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Review Article

Glioblastoma diagnostics and prognostic biomarkers: Current status in medicine and exosome derivation

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ABSTRACT

Glioblastoma multiforme is the most fatal form of brain tumor, distinguished as an aggressive growth, and assists annexing by cell relocation and mortification of extracellular matrix with average survival rate of approximately 6–14 months. Most patients suffer from recurrence due to molecular heterogeneity of glioblastoma and deregulation of many signaling pathways involved in proliferation, survival, and apoptosis. A number of potential diagnostic, prognostic, and predictive biomarkers have been explored, i.e., ATRX, glial fibrillary acidic protein (GFAP), Ki67, p53, and isocitrate dehydrogenase markers, which helps in the diagnosis of patients with glioblastoma. The contemporary treatment of glioblastoma involves surgery succeeded by radiotherapy, chemotherapy, and adjuvant therapies; however, current therapies display limited clinical success. Furthermore, in future, exosomes will play exponentially multiple roles in the intercellular communication in both normal and disease conditions. Several features of exosomes present them as pioneering indicators to recognize cancer biomarkers for early diagnosis of the diseases and uses as therapeutics.

1. Introduction

In this advanced molecular era, formulating the concept for CNS tumor diagnoses based on WHO classification for the first time using molecular framework besides histology to describe the presence of various tumor entities is possible. In 2016, WHO criteria for CNS dispense significant reconstitution of diffused gliomas, other embryonal tumors, and medulloblastomas and integrate new entities that are defined by both histological and molecular features, including isocitrate dehydrogenase (IDH)–wild-type glioblastoma; IDH-mutant glioblastoma; H3-K27 M mutant; diffuse midline glioma; edulloblastoma; REL-associated protein (RELA) fusion–positive ependymoma; Wingless/Integrated (WNT)-activated medulloblastoma; and C19MC-altered, sonic hedgehog (SHH)-activated embryonal tumor with multilayered rosettes. The 2016 edition has included newly acknowledged neoplasms and has deleted some patterns, entities, and variants that do not have biological and diagnostic relevance. Other notable changes include the addition of brain invasion as a criterion for atypical meningioma and the introduction of a soft tissue-type grading system for the now combined entity of solitary fibrous tumor/hemangiopericytoma—a departure from the manner by which other CNS tumors are graded.[1] The reformed update (2016 CNS WHO) thus breaks with the age-old principle of diagnosis which was totally dependent on microscopy but now includes molecular parameters into the classification of CNS tumor entities.[1] The 2016 upgradation holds a number of differences from the CNS WHO 2007. The use of "integrated"[2] includes both phenotypic and genotypic parameters for CNS tumor classification, which adds a new level of objectivity that had been missing from some particulars of the diagnostic methods in the past. Glioma grade I and II tumors, i.e., pilocytic astrocytoma and diffuse astrocytoma, are slow-growing tumors, whereas grade III and IV tumors, i.e., anaplastic astrocytoma and glioblastoma multiforme (GBM), are fast-growing gliomas[3,4]

Low-grade tumors include grades I and II tumors, which are not very vigorous and are generally related with long-term endurance.

High-grade tumors include grades III and IV tumors, which develop very rapidly, cause more damage, and are harder to treat. These tumors are also considered malignant,[1] as shown in Table 1.

GBM is a type IV tumor, which is highly aggressive and characterizes approximately 14–15 months after recognition. It is one of the critical public health issues. GBM drags the glial cells in the

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Table 1
Characteristics of different types of glioma.

Grades	Tumor type	Tumor differentiation	Characteristics
Grade I	Pilocytic astrocytoma	Benign	Grows slowly Typical appearance, long-term continuity, curable
Grade II	Diffuse astrocytoma	Benign or malignant in some cases	Develops slowly Moderately atypical appearance Spreads to nearby tissues
Grade III	Anaplastic astrocytoma	Malignant	Develops more quickly Atypical appearance of cells spreads to other tissues
Grade IV	Glioblastoma multiforme	Malignant	Atypical cells that replicate rapidly Aggressive form Forming new blood vessels to sustain fast growth Difficult to treat

brain and decreases brain function.[5] Predominantly, unfavorable growth of tissues is seen in the brain and spinal cord tumors. These undesired masses rapidly progress and interfere in the functions of the brain.[6] In this review, we focus on comprehensive overview of glioblastoma. GBM is a recurrent tumor with global prevalence of less than 10 per 100,000 people.[7] Malignant glioma is the main cause of 2.5% of deaths and the third leading reason of death from cancer in people aged 15–35 years. In 2016, the number of incidence of GBM cases in India was 23000 and that of widespread cases was 49000.[8,9] The prevalence of GBM is higher in men than in women (1.6:1). Patients with a less vigorous GBM have longer endurance with consequent morbidity, but therapy is uncommon.[10] Patients with GBM may present with various signs and symptoms that are developed by three mechanisms: via direct effect as a consequence of necrosis: secondary effect resulting in moderate increase in tumor size and edema;[11,12] and depending on the tumor location, symptoms such as simple partial, complex partial, or generalized seizures appear.

There are some routine immunohistochemistry (IHC) markers, including ATRX, IDH1/2, p53, GFAP, Ki67, which help in the diagnosis of GBM. Various radiological tests and scans, i.e., magnetic resonance imaging (MRI), computed tomography (CT), or biopsy, are used to detect GBM. Therapies such as chemotherapy, surgery, and radiation therapy are used in the treatment of GBM.

Furthermore, exosomes including oncosomes and microvesicles have entered the domain of diagnosis and exuded from a variety of cells into biofluids or neighboring tissues as lipid membrane structures.[13] In this article, we review the potential of glioblastomas and their diagnostic and prognostic biomarkers. Therefore, the aim of the present review is to give a clinical outlook of GBM for nonclinicians.

2. Epidemiology of gliomas

Global incidence of gliomas is less than 10 per 100000 people. Approximately 459,000 individuals in the United States are living with a primary brain or CNS tumor diagnosis, 138,000 with malignant tumors, and 550,000 with benign tumors. Almost 20–50% cases of primary brain tumors latterly develop in the secondary form.[8] Similarly, 200,000 Americans are detected with a brain tumor yearly. Generally, spinal cord tumors are uncommon than brain tumors. Brain tumors affect people of all ages, whereas spinal cord tumors are commonly seen in the youth and adolescents. Almost 4200 CNS tumors are detected yearly in adolescents. Malignant gliomas are responsible for 2.5% of deaths, and this is the third main cause of death from cancer in persons aged between 15–34 years.[9,10] Since 1960s, more than 116 cases of glioma have been reported from radiation exposure, and it was estimated that the risk of developing a glioma increases when using radiotherapy. There is another report that low doses of radiation, which are used to treat tinea capitis and skin hemangioma in infants, have also been associated with relative risks for gliomas. Malignant brain tumors account for 1.4% of the total cancer cases, which cause permanent injury to the brain, are commonly resistant to most treatments, and result in high morbidity. Different studies analyzed the effects of ionizing radiation after the exposure of atomic bomb irradiation in Nagasaki and Hiroshima, in which an increased incidence of all brain tumor types, including gliomas, was found. No evidence was found between the risk of developing GBM and routine exposure to diagnostic radiation in both children and adults.[11,14] Other studies found that patients who receive treatment for acute lymphoid leukemia and who get exposed to other environmental factors such as smoking, as well as pesticide, who have severe head injury, who experience occupational risk factors were easily prone to develop gliomas.[15,16] Previous studies on animals show that some of the pesticides and other agricultural chemicals that combine with copper sulfates induce cancer in animals as well as in humans.

3. Sign and symptoms

Location of the tumor where the symptoms appear in a person suffering may vary from person to person. For instance, if a person has a tumor in the frontal lobe, changes in the mood or personality or paralysis on one side of the body can be seen; if a person has a tumor in the temporal lobe, he/she can face problems with sensation, motor skills, and writing; and if the tumor is in the cerebellum or in the occipital lobe, it causes trouble in coordination, vision, and balance.[17,18] Thus, cancer develops in any part of the brain or spinal cord; as it grows, it can place an increasing amount of pressure on the surrounding structures. In many cases, this pressure causes headaches, drowsiness and disorientation, changes in appetite, weakness, confusion, dizziness, and unusual behavior. In case if any of these symptoms develop in the body, a physician may recommend the combination of imaging and laboratory tests.[19,20]

4. Pathogenesis

Ninety-five percent of GBM tumors arise in the supratentorial region, and the most recurrent location for GBM is the cerebral hemisphere, with only a less percentage of tumors arising in the brainstem, cerebellum, and spinal cord.[21,22] Macroscopically, GBM is fully heterogeneous, promoting necrosis, multifocal hemorrhage, and formation of cystic and viscid region.[22] Current evolution in genomic technology has enhanced understanding of key molecular alterations that trigger GBM. The morphological basis is not always a specific prognostic measure of any individual case. Hence, there is a requirement for establishing better prognostic markers, with the objective of anticipating tumor behavior, and one such

corresponding method is an evaluation of proliferative indication of the tumor.[23,24]

5. Clinical characteristics of glioblastomas

Around half of the patients with GBM majorly present with a short clinical history that varies between 3-6 months; however, for a tumor growing from a low-grade astrocytoma, the clinical history reaches over a number of years. Patients with GBM may present with various signs and symptoms (headache, vomiting, seizures, etc.), which are developed through three mechanisms:

- Via direct effect, brain tissue is deteriorated as a consequence of necrosis, which gives rise to symptoms such as focal neural deficit (40–65%) and intellectual impairments. Signs and symptoms originated from the malignancy-dependent sections of the brain affected by the tumor.[25] For example, patients who have hearing and ocular complications show that a tumor is situated in the temporal lobe area, whereas 20–45% of patients present with a personality change as a result of a tumor situated in their frontal lobe, thus diminishing intellectual functions. If the tumor is large with remarkable mass, it culminates imbalance in walking and defecation.
- By secondary effects of elevated intracranial pressure, which is a direct result of moderate increase in tumor size and increased edema adjacent to the tumor, which leads to a shift in intracranial contents that causes headaches, a hallmark characteristic of GBM in 30–50% of patients.[25,26] Conventionally, headaches are unilaterally constrained with cumulative severity, having no distinct pain pattern. These headaches may also be related with vomiting and papilledema, which is now occasionally seen due to recognition of the disease at an earlier phase Clinical manifestations of GBM with their percentages are given in Table 2.

6. Diagnosis

Symptoms indicate the presence of gliomas in patients. If any of the signs become visible or noticeable, then a clinician asks the patient about his/her medical records and prescribes related detection tests to diagnose the causes.

6.1. Neurological screening

The functions of the nervous system including physical and intellectual surveillance of patient analyzes brain, and if retaliation from the patient's brain is not rational, then numerous imaging tests will be recommended with suggestion to refer a neurologist or oncologist for further consideration.[25,26]

6.2. Imaging tests

Clinicians prescribed the imaging tests for recognizing the type of tumor when a brain tumor is suspected. Different imaging studies that are essential for the diagnosis include the following:

Table 2
Manifestation of the numerous clinical symptoms involved in GBM.

Clinical symptoms	% in glioblastoma
Seizures	27.3
Headache	79.5
Vomiting	45.5
Neurological deficit	88.6

GBM, glioblastoma multiforme.

6.2.1. Computed axial tomography

Computed tomography shows a combined two-dimensional image of the soft tissue, bone, and blood vessels along multiple narrow X-ray beams.

6.2.2. Magnetic resonance imaging

MRI is performed with the help of a large magnetic field and radio waves; it provides a clear and definite three-dimensional image of the brain tumor.[27] CT scan or MRI of the brain is conducted using an intravenous contrast dye. The dye is ingested by the tumor which makes it recognizable and also helps to estimate the size of tumor.

6.2.3. Positron emission tomography

It is used for brain mapping and to prognosticate hypermetabolic activities with malformation of tumor cells or scar tissue.

6.2.4. Magnetic resonance spectroscopy

It assesses the quantity of metabolites in the body, recognizes the type of a tumor by irregular patterns of activity, determines the response for cancer therapies, and defines aggression of tumor.

6.2.5. Spinal tap or lumbar puncture

It determines an infection or tumor cells by measuring pressure in the spinal canal and brain with the help of a specific needle in the lower back.[27,28]

6.2.6. Diagnostic surgery

For identifying brain tumors, surgery can be performed. Ideally, a neurosurgeon completely abolishes tumor cells; if it is not possible, he/she then eliminates as much tumor cells as possible without harming the brain's neurologic activities.[29,30] For distinguishing the tumor type and grade and for treatment advisory, biopsy can be performed in cases where incision is not possible.

6.3. Biopsy

In general, biopsy is a surgical method in which a minute quantity of a tumor cell specimen is observed under a microscope. With the help of biopsy, the type of tumor, i.e., whether it is benign or malignant, is identified. Biopsy can be performed as an individual procedure or at the time the tumor is removed if treatment is the only choice. The tumor width at the time of detection is usually approximately 4 cm.[30,31] Overall schematic of GBM diagnosis and treatment is given in Fig. 1.

7. Treatment

Despite significant efforts, GBM treatment is still the most stimulating task in clinical oncology. Over the last decade, a variety of different treatments was evaluated with very less success.[32]

Major threats in therapy of GBM are associated with the locality of the disease and its complicated and diverse biology. Progresses in surgical approaches, radiotherapy, and ancillary chemotherapy have shown moderate advancements in survival and quality of life of the patients with GBM, but the prophecy is still distressing.[33,34] Despite that, much more remarkable steps need to be made to see positive consequences, corresponding to those seen in certain other cancers that can now be treated profitably. The contemporary degree of care for patients with high-grade glioma not only involves therapeutic management, i.e., antitumor therapy, but also is comprehensive of providing effective supportive care to the patient.[34] Dexamethasone is the preferred corticosteroid in these patients because of its low mineral corticoid action. Because of its low venomous profile and no drug-to-drug interactions with

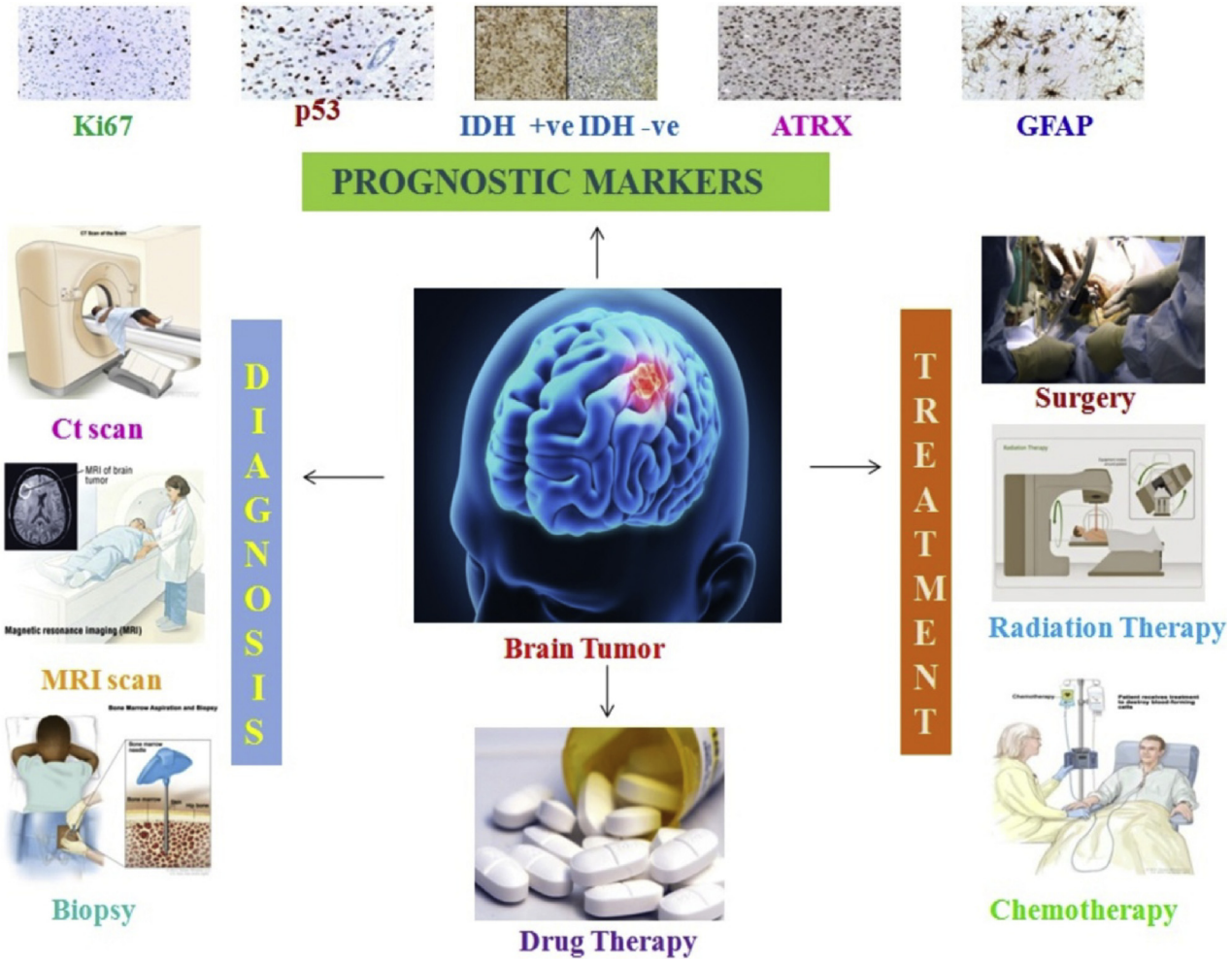

Fig. 1. Overall diagram of diagnosis and treatment of GBM. GBM, glioblastoma multiforme; CT, computed tomography; MRI, magnetic resonance imaging.

annihilator agents, levetiracetam is frequently recommended to patients with seizures. Specific therapeutic management includes surgery or surgical resection of the tumor together with radiation and consequent ancillary temozolomide (TMZ) therapy.

7.1. Surgery

The degree of surgical resection is based on the location and eloquence of the brain area involved.[35] GBM is a locally very incursive tumor that does not heal thoroughly by surgery, and deterioration occurs in approximately 75% of cases usually within 2–3 cm of the edge of the original wound. However, in newly diagnosed patients, the area of surgical resection clasps prognostic value, but again, recurrence of tumors is seen in case of surgical intercession in patients usually having poor prognosis.[35,36]

7.2. Radiation therapy

Radiotherapy can be used to kill leftover tumor cells following by surgical treatment. It has been shown to enhance life probability of patients having high-grade gliomas. Brachytherapy and stereotactic radiosurgery are found to be efficacious therapies for adjacent degenerative GBM, but they have ambiguous roles in considering newly diagnosed GBM. Subclass of patients who have undergone a gross total resection may get a continuity advantage after undergoing stereotactic radiotherapy.[37,38] Instead, hyperfractionated radiotherapy has shown that continuity results in GBM may actually be adverse in certain subclasses.[38] Intensity-adjusted radiotherapy and boron neuron capture therapy are few of the contemporary radiation-based therapy procedures, which have previously been carried out in patients with high-grade gliomas to assess their effectiveness.[38,39] Treatment with these therapies revealed less toxicity and less exposure to standard tissues, and outcomes demonstrate that these are not inferior to conventional radiotherapy being used for patients with brain tumors.

7.3. Chemotherapy

To ameliorate the survival of patients, various annihilator agents have been examined for their advantage in the treatment of GBM, for example, alkylating agents such as TMZ are used as a methylating agent, and carmustine or bis-chloroethylnitrosourea (BCNU) and lomustine (CCNU) have manifested some advantages and have been applied clinically in most patients with GBM.[39–42] Chemotherapeutic agents with their roles and side effects are given in Table 3.

7.4. Recombinant immunotoxin therapy

Adjacent conventional therapies involving specified toxins, i.e., immunotoxins, demonstrate a new class of anticancer prospect and provide high limpidity for tumor cells that particularly over treated the cell surface or extracellular matrix protein objectives.

Table 3
Various chemotherapeutic agents with their defined roles and side effects.[43–45]

Chemotherapeutic agents	Role	Side effects	Brain tumor type
Lomustine (BCNU)	Highly lipid-soluble drug, alkylates both RNA & DNA, and able to cross the blood −brain barrier	Nausea, pulmonary fibrosis, and bone marrow suppression	Malignant glioma, astrocytoma, glioblastoma, oligodendroglioma, and adult medulloblastoma
Carmustine (CCNU)	Alkylating agent, cross-links DNA resulting in disruption of DNA function, arrests cell cycle and apoptosis, highly lipophilic, and crosses the blood−brain barrier	Nausea, bone marrow suppression, and pulmonary fibrosis	Malignant glioma, specifically glioblastoma
Bevacizumab (Avastin)	It slows the tumor growth	Gastrointestinal cleft, allergic reactions, and vision loss	Anaplastic glioma and glioblastoma
Procarbazine	It inhibits the protein, RNA, and DNA synthesis; alsoinhibits transmethylation of methyl groups of methionine into *t*-RNA; and crosses the blood−brain barrier	Confusion, tiredness, seizures, fever, body aches, chills, and chest pain	Anaplastic glioma, glioblastoma, oligodendroglioma, astrocytoma, and lymphoma
Temozolomide (TMZ)	Methylating agent; methylation damages the DNA and triggers the death of tumor cells. Some of the tumor cells repair DNA damage but some diminish the efficacy of TMZ by expressing a protein AGT or MGMT gene, which prevents synthesis of AGT enzyme by which tumors are more sensitive to killing by TMZ	Liver problems, low WBC count, Bone marrow suppression, seizures, and chest pain	Malignant glioma and glioblastoma

WBC, white blood cell.

Recombinant immunotoxins (RITs) exemplify a favorable process for GBM treatment because of their remarkable attributes over monoclonal antibodies (mAbs) and acknowledged chemotherapeutics.

- First, RITs are significantly smaller in molecular size, which makes it more effective to probe into solid tumors than mAbs.
- Second, RITs retain the particularity of mAbs, but distinct mAbs are immensely strong and have unknown mechanisms of drug resistance.
- Third, RITs can efficaciously destroy quiescent, nondividing cells and are different from conventional chemotherapeutics.
  •Finally, RITs have slight cross-resistance with another agent and are also effectual in healing chemorefractory cancer. Production of volatile domain fragments of mAbs was first reported in 1988, and RIT progression is one of the most proliferating fields in contemporary years.[46] A large variety of RITs have also been produced in GBM treatment, and different RITs have entered clinical trials. Despite that, various issues remain considerable barriers to attaining effective treatment.

8. Signaling pathways related to glioblastoma

The mechanisms of GBM incidence and development remain largely unknown, and current progress in the better understanding of the signaling pathways underlying GBM pathogenesis has led to new therapeutic development that approaches targeting multiple oncogenic signaling abnormalities related with GBM.

8.1. p53

A widely known tumor suppressor p53 responds to various genotoxic and cytotoxic stresses by prompting cell cycle arrest and apoptosis and also responds to DNA damage. It plays an important role as a large transcription factor and in tumor suppression. More than 2500 genes are regulated by p53 pathway,[47,48] out of which most are involved in tumor development, tumorigenesis, and tumor invasion. GBMs are divided into primary and secondary subtypes. In the development of secondary GBMs, p53 plays a significant role and are often initial detectable genetic alteration in primary brain tumors; approximately 65% of p53 precursors are found in low-grade diffuse astrocytomas. Although less frequent, in primary gliomas, mutations in the p53 pathway are also detected as reported in the literature.[49–51]

8.2. pRB

pRB plays a key role in cell cycle progression inhibition by binding and hindering transcription factors of E2F family. The RB gene encodes pRB signaling pathway, regulated by the cyclin-dependent kinases (CDKs) complex.[52] In more than 20% of high-grade gliomas, mutations in pRB signaling pathway are detected. In GBM, the loss of pRB expression,[53] whereas in transition from low- to intermediate-grade gliomas[52,54] In glioblastomas, the inactivation of CDKN2B is common in addition to amplification of CDK4 and CDK6 that play crucial roles in astrocytic tumorigenesis and glioma progression.[55]

8.3. PI3K-PTEN-Akt-mTOR pathway

Normal cellular functions including cellular proliferation, apoptosis, cell invasion, and mobility is regulated by phosphoinositide 3-kinase (PI3K)-phosphatase and tensin homolog-v-akt murine thymoma viral oncogene homolog (Akt)-mammalian target of rapamycin (mTOR) pathway that can also be important in tumorigenesis. The first intracellular member of this signaling pathway phosphatidylinositol 3-kinase (PI3K) complex activation is regulated by many growth factors in coexistence with their receptors, such as epidermal growth factor (EGF) and its receptor (EGFR). Akt gets activated by phosphatidylinositol-3,4,5-triphosphate generated by activated PI3K. Several signaling cascades are triggered by AKT to regulate cell survival and growth. The activation of PI3K by the deletion of PTEN induces CNS axon regeneration through the activation of mammalian target of rapamycin (mTOR) signaling.[56] In the development of GBM, indigenous activation and mutation of the PI3K signaling pathway play important roles.[57–59] In about 70% of GBMs, either by deletion of phosphatase and tensin homolog, located on chromosome TEN (PTEN) or amplification of EGFR and/or vascular endothelial growth factor receptor (VEGFR)/platelet-derived growth factor receptor (PDGFR) alpha,[60] the PI3K is altered. The most frequent signaling mutations in GBM are overexpression of EGFR and activation of the PI3K pathway.[61,62] In GBM, average amplification rate for EGFR is

approximately 35% and amplification rate for the EGFRvIII mutant is approximately 40%.[47] PTEN plays the role of a tumor suppressor gene, and PTEN mutations lead to the development of many cancers, including GBM. PTEN negatively regulates the PI3K/AKT/PKB pathway[63,64] by the reduction of intracellular levels of phosphatidylinositol-3,4,5-triphosphate via blocking Akt signaling. It is likely that induced expression of functional PTEN may serve as a therapeutic strategy in GBMs with amplified or mutated EGFR-PI3K-Akt-mTOR pathways.

8.4. RAS/MAPK

Human RAS genes (rat sarcoma) are transforming oncogenes, including three highly related genes called H-Ras, N-Ras, and K-Ras. The activation and deactivation of RAS is controlled by the G protein family binding to guanosine triphosphate (GTP) or guanosine diphosphate (GDP), respectively, as RAS belongs to the G protein family.[65,66] Direct binding of activated RAF kinase with activated RAS then regulates downstream signaling pathways such as the mitogen-activated protein kinase (MAPK) pathway.[67–69] In multiple cases of high-grade astrocytoma and GBM, the overexpression of RAS was detected.[70–72] The implication of a positive correlation between expression of PDGFR and receptor tyrosine kinase (RTKs) and glioma pathogenesis was led through the overexpression of other growth factor receptors that regulate RAS such as PDGFR or RTKs besides EGFR. The abnormal activation of the RAS/MAPK pathway could be a potential target for glioma treatment.

8.5. STAT3

Signal transducers and activators of transcription protein complexes (STAT) are a family of cytoplasmic proteins with Src homology-2 (SH2) domains that act as transcription factors. STAT proteins regulate cellular responses to cytokines and growth factors through transducing signals from the plasma membrane to the nucleus, thus activating transcription of target genes and inhibiting cellular activities.[73] Activated by EGF, STAT3, one of the STAT family proteins, is unregulated in multiple tumors, including GBM.[74] In recent studies, STAT3 is found to have oncogenic roles in GBM.[75]

9. Prognostic marker

There are certain prognostic factors that are identified for glioblastoma treatment.[76] These are routinely IHC prognostic markers observed in the GBM including the following ones:

9.1. Glial fibrillary acidic protein

Glial fibrillary acidic protein (GFAP) is the hallmark intermediate filament (IF; also known as nanofilament) protein in astrocytes, a main type of glial cells in the central nervous system (CNS). The study reported that GFAP released from astroglial tumors, especially malignant gliomas, could be used as a prognostic and diagnostic marker for GBM.[77,78] GFAP is highly distinct for cells with astrocytic separation and is widely used as an authentic marker in the immunohistochemical diagnosis of brain tumors.

9.2. Isocitrate dehydrogenase

Approximately 70%–80% of secondary GBMs have somatic variation in the IDH1 gene, which are absent in primary GBMs. Wild-type IDH1 protein is seen in the cytoplasm, endoplasmic reticulum, and peroxisomes and circulates in the pathway of oxidative decarboxylation of isocitrate to α-ketogluterate. Variations in IDH1 related with glioblastoma map to the highly maintained residue R132 in the enzyme active site, which usually results in an Arg to His replacement, although other substitutions can also occur. The IDH1-R132 mutation occurs in 50–80% of grade II and III oligodendrogliomas and astrocytomas but rarely seen in primary GBMs. To a lesser degree, glial tumors have somatic variation in the analogous codon (codon R172) of the IDH2 gene.[79] It is quite unknown how a tumor's biology is strained by IDH1/2 variations. IDH1/2 mutations result in genome-wide epigenetic changes in human gliomas. Another theory is that the mutations decrease the ability of cells to produce NADPH and accordingly lower the capacity of the cell to scavenge oxygen species, which makes the tumor cells more sensitive to irradiation and chemotherapy.[77,79] This increased susceptibility to treatments results in increased patient survival.

Many studies have found that IDH1-R132 and IDH2-R172 mutations are connected to the genomic profile of the tumor and are major prognostic markers in grade II to IV gliomas. However, other studies have not found an alliance of IDH1/2 variations with prognosis in low-grade tumors.[77] Therefore, the prognostic importance of these genetic markers for survival is not clearly understood.

9.3. ATRX

ATRX mutation in gliomas was first found in adolescents and young adults aged 11–30 years. In adults, i.e., those aged >30 years, ATRX is mutated less commonly in primary GBMs, but it is constantly seen in lower grade (WHO grade II/III) and secondary GBMs. ATRX mutation is found in about 15 types of human cancers, including neuroblastoma, osteosarcoma, and pancreatic neuroendocrine (PanNET) tumors. However, the role of ATRX in tumorigenesis remains unspecified.[78,80] The ATRX proteins plays a significant epigenetic role, depositing histones at heterochromatin and telomeric DNA. Loss of ATRX facilitates tumor growth rate and reduces median survival, uncovering the impact of ATRX loss on glioma tumor proliferation.

9.4. Ki67

The proliferative index is a strong biologic marker that evaluates the growth of a neoplasm significantly and thus sustains in recognizing the prognosis for patients with a neoplasm. A variety of methods have been used to estimate the proliferative index of CNS tumors.[81] Of these, one of the most efficient methods is the Ki-67 labeling index (Ki-67 LI). The value of Ki-67 LI in the evaluation of cell proliferating activity has been widely recorded for various human tumors, including the brain neoplasms. To estimate the Ki-67 LI in association with the WHO histological grades of astrocytomas to anticipate the biological behavior, which gives direction for using Ki-67 LI as supplement to the routine histology in grading astrocytomas promptly.[81,82] The expression level of different routine markers that are used in the diagnosis is shown in Table 4. In this analysis, we have recruited 83 patients with GBM at 1 year from Sir Ganga Ram Hospital in which 59 patients (71.1%) showed positivity in p53, 44 patients (53.0%) showed positivity in GFAP, and all patients showed positivity in Ki67. Wild-type IDH shows 15.7% positivity (13 patients), and ATRX shows 41.0% positivity in GBM, which are described in Table 4.

Table 4
Expression status of routine markers in GBM.

Expression status	p53	GFAP	Ki67	Wild-type IDH	ATRX
Negative	28.9%	47.0%	0.0%	84.3%	59.0%
Positive	71.1%	53.0%	100.0%	15.7%	41.0%

GBM, glioblastoma multiforme; IDH, isocitrate dehydrogenase.

9.5. p53

Gliomas, specifically the astrocytic type, can become progressively anaplastic over time. The p53 gene plays a crucial role in this progression. Low-grade tumors with p53 variations sustain a clonal extension of the p53-mutated tumor cells when those tumors develop to a higher grade. Other studies revealed that although p53 mutation does not estimate the tumor progression, tumors that do progress tend to have p53 mutations early in their course, and the p53 variations may be related with the reduced time for development.[83] Astrocytes are found to be more sensitive to transformation stimuli, which further shows that a mutated p53 gene plays consequent role in tumor development. Not all gliomas that progress have p53 variations. p53 mutation was present in low-grade tumors, by which a tumor develops through a transitional step to complement anaplastic astrocytomas before ending as a GBM. Wild-type p53 still develops directly to complement GBM.[83]

Differentiation in p53 expression also arises between the occasional gliomas developing to GBM and de novo GBM seen generally in older patients. These patients retaining de novo glioblastomas have rather low rate of p53 mutation. In another way, those patients with secondary glioblastomas that occur from a formerly diagnosed lower grade glioma have a high rate of mutation, which also demonstrates a part for p53 in tumor development.[83,85]

This differentiation also reflected the fact that younger adult patients with GBM are more likely to have p53 mutations. Generally, these patients are more vulnerable to have progressive secondary GBM rather than de novo glioblastomas and tend to live longer.[84] Various routine markers have their different roles and degree or percentage in GBM (Table 5).

10. Future prospective

10.1. Exosome as an originator of biomarkers

Exosomes are tiny, approximately 30–140 nm, membrane-enclosed molecule derived from cells of almost all eukaryotic cells because they are found in all body fluids such as urine, blood, saliva, and even pathogenic cells. Exosomes are formed while early endosomes emerge, but specific mechanism of exosome biogenesis is still not well elucidated and familiar. Early endosomes are a direct consequence of the primary endocytic phenomenon at the plasma

Table 5
Different routine markers with specific roles and their percentage in GBM.[83,84]

Routine marker	Role	Percentage in GBM
Ki67	Evaluates the growth of a neoplasm and cell-proliferating activity	100.0
IDH1	Mutations decrease the ability of cells to produce NADPH and accordingly lower the capacity of the cell to scavenge oxygen species, which makes the tumor cells more sensitive to irradiation and chemotherapy	0.0
GFAP	GFAP released from astroglial tumors, especially malignant gliomas, could be used as a diagnostic biomarker for GBM and as an authentic marker in the immunohistochemical diagnosis and detection of brain tumors	100.0
ATRX	ATRX protein plays a significant epigenetic role, depositing histones at heterochromatin and telomeric DNA	77.3
p53	Tumor development; low-grade tumors with p53 variations sustain a clonal extension of the p53-mutated tumor cells when those tumors develop to a higher grade	100.0

GBM, glioblastoma multiforme; IDH, isocitrate dehydrogenase.

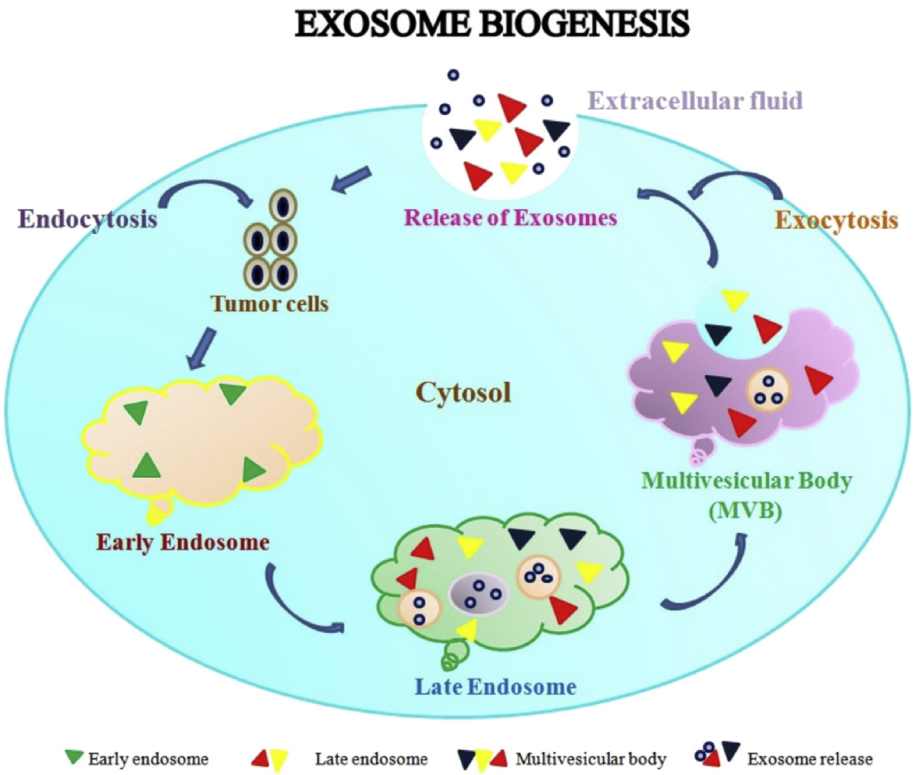

Fig. 2. Biogenesis of exosomes in GBM. GBM, glioblastoma multiforme.

membrane. The endosomal surface folds inward, and it further buds off from the membrane and provokes exosomes.

The amount of exosomes and their roles alter with the cells of origin. For instance, exosomes liberated from cancer cells enclose a wide array of RNA, proteins, lipids, and DNA, which promote tumor growth by modifying multiple trademarks of cancer.[85] Therefore, exosomes may describe characteristics of oncogenesis, including genetic vulnerability, variations of the microenvironment, tumor growth, cellular incursion, migration, progression, and immune defiance. Exosomes develop from the endosomal section and are discharged into the extracellular space but can also dissociate from the plasma membrane of the cell. Exosomes have been confined to multiple body fluids such as cerebrospinal fluid and plasma. They play a role in intercellular communication and regulate the microenvironment to change the immune response.[13,86] Biogenesis of exosomes in gliomas is shown in Fig. 2.

10.2. Applications of exosomes

- Exosomes play multiple roles in the intercellular communication, waste management, elucidation of antigen, and rectification of proinflammatory cytokine release.
- Exosomes also play a role in normal, physiological sequences (stem cell preservation, restoration of tissue, blood coagulation, and immune administration).
- Function of exosomes in infectious state, i.e., pathological processing, aids to unveil exosomes feasible and subsequent applications as remedial targets or presumptive, as therapeutic agents.[87]
- The biological role of exosomes is that they act as proteins that are discharged out from reticulocytes throughout the process of maturation in red blood cells.
- Advantageous roles in coagulation, inflammation, and angiogenesis were also delineated.
- Exosomes also carry the specific nucleic acids, work as cargo, and spread several pathogens such as viruses and prions from one cell to the other cell.[88]

11. Summary

A glioblastoma is an aggressive recurrent tumor, poor prognosis of which is related with poor aspects of life. Enhancing health care through customized drugs is an ideally booming field. Drugs for tumor treatment can be configured to individuals' feature, biotic trademarks, and response to a particular therapy. Therefore, a number of potential diagnostic, prognostic, and predictive biomarkers have been explored, but we still need a novel circulatory biomarker with an effective approach. Diagnosis of GBM includes the biopsy and diagnostic surgery, which includes craniotomy. Chemotherapy and radiation therapy both are used for treatment of the tumor either singly or in combination. Exosomes embrace a mark in the development of effective customized remedial techniques given their use for biomarker detection and personalized diagnostic potential. Exosomes are provocative new chapter in therapeutics with an innovative programme for tumor treatment.

Conflicts of interest

Authors have nothing to disclose.

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