compared with traditional treatment options. In addition, some patients showed long-term survival characteristics, indicating that DC vaccines may have more lasting efficacy in specific patient groups [62].

2. Progression-free survival (PFS) is used to evaluate whether the disease worsens within a certain period of time. In many DC vaccine trials, the extension of PFS is often regarded as an indirect proof of efficacy. For example, a study of patients with recurrent GBM found that the median PFS of patients treated with DC vaccines reached 8 months, while that of the control group was only 4 months, indicating that the vaccine may slow the progression of the tumor [63].

3. The immune response rate is an important biological indicator of the efficacy of DC vaccines, which is usually evaluated by detecting the proportion or functional status of tumor-specific T cells in the patient's peripheral blood. For example, flow cytometry and ELISPOT experiments are often used to measure the activity of CD8+ cytotoxic T cells and their specific response to tumor antigens. In addition, patients treated with DC vaccines often show higher effector T cell infiltration and lower levels of immunosuppressive factors in the tumor microenvironment, which also reflects the immunomodulatory effect of the vaccine [64].

4. Improved quality of life is an important clinical endpoint, especially in the treatment of aggressive tumors such as glioblastoma. Many trials have shown that DC vaccine treatment has fewer side effects than traditional treatments, and patient's functional status and quality of life can be better maintained. This low toxicity and high efficacy further support the application prospects of DC vaccines in tumor treatment [65].

4.3 Combination therapy

Combination therapy is an important strategy to enhance the efficacy of dendritic cell (DC) vaccines, aiming to overcome the limitations of single therapy and achieve synergistic effects in anti-tumor immunity [66]. In recent years, studies have shown that the combination of DC vaccines with other treatments can significantly enhance the immune responses and clinical efficacy of patients.

1. Combination with radiotherapy and chemotherapy: Radiotherapy and chemotherapy can not only directly kill tumor cells but also promote antigen uptake and activation of DC by releasing tumor antigens. For example, radiotherapy-induced tumor cell death will release more antigens, thereby enhancing T cell responses induced by DC vaccines [67]. In addition, low-dose chemotherapy (such as cyclophosphamide) can also reduce the proportion of regulatory T cells (Treg), alleviate the immunosuppressive effect of the tumor microenvironment, and provide more favorable conditions for DC vaccines [68].

2. Combination with immune checkpoint inhibitors: Immune checkpoint inhibitors (such as anti-PD-1/PD-L1, anti-CTLA-4 antibodies) enhance the T cell activity induced by DC vaccines by relieving the inhibition of effector T cells [69]. Studies have shown that this combination can not only significantly increase the

number of tumor-infiltrating T cells but also prolong the patient's progression-free survival (PFS) and overall survival (OS) [70].

3. Combination with oncolytic viruses or other immunotherapies: Oncolytic viruses can activate DCs and enhance the efficacy of vaccines by directly lysing tumor cells and releasing danger signals [71]. In addition, combining DC vaccines with CAR-T cell therapy and using DCs to activate more effector T cells can significantly enhance the anti-tumor response [72].

Through multimodal combination therapy, the anti-tumor potential of DC vaccines can be maximized, which also provides a broader space for future personalized immunotherapy strategies.

5. Effects of glioblastoma microenvironment on DC vaccines

5.1 Multiple effects of immunosuppressive factors

There are a variety of immunosuppressive factors in the glioblastoma (GBM) microenvironment, which interferes with the function of dendritic cells (DC) through multi-level mechanisms, thereby weakening the anti-tumor effect of DC vaccines [73]. These immunosuppressive factors mainly include cytokines, metabolites, and enzyme molecules, which work together to build a complex immune escape network. Cytokines such as TGF-β and IL-10 are highly expressed in the GBM microenvironment. They inhibit the maturation and antigen presentation ability of DC, making it difficult for DC to activate specific T cells. TGF-β also downregulates the expression of costimulatory molecules on the surface of DC, such as CD80 and CD86, thereby further reducing the efficiency of immune activation [74]. In addition, VEGF (vascular endothelial growth factor) not only promotes tumor angiogenesis but also interferes with the differentiation process of DC from monocytes [75]. Metabolites also pose a threat to DC function. For example, lactate produced by GBM cells weakens the metabolic activity of DCs by acidifying the microenvironment, while metabolites generated by the breakdown of tryptophan by indoleamine 2,3-dioxygenase (IDO) can induce DC tolerance and prevent them from activating effector T cells [76]. In addition, oxidative stress molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are expressed at high levels in GBM, which directly reduces the antigen uptake and processing capacity of DCs by damaging the DC cell membrane and DNA structure [77]. The multiple effects of these immunosuppressive factors significantly limit the efficacy of DC vaccines. Therefore, inhibitors targeting these factors or combined treatment strategies have become an important direction for improving the efficacy of DC vaccines [78].

5.2 Interaction between myeloid-derived suppressor cells and DC vaccines

Myeloid-derived suppressor cells (MDSCs) are immunosuppressive cells that exist in large numbers in the microenvironment of glioblastoma (GBM) and can significantly interfere with the function of dendritic cells (DC) vaccines [79]. MDSC inhibits DC maturation, antigen presentation ability, and T cell activation through multiple mechanisms, thereby weakening the anti-tumor effect of DC vaccines. First, MDSC interferes with DC maturation and function by secreting immunosuppressive

factors such as IL-10 and TGF-β. These factors can downregulate the expression of costimulatory molecules (such as CD80 and CD86) on the surface of DC, while reducing the presentation ability of major histocompatibility complex (MHC), making DC unable to effectively activate T cells [80]. In addition, vascular endothelial growth factor (VEGF) secreted by MDSC also inhibits DC differentiation, further reducing the number of functional DCs [81]. Second, MDSC inhibits DC function through metabolic pathways. For example, reactive oxygen species (ROS) and reactive nitrogen species (RNS) secreted by MDSC directly damage DC cell membranes and internal molecular structures, weakening their antigen uptake and processing capabilities [82]. IDO (indoleamine 2,3-dioxygenase) is another immunosuppressive enzyme highly expressed by MDSC, which induces DC tolerance by consuming tryptophan and prevents it from activating effector T cells [83]. In addition, MDSC further amplifies the immunosuppressive effect by synergizing with regulatory T cells (Treg). Treg can inhibit the immunostimulatory effect of DC through a negative feedback mechanism, while MDSC strengthens this inhibitory effect by promoting the proliferation of Treg [84].

In order to overcome the negative effects of MDSC on DC vaccines, researchers are exploring a variety of intervention strategies, such as using low-dose chemotherapy or inhibitors targeting MDSC to reduce the number or function of MDSC [85]. In addition, combining immune checkpoint inhibitors or metabolic regulators can further alleviate the inhibitory effect of MDSC, thereby improving the efficacy of DC vaccines [86]. Through multi-faceted combined treatment, it is expected to break through the bottleneck of MDSC-mediated immunosuppression and bring more effective immunotherapy options to GBM patients [87].

5.3 Immunosuppressive effects of regulatory T cells (Treg)

Regulatory T Cells (Treg) are important immunosuppressive cells in the glioblastoma (GBM) microenvironment and play a significant role in the immune activation ability of dendritic cell (DC) vaccines [88]. Tregs weaken the function of DCs and inhibit the activation of effector T cells through direct and indirect mechanisms, thereby helping tumors evade immune surveillance. In the GBM microenvironment, the number of Tregs is usually significantly increased, which is mainly attributed to tumor-secreted immune regulatory factors such as TGF-β and IDO (indoleamine 2,3-dioxygenase), which induce conventional T cells to differentiate into Tregs or promote Treg expansion [89]. The increased Tregs not only suppress the immune response locally in the tumor but also weaken the effect of DC vaccines through systemic immune regulation mechanisms [90]. The direct inhibitory effect of Tregs on DC function includes inhibiting the expression of costimulatory molecules (such as CD80 and CD86) on the surface of DCs through cell contact, thereby reducing the ability of DCs to activate effector T cells [91]. In addition, the immunosuppressive factors IL-10 and TGF-β secreted by Tregs can further weaken the antigen presentation efficiency of DCs, making it difficult to induce potent anti-tumor immune responses [92]. In terms of indirect mechanisms, Tregs regulate the immune balance of the tumor microenvironment, maintain high levels of immunosuppressive factors, and promote the function of myeloid-derived suppressor cells (MDSCs), thereby forming a synergistic suppression network and further weakening the efficacy of DC vaccines [93]. To overcome the immunosuppressive effects of Tregs, researchers are developing targeted treatments for Tregs, such as using low-dose chemotherapy to reduce the number of Tregs or combining immune checkpoint inhibitors (such

as anti-CTLA-4 antibodies) to relieve their suppressive effects [94]. The combined application of these strategies with DC vaccines is expected to improve the intensity and sustainability of anti-tumor immune responses [95].

5.4 Limitation of immune cell infiltration by the blood-brain barrier

The blood-brain barrier (BBB) is a major obstacle in the treatment of glioblastoma (GBM). Its structure is composed of tightly connected brain endothelial cells, a basement membrane, and astrocyte foot processes, which strictly restrict the entry of foreign substances and immune cells [96]. This barrier function has a significant impact on the efficacy of dendritic cell (DC)-based vaccines. In healthy tissue, the BBB effectively blocks the invasion of peripheral immune cells into the central nervous system. However, in GBM, although tumor growth and angiogenesis may lead to local disruption of the BBB, the infiltration of immune cells such as DCs and effector T cells is still significantly restricted [97]. Even if a DC vaccine is delivered via intravenous injection, the vast majority of DC will be kept out of the tumor by barriers [98]. To overcome this limitation, researchers have developed various strategies, such as using carriers such as liposomes and nanoparticles to carry DCs or their secreted factors to penetrate the BBB [99]. In addition, direct injection of DC vaccines into the brain or meningeal area can also effectively bypass the barrier and increase the concentration of DC in the local tumor [100]. While breaking through the BBB barrier, these methods provide more possibilities for the application of DC vaccines in the treatment of GBM [101].

5.5 Microenvironment reprogramming and immunotherapy synergistic strategy

Microenvironment reprogramming is an important strategy to improve the therapeutic effect of glioblastoma (GBM). By changing its immunosuppressive microenvironment, the efficacy of vaccines based on dendritic cells (Dendritic Cells, DC) can be significantly enhanced [102]. The immunosuppressive microenvironment of GBM is mainly composed of regulatory T cells (Treg), myeloid-derived suppressor cells (MDSC), and immunosuppressive factors (such as TGF-β, IL-10), etc., which together weaken the anti-tumor function of the immune system. Function. Microenvironmental reprogramming targeting these factors combined with DC vaccines can produce synergistic anti-tumor effects [103].

1. Targeting immunosuppressive factors

   The use of drugs that inhibit key factors such as TGF-β, IL-10, or IDO can weaken the immunosuppressive effect of the GBM microenvironment. For example, TGF-β inhibitors not only enhance the antigen-presenting ability of DCs but also restore the activity of effector T cells (Teff) by blocking its signaling [104]. In addition, VEGF inhibitors can reduce the recruitment of immunosuppressive cells and promote DC differentiation and maturation [105].

2. Regulate myeloid-derived suppressor cells (MDSC) and regulatory T cells (Treg)

   Low-dose chemotherapy (such as cyclophosphamide) has been shown to selectively reduce the number of Tregs and MDSCs, weakening their inhibitory effects on DCs and effector T cells [68]. CXCR2 inhibitors targeting MDSCs significantly improved DC vaccine-induced immune responses by blocking their

migration pathways [106]. At the same time, immune checkpoint inhibitors (such as anti-PD-1/PD-L1 antibodies) further amplify the efficacy of DC vaccines by lifting the suppression of effector T cells by Tregs [107].

3. Combining oncolytic viruses and nanotechnology

   Oncolytic viruses can not only directly kill tumor cells but also release danger signals and promote DC activation and antigen presentation functions [108]. At the same time, using nanoparticles or liposomes as carriers to deliver antigens and immune stimulants can improve the persistence and activity of DCs in the GBM microenvironment and enhance the immunogenicity of vaccines [109].

4. Combined with other immunotherapies

   DC vaccines combined with CAR-T cell therapy or antibody drugs can produce multi-level immune responses. For example, T cells activated by DC vaccines can enhance the effect of CAR-T therapy, and antibody drugs (such as bispecific antibodies) can further recruit effector T cells to the tumor site to form a synergistic anti-tumor effect [110].

Through the strategy of microenvironment reprogramming and combined immunotherapy, not only can the immunosuppression of the GBM microenvironment be overcome, but also the therapeutic efficiency of DC vaccines can be improved. This comprehensive treatment model brings new hope for the treatment of highly invasive tumors such as GBM and shows broad clinical application prospects.

6. Challenges and unresolved issues

Dendritic cell (DC) vaccines have made significant progress in cancer immunotherapy, but they still face challenges that limit their widespread application, especially in glioblastoma (GBM). These challenges span immunobiology, manufacturing, clinical efficacy, and patient-specific factors, requiring multidisciplinary solutions [111].

6.1 Immunosuppressive microenvironment of GBM

GBM has a highly immunosuppressive microenvironment, which is the main obstacle to the efficacy of DC vaccines. Regulatory T cells (Treg), myeloid-derived suppressor cells (MDSC), and immunosuppressive factors (such as TGF-β, IL-10, IDO) jointly weaken the function of DC and the activity of anti-tumor T cells [112]. In addition, tumor cells can change the acid-base balance of the local environment through metabolic regulation (such as lactate production), further suppressing the immune response [113]. These complex immune escape mechanisms make it difficult for a single DC vaccine to achieve optimal results in GBM treatment [88].

6.2 Limitations of the blood-brain barrier (BBB)

The blood-brain barrier is another major challenge in GBM treatment. Although DC vaccines can enter the patient's body through a variety of delivery routes (such as

intravenous injection, intra-lymph node injection), their ability to penetrate the BBB to reach the tumor site is limited [101]. This barrier not only limits the efficiency of vaccine delivery but also affects the activity of effector T cells in the local tumor [114].

6.3 Complexity and quality control of DC vaccine preparation

The preparation process for DC vaccine is complex, requiring the isolation of monocytes from the patient's peripheral blood and differentiation, maturation, and antigen loading in vitro [115]. The whole process is time-consuming, costly, and has large individual differences. In addition, the quality control of DC maturation status and antigen presentation ability remains a difficult point. The lack of standardized processes and unified quality assessment indicators makes it difficult to compare the results between different studies [116].

6.4 Individual differences among patients

The immune status and tumor heterogeneity between GBM patients are significant, which affects the therapeutic effect of DC vaccine. For example, the tumor mutation load and antigen expression profile of different patients vary greatly, making it impossible for universal antigens to meet the needs of all patients [10]. In addition, some patients have poor responses to DC vaccines due to low immune system function or immunosuppressive treatment (such as steroids) [117].

6.5 Lack of efficacy evaluation and biomarkers

The indicators currently used to evaluate the efficacy of DC vaccines mainly include overall survival (OS) and progression-free survival (PFS), but these indicators take a long time to obtain results and may be affected by other treatments [118]. In addition, the lack of reliable biomarkers to predict patients' ability to respond to DC vaccines limits the monitoring of efficacy and the implementation of personalized treatment [55]. However, some CD markers of PBL cells verified in GBM patients treated by different immunotherapies permit partially predicting patients' response to immunotherapy vaccination, such as CD8 CD11b (−), CD28, or CD25 [119].

6.6 Limitations of monotherapy

DC vaccines have limited effectiveness as monotherapy, especially in tumors with complex immune escape mechanisms such as GBM. Although some patients showed prolonged survival, the overall response rate was low. This limitation suggests that DC vaccines need to be used in combination with other treatments (such as immune checkpoint inhibitors, chemoradiotherapy, or CAR-T cell therapy) to enhance their anti-tumor activity [120].

6.7 Inadequate scale and design of clinical trials

Most of the current clinical trials on DC vaccines are small in scale, and the results lack statistical significance. In addition, the selection of control groups and efficacy evaluation criteria in the trial design are inconsistent, which increases the difficulty of data interpretation [121]. These problems limit the possibility of DC vaccines being promoted and applied on a larger scale [122].

6.8 Ethical and cost issues in preparation and application

The individualized preparation and clinical application of DC vaccines are costly, making them difficult to promote in areas with limited medical resources [123]. In addition, the preparation process involves the collection of patient tumor tissue and peripheral blood, which may raise ethical issues [29].

7. Future development direction

The future of dendritic cell (DC) vaccines lies in achieving breakthroughs on multiple fronts, providing promising advances in glioblastoma (GBM) treatment. The rapid development of precision medicine will push DC vaccines toward personalization. High-throughput genetic sequencing and bioinformatics can identify tumor-specific neoantigens to develop tailored vaccines for individual patients [124]. Advanced gene editing technologies such as CRISPR-Cas9 will further enhance DC antigen presentation and immune activation, significantly improving therapeutic efficacy [125]. Delivery technologies will also advance, with smart carriers such as nanoparticles and bio-scaffolds improving vaccine permeability across the blood-brain barrier (BBB) and extending DC retention in the body [126]. Combination therapy is expected to be a key direction in the future, as combining DC vaccines with immune checkpoint inhibitors, radiotherapy, chemotherapy, oncolytic viruses, or CAR-T cell therapy can produce synergistic anti-tumor effects [127]. Furthermore, reprogramming of the microenvironment—using small molecule inhibitors to target myeloid suppressor cells (MDSCs) and regulatory T cells (Tregs)—will optimize the local immunosuppressive milieu and enhance vaccine efficacy [128]. Standardized preparation protocols and evaluation systems will accelerate clinical translation. At the same time, cost control and automation in production will reduce barriers and enable global accessibility. With technological advancement and interdisciplinary collaboration, DC vaccines are expected to provide more precise, efficient, and generally applicable solutions for glioblastoma treatment [129].

8. Conclusion

Dendritic cell (DC)-based vaccines, as a cutting-edge technology for tumor immunotherapy, have shown great potential in the treatment of malignant tumors such as glioblastoma (GBM). Its unique antigen presentation ability makes DC an important bridge connecting innate immunity and adaptive immunity, providing a key pathway for activating specific T cell immune responses. However, the immunosuppressive microenvironment of GBM, the limitations of the blood-brain barrier, and the challenges of DC vaccine preparation and quality control still restrict the further improvement of its efficacy. Early clinical trials have demonstrated the safety of DC vaccines and their efficacy in some patients, but the overall response rate and sustainability of efficacy still need to be addressed by optimizing microenvironment remodeling strategies, improving delivery technologies, and combining treatment models. In the future, DC vaccines will develop in the direction of personalized, engineered, and multimodal treatment. The integration of new antigen discovery technology, gene editing technology, and intelligent delivery systems will further enhance the specificity and effectiveness of DC vaccines. At the same time, the application of

standardized preparation processes and biomarkers will help the clinical promotion and efficacy monitoring of vaccines. Through multidisciplinary collaboration, DC vaccines are expected to break through existing bottlenecks, provide more efficient and precise treatment options for GBM patients, and push cancer immunotherapy to new heights.

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Chapter 6

Glioblastoma Management in the Post-COVID-19 Era: Challenges, Strategies, and Adaptations

Xiaohui Ren and Ling Qin

Abstract

The COVID-19 pandemic significantly disrupted glioblastoma (GBM) management, affecting its pathophysiology, diagnosis, treatment, and prognosis. GBM, an aggressive brain tumor with poor survival rates, presented unique vulnerabilities during the pandemic due to its rapid progression and patients' compromised immunity. SARS-CoV-2 may also influence GBM progression by binding to overexpressed receptors on glioblastoma cells, potentially activating oncogenic pathways. This interaction highlights the potential for COVID-19 to exacerbate tumor aggressiveness. Diagnostic delays resulted in increased tumor volumes, while treatment pathways adapted with minimally invasive surgeries, hypofractionated radiotherapy, and telemedicine to ensure continuity of care. Despite these innovations, survival outcomes varied globally, with centers adopting flexible protocols reporting fewer adverse impacts. Vaccination emerged as a critical tool, with prioritization for GBM patients and minimal adverse effects, providing protection against severe COVID-19. This paper explores the multifaceted impact of COVID-19 on GBM, detailing disruptions and strategies that paved the way for resilient neuro-oncological care during and after the pandemic.

Keywords: glioblastoma, COVID-19 pandemic, treatment, telemedicine, vaccination

1. Introduction

Glioblastoma multiforme (GBM) is recognized as one of the most common primary brain malignant tumors in adults, accounting for 60–70% of high-grade gliomas [1]. The 5-year survival rate of GBM is only approximately 5%, and the risk of mortality in GBM patients may be even higher in the setting of the COVID-19 pandemic [1, 2]. Coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has accumulated 704,753,890 confirmed cases globally until April 2024 (https://www.worldometers.info/coronavirus/). Given the susceptibility to SARS-CoV-2, most people around the world are considered to be reinfected by SARS-CoV-2 (Omicron variant) pandemic [3]. The ongoing COVID-19 pandemic continues to pose significant risks, particularly for vulnerable population globally. Efforts to control the pandemic have led to substantial restrictions on daily

lift, raising concerns about mental health challenges. According to the U.S. Centers for Disease Control and Prevention, "individuals with certain underlying medical conditions face an increased risk of developing severe illness from COVID-19." Patients with GBM due to reduced immune function and weakened systemic immune system owing to antitumor treatments like radiation, chemotherapy, or surgery [4–6], are more likely to contract SARS-CoV-2 compared to those without tumors. The mentioned antitumor treatments permit, although only partially, to maintain the immune response.

The lungs are widely recognized as the primary site of SARS-CoV-2 infection; however, some studies suggest that the virus may also invade the central nervous system (CNS), leading to neurological disorders such as headache, dizziness, and impaired consciousness as well as cranial nerve-related symptoms [7]. Additionally, cognitive impairments, usually termed "brain fog", are common among individuals with long-term COVID-19 symptoms and represent one of the most frequently reported post-infection sequelae [8, 9]. Evidence increasingly confirms SARS-CoV-2's involvement in the CNS with severe neurological symptoms occurring in 36.4% of affected patients [10].

2. The mechanism of SARS-CoV2 entry into CNS

The potential mechanism of the neurological symptoms associated with COVID-19 has been controversial until the strong evidence of COVID-19 neuro-invasion and neurovirulence supported by SARS-CoV-2 RNA appearance in cerebrospinal fluid and brain autopsy tissue [11]. SARS-CoV-2 may reach and impact the CNS through various pathways, including the olfactory route, axonal retrograde transport or interactions with the blood-brain barrier (BBB) or blood-cerebrospinal fluid (CSF) barrier [12, 13]. The BBB, consisting of tight junctions between adjacent endothelial cells, is essential in limiting paracellular viral movement and is recognized as a major pathway for coronavirus to access the CNS [13]. Importantly, SARS-CoV-2 RNA has been detected in various brain regions, such as cortical neurons, olfactory nerve, frontal lobe and brainstem [14–16]. It is also widely known that glial and neuronal cells express ACE2 on their surface, supplying a potential target for COVID-19 infection [17]. SARS-CoV2 enters host cells *via* spike (S) proteins. After binding to the angiotensin-converting enzyme 2 (ACE2) on target cells, the S protein is cleaved into S1 and S2 by TMPRSS2 and host cell proteases such as cathepsin L (CTSL) [18], which facilitates viral entry into the cell *via* membrane fusion and endocytosis. This cleavage of S protein is critical for viral activation and subsequent infection [19, 20]. Interestingly, although ACE2 and TMPRSS2 have been considered as the two widely accepted receptors during SARS-CoV-2 cell entry, it has also been reported to be relatively low in endothelial cells of human brain in some studies [21, 22]. The coronavirus receptors Alanyl aminopeptidase (ANPEP), Tyrosine-protein kinase receptor UFO (AXL), and Glutamyl Aminopeptidase (ENPEP) were detected in the olfactory region and endothelial cells of human brain, indicating that SARS-CoV-2 cell entry in human brain might require these three receptors rather than only rely on ACE2 and TMPRSS2.

Additionally, the structural damage to the BBB among brain tumor patients elicits the SARS-CoV-2 infection to the CNS. A Q-albumin index >0.008 g/dl always indicates BBB dysfunction and has been proven in specific brain tuomor [23]. Brain tumors can compromise the integrity of the BBB by secreting proinflammatory

mediators, vasoactive cytokines, and growth factors, which can influence tight junctions and enhance permeability [23, 24]. Numerous studies have shown downregulation of tight junction protein expression of BBB in specific brain tumors, including astrocytomas and glioblastoma multiforme [24].

3. The evidence of direct deleterious role of COVID-19 on GBM

In glioma tissue extracted from a COVID-19 patients, ACE2 expression was found to be higher in GBM compared to the surrounding GBM-adjacent tissue [25]. The glioma tissues derived from patients who has experienced COVID-19 months before surgery also show that SARS-CoV-2 entry factors are expressed in glioma tissues [26]. Besides these known facts above, glioma cells possess surface receptors such as epidermal growth factor receptors (EGFR), vascular endothelial growth factor receptors (VEGFR) and hepatocyte growth factor receptors (HGFR/c-MET), all of which play crucial roles in tumor progression and invasion [27]. It has been established that the viral S protein molecule can bind to the other overexpressed receptor molecules in glioma cells, including epidermal growth factor receptors (EGFR) and vascular endothelial growth factor receptors (VEGFR). Notably, the COVID-19 S protein exhibits a binding affinity for EGFR, VEGFR and c-MET receptor proteins, comparable to its affinity for ACE2. The high affinity of viral S protein toward EGFR and VEGFR tends to propose a path to investigate the glioma oncogenesis role of COVID-19 [28]. Insulin-like growth factor-I (IGF-I), which is the principal growth factor of cancer development [29], could affect glioma progression as well [30, 31]. The capability of these receptors to activate downstream signaling pathways that involved in oncogenesis highlights their potential as promising targets for molecular therapeutic interventions.

Otherwise, immunohistochemical staining of the GBM tissue showed that both the mRNA and the protein levels of ANPEP and ENPEP were significantly increased compared to normal brain tissue in GBM [32]. The presence of ANPEP and ENPEP in endothelial cells of the BBB facilitates the possibility of SARS-CoV-2 cell entry into the brain. Protein-protein docking studies suggest that ANPEP or ENPEP could bind to the receptor binding domain (RBD) of the virus, potentially aiding in SARS-CoV-2 infection and increasing the susceptibility of GBM to the virus. Notably, GBM cell lines derived from recurrent glioblastoma exhibit higher malignancy and elevated mRNA levels of ANPEP and ENPEP, further supporting their possible oncogenic roles in GBM. Additionally, ANPEP and ENPEP expression levels were showed to increase with age and correlate with poor prognosis [32]. A protein interaction network analysis revealed that ANPEP, ENPEP, and ACE2 can form a protein complex, suggesting that ANPEP and ENPEP may contribute directly to SARS-CoV-2 communication in brain, alongside ACE2. However, further investigation is needed to understand how ACE2 and other coronavirus receptors influence the pathogenicity of GBM and how SARS-CoV-2 infection impacts the clinical outcomes of GBM patients.

4. The impact on diagnosis, treatment and prognosis of glioblastoma during COVID-19

The COVID-19 pandemic caused significant disruptions in the timely diagnosis and treatment of glioblastoma (GBM). In the setting of brain cancer, a 16% reduction in the diagnosis of new cases was observed. Analysis of data from the Patient Register

revealed a 6% overall decline in the number of newly diagnosed cancer cases from March to December 2020 compared to the corresponding period in 2019 [33]. Missed diagnoses were estimated at 70 cases in 2020 due to healthcare disruptions in Belgium. Additionally, patients presenting with WHO performance scores indicating severe symptoms increased by 10%, suggesting delayed presentations [34]. These delays or missing status impacted tumor size, treatment pathways and, in some cases, survival outcomes. The increased tumor volumes before diagnosis had been demonstrated. In a study of 236 neuro-oncology patients in Germany, median GBM tumor volume at the time of diagnosis rose from 15.7 cm^3 pre-COVID-19 to 21.7 cm^3 during COVID-19 [35]. In Belgium, the age-standardized incidence rate for malignant brain tumors dropped by 8% in 2020 for patients aged 50 and older. A significant decline of 37% was noted in April 2020 during the first COVID-19 wave [34]. This decline was attributed to the cancelation of routine appointments and imaging services, as well as patients avoiding hospitals due to fear of COVID-19 exposure. The median time from symptom onset to imaging was extended, although specific delays varied across studies. Diagnostic imaging services, particularly MRIs, were deprioritized in favor of emergency procedures during COVID-19 surges. This delay in imaging pushed back critical diagnostic steps for GBM, which often progresses rapidly [34]. In Canada, delays in surgical interventions resulted in a higher reliance on biopsies (21.5% during COVID-19 vs. 12.9% pre-COVID-19, $p = 0.037$) and fewer gross total resections (36.7% during COVID-19 vs. 56.5% pre-COVID-19, $p < 0.05$) [36]. Fewer patients underwent aggressive surgical resection during COVID, with an increased reliance on biopsy-only interventions. A similar trend was observed in Germany, where diagnostic or limited surgical interventions became more common as elective surgeries were deprioritized [35]. Otherwise, the time from surgery to oncology consultation and the initiation of radiation therapy remained consistent at about 6 weeks [35]. Hypo-fractionated radiotherapy regimens were increasingly used to reduce the frequency of hospital visits. In Canada, the proportion of patients treated with palliative radiotherapy rose from 9.4% pre-COVID-19 to 15.2% during the pandemic [36]. As the comprehensive therapy had been proven to be the most critical determinant of GBM survival [37], the proportion of GBM patients receiving combined surgery, chemotherapy, and radiotherapy fell, directly impacting survival.

COVID-19-positive GBM patients had a 2.18 times higher risk of mortality and a 0.43 odds ratio of receiving surgery compared to non-infected counterparts [38]. However, in the Surveillance, Epidemiology, and End Results (SEER) cohort (USA), survival outcomes of GBM patients did not show significant differences between the pre-COVID-19 (2018–2019) and COVID-19 periods (2020), with median survival times remaining consistent. This highlights the resilience of specialized centers in mitigating the pandemic's effects [37]. Another neuro-oncological care center in USA during the COVID-19 pandemic had also not showed adverse patient outcomes [39]. This suggests that adaptive physician-led changes were successful. In contrast, two-year observed survival (OS) for malignant brain tumors in Belgium decreased by 4% in 2020 compared to 2015–2018, with some recovery evident by mid-2020 [34]. The controversy on prognosis of GBM reflected the heterogeneous clinic conditions during the GBM diagnosis and treatment.

5. The impact of COVID-19 pandemic on neurosurgery clinic scenarios

The COVID-19 had tremendously impacted various aspects of neurosurgery, disrupting traditional workflows, decision-making, patient management and even

clinical trials across the world. The healthcare resources had been compelled to reallocate and adjust timely to avoid infection, prioritizing COVID-19 treatment while limiting access to neurosurgical procedures. The management of brain tumors underwent substantial modifications. Elective surgeries were widely deferred to preserve intensive care unit (ICU) capacity and protect both patients and staff from potential virus exposure. In the United Kingdom, COVID-19 significantly affected patients needing surgery for malignant brain tumors, leading them to opt for alternative treatments or in most cases forgo surgery or any treatment altogether [40]. At Mount Sinai in New York, elective neurosurgical operations were widely deferred, focusing only on urgent cases due to resource constraints such as ICU beds and personal protective equipment (PPE) [41]. This was a common phenomenon observed in other institutions. In Poland, a study revealed a 10.5% decrease in the total number of brain tumor surgeries in 2020 compared to previous years, with elective procedures significantly more affected than acute ones [42]. Similar trends were observed in Germany, where hospital admissions for brain tumor care dropped from 8.2 daily cases pre-pandemic to 7.3 during the pandemic, and the average length of stay also decreased from 10.2 days (pre-pandemic) to 9.5 days during the pandemic., reflecting reduced access to healthcare [43]. Other aspects of neuro-oncology clinical activities particularly encountered the strain, as multidisciplinary tumor boards faced challenges in maintaining continuity of timely care. Clinical trials and investigational treatments were also heavily impacted globally, with over 60% of trials suspended during the peak of the pandemic. The global survey had showed that 67% of practitioners suspended clinical trials, including 62% suspending Phase III trials [44]. This disruption significantly impacted access to investigational therapies and the pace of research.

Nevertheless, some regions, such as Normandy in France, maintained consistent tumor board activities, even increasing the proportion of surgical resections the proportion of tumor resections increased to 81.4% (n = 79/174) during lockdowns, compared to 64.5% (n = 408/1366) during non-lockdown periods [45]. At the University of California San Diego, neurosurgery experienced only a slight reduction in operative volume while consistently meeting critical neurosurgical demands [46]. German data also showed a moderate rise in the rate of brain tumor resection. Meanwhile, frailty among neurosurgical patients also shifted, with German data showing a reduction in frailty scores during the pandemic. This may be attributed to prioritization of less frail patients for limited surgical slots, leading to the rate of brain tumor resections increased from 29.9% in pre-pandemic to 36.6% during the pandemic [43].

In the realm of specific surgeries like pituitary tumor resections, trans-sphenoidal approaches were largely avoided due to high viral transmission risks. An increased risk of contagion among ear, nose and throat surgeons has been revealed in China [47, 48]. This compelled patients to suspend trans-sphenoidal neurosurgery for lesions in the pituitary fossa and anterior skull base. In Australia, the number of trans-sphenoidal surgeries for pituitary tumors sharply declined as a result. Alternative approaches, such as craniotomies, had been considered for the most urgent cases despite their higher risk profile [49].

These disruptions highlight the need for adaptable systems and innovative strategies, such as telemedicine and adjusted treatment protocols, to ensure the resilience of neurosurgical care in the face of global health crises [50, 51]. The pandemic underscored the critical balance between managing infectious disease crises and maintaining essential non-COVID healthcare services.

6. GBM treatment strategy under the long-term stress of COVID-19

The management of glioblastoma (GBM) during and post-COVID-19 pandemic has undergone significant adaptation to balance effective treatment with resource constraints and infection control. The comprehensive exploration of GBM treatment strategies based on the previous experiences has been described here. Concerning surgical treatment strategy, surgeries for GBM during the pandemic were prioritized based on urgency. Elective surgeries were postponed, and essential surgeries for patients with severe neurological deficits or life-threatening conditions, such as significant mass effect, were expedited. Emergency cases were performed within 24–48 hours, with enhanced personal protective equipment (PPE) for all staff. Semi-urgent cases were delayed by 2–4 weeks when the operation was safely feasible [52, 53]. At Mount Sinai in New York, only urgent surgeries proceeded, with a focus on gross total resections whenever possible to maximize survival benefits [41]. In Italy, the "Hub and Spoke" model centralized surgeries to specific COVID-free hospitals, ensuring safety and continuity of care for GBM patients. This model enabled 67 surgeries in 3 weeks, demonstrating the efficiency of centralized care [54]. Moreover, minimally invasive surgical approaches were preferred to reduce ICU stay and postoperative complications. A center in Santa Monica, California, implemented a streamlined protocol emphasizing minimally invasive craniotomies and endonasal surgeries, reducing ICU utilization from 54 to 29% [55]. Awake craniotomies became a key technique in some institutions, allowing for outpatient or limited inpatient monitoring, minimizing resource use [55–57]. Finally, when resources were constrained, diagnostic biopsies were sometimes chosen over extensive resections. A case study during the pandemic showed that a stereotactic biopsy was performed instead of a craniotomy for a 75-year-old GBM patient with multiple comorbidities to reduce the risk of ICU admission and facilitate immediate adjuvant therapy [41]. Other modified protocols, such as rapid preoperative COVID-19 testing, strict zoning in operating theaters, and early discharge planning, minimized patient exposure [52].

With regards to radiotherapy strategy, hypofractionated radiotherapy regimens were widely adopted in order to reduce hospital visits and potential exposure to COVID-19. Older or frail patients received 40 Gy in 15 fractions instead of the standard 60 Gy in 30 fractions. This shorter regimen demonstrated similar outcomes for elderly GBM patients. A study highlighted the use of 25 Gy in five fractions for palliative cases with relatively small volume disease, ensuring timely care while minimizing hospital visits [58]. Besides that, radiation centers implemented rigorous infection control protocols, including pre-treatment COVID-19 testing, daily screen for symptoms and reduction patient contact in waiting areas [59].

Chemotherapy regimens were tailored to reduce immunosuppression risks and hospital visits. Temozolomide (TMZ) continued to be the cornerstone of GBM chemotherapy. However, its use was carefully evaluated for patients without methylated methylguanine-DNA-methyltransferase (MGMT) promoters, given their lower responsiveness. TMZ was avoided in unmethylated cases when risks outweighed benefits [60]. Oral chemotherapy agents were preferred to minimize the need for infusion visits. In cases where radiotherapy was delayed, chemotherapy was used as a bridging therapy to control tumor progression until definitive radiotherapy could be initiated [58]. Chemotherapy toxicities were monitored through telemedicine to reduce clinic visits, with conservative dosing strategies employed to prevent hospitalizations [59].

As for tumor treating fields (TTFields), it provided a portable, home-use alternative, requiring minimal hospital visits. This therapy showed no known immunosuppressive effects and maintained efficacy when combined with TMZ [61]. Device setup and troubleshooting were conducted through telemedicine. Monthly device check-ins transitioned to virtual platforms, reducing patient-provider physical interactions [61].

The treatment of GBM during and after COVID-19 features flexibility, innovation, and a commitment to patient safety. The integration of reasonable surgery, oral chemotherapy, shorter radiotherapy courses, telemedicine and home-based therapies like TTFields ensures that GBM patients continue to receive high-quality care despite ongoing healthcare challenges in the future. These strategies not only mitigate pandemic-related risks but also pave the way for more efficient and patient-centered neuro-oncological care in the post-COVID ear. The comprehensive counseling emphasized infection prevention and home-based symptom management. For terminal cases or high-risk patients, care plans focused on palliation, avoiding aggressive interventions unless necessary.

7. The role of telemedicine in GBM management during and post-COVID-19

Multidisciplinary collaboration vital responsible artificial intelligence (AI) solutions against COVID-19 itself or its related clinical issues has been widely used during pandemic [62]. The AI interplay tool leverages COVID-19-related information from social media to protect vulnerable patients timely. Besides that, the telemedicine, which partially or totally employs AI, has emerged as a pivotal tool in managing GBM patients during and after the COVID-19 pandemic, ensuring continuity of care while minimizing infection risks and travel burdens. At large institutions, telemedicine replaced nearly 70% of in-person follow-ups during the pandemic's peak, helping recover patient volumes that had dropped by over 50% due to site closures. The virtual consultations for chemotherapy monitoring, consent, and follow-up were widely used for 58.17% of GBM patients in a study at the University of Miami. The urgent symptom evaluations for complications, such as seizures or edema, avoid emergency room visits unless absolutely necessary [63, 64]. Those procedures above ensure the continuity of GBM patients' care. In order to supply patient-centered care, patients living 100–200 miles from the treating center can chose telemedicine for 80% of their follow-ups, reducing travel costs and logistical burdens. Vulnerable or older patients particularly benefited, with over 60% opting for telemedicine due to reduced exposure to hospital-acquired infections. Besides that, the platforms integrated interpreters and caregiver support, enhancing accessibility for diverse patient populations. Telemedicine virtual platform has also facilitated other neuro-oncology practices, such as multidisciplinary care, postoperative and follow-up care, medical education and clinical trial consent as well [65].

Telemedicine has transformed GBM management by increasing access, reducing patient burden, and maintaining high standards of care. While challenges like technological limitations and reimbursement policies remain, the integration of virtual platforms and innovative tools will continue to enhance neuro-oncology practices. This model represents a sustainable approach to delivering patient-centered care in the post-pandemic era.

8. The impact of COVID-19 vaccination on GBM management

Vaccination against COVID-19 had proven crucial for GBM patients during the pandemic, providing essential protection for an already vulnerable population. GBM patients, particularly those undergoing chemotherapy, faced heightened risks of severe COVID-19 due to compromised immunity. Governments prioritized these patients for early vaccination. In an international survey, 55.2% of brain tumor patients were prioritized by their government. The brain tumor was also the most common reason reported in the survey for prioritization to receive the COVID-19 vaccine (30.8%), followed by age (16.4%), and other medical conditions (9.5%) [66]. Concerning GBM, about *26.4% of GBM patients* were on active treatment (chemotherapy, radiotherapy, or combination therapies) when they got vaccinated, highlighting the focus on concurrent care and immune protection. The low adverse event rates made COVID-19 vaccination popular among brain tumor patients. In a study of 102 brain tumor patients, *no severe adverse events* like anaphylaxis were observed. Mild reactions included *fatigue (3%), fever (3%), and local injection site pain (2.9%)*, resolving within few days. The vaccines did not affect GBM disease progression or outcomes, boosting their use during tumor active treatment [67]. Vaccination was recommended during stable periods of the tumor treatment cycle to maximize immune response. The interval between chemotherapy and vaccination was tailored (1–28 days) to reduce interactions. High Seroconversion Rates (90%, n = 102) were showed in solid tumor patients, including GBM cases, even under active treatment. Only four vaccinated GBM patients (3.9%, n = 102) contracted COVID-19 without symptoms, compared to 50% infection rates in unvaccinated GBM patients [67]. Meanwhile *89.9% of caregivers* were vaccinated, reducing the risk of transmission to GBM patients and allowing caregivers to safely accompany patients to appointments [66]. With minimal adverse effects, significant protection from severe disease, and seamless integration into existing treatment protocols, vaccination strategies have effectively mitigated risks for this vulnerable population.

9. Conclusion

The COVID-19 pandemic imposed unprecedented challenges on glioblastoma management, from pathophysiology, delayed diagnoses to altered treatment protocols. Diagnostic delays increased tumor volumes, and healthcare resources were reallocated to manage the crisis, limiting access to essential neuro-oncological care. Innovations such as telemedicine, hypofractionated radiotherapy, and minimally invasive surgical techniques mitigated some of these disruptions, ensuring patient safety and continuity of care. Vaccination played a pivotal role, providing significant protection to immunocompromised GBM patients and reducing COVID-19-related complications. The resilience and adaptability demonstrated by healthcare systems underscore the need for integrated approaches and innovative care models in managing GBM during global health crises. These experiences provide a framework for enhancing patient-centered neuro-oncology care in a post-pandemic world.

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Glioblastoma multiforme, the most malignant type of brain tumour (with an incidence of 4–8 cases per 100,000 people), typically results in a fatal outcome, with an average survival time of about one year. The pathogenesis involves tumour development through various genetic pathways and the abnormal deregulation of growth factors, particularly IGF-I, eGFR, and PDGF. Current therapeutic strategies—including surgery, radiation, and chemotherapy—can extend survival to around 14 months, occasionally up to 18 months. More recent advancements in therapy, especially cancer gene therapy (e. g., anti-IGF-I approaches) and immunotherapy (e. g., CAR-T and checkpoint inhibitor therapies), have further increased survival times to approximately two years. Despite these promising developments, the fight against glioblastoma is ongoing and continues to require extensive research in both diagnostics and treatment. Examples of these efforts are highlighted throughout this book. The chapters emphasize the critical role of microsurgery in maintaining patient safety. In terms of innovative diagnostics, early tumour detection and precise monitoring are increasingly supported by the use of Raman spectroscopy combined with liquid biopsy. Advances in immunotherapy are also explored, including explanations of their mechanisms and the development of new vaccines, particularly those prepared using dendritic cells. Finally, considering the ongoing relevance of the COVID-19 pandemic, the book includes an important discussion on managing glioblastoma during this global health crisis.

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