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REVIEW ARTICLE

New Perspectives on Multifaceted Treatment Strategies for Glioblastoma

Natalie Hwang Chun Hwee

Hwa Chong Institution, 661 Bukit Timah Road, Singapore 269734, Singapore; nataliehwang2007@gmail.com

ABSTRACT: Glioblastoma multiforme (GBM) is a World Health Organization grade IV glioma and the most prevalent malignant brain tumor in adults. GBM originates from low-differentiated glial cells with nuclear atypia. It is often associated with a poor overall prognosis. Even though the metastasis rate of glioblastoma is very low of around 0.5%, the median overall survival period is unfortunately less than 2 years. The most common causes of glioblastoma include genetic mutations of genes, certain environmental factors, and lifestyle habits. Traumatic head injury is a speculated cause of glioblastoma that needs further research. The standard treatment for GBMs is surgical resection followed by radiation therapy and chemotherapy. However, glioma cells have shown robust DNA repair and self-renewal capabilities. They are also known for their highly infiltrative nature and resistance towards chemotherapy drugs. Consequently, approximately 90% of GBM patients suffer a recurrence within two years of the diagnosis. Here, we discuss how recent research has led to the innovation of targeted gene and immunotherapy as well as the mechanisms and results of the new treatment methods, which are aimed at overcoming chemoresistance and relapse of the cancer.

KEYWORDS: Biomedical and Health Sciences, Genetics and Molecular Biology of Diseases, Pathophysiology, Neurobiology, Cellular Studies.

Introduction

Glioblastoma (GBM), previously known as glioblastoma multiforme, is the most malignant type of brain tumor that is highly prevalent among adults. Despite many advances in treatment modalities, it remains largely incurable. Gliomas are a group of heterogeneous primary brain neoplasms that differ in level of malignancy, histology, and genomic alterations. They can come from neural stem cells, making up 30% of the central nervous system tumors and 80% of malignant central nervous system tumors. Even though there are many treatments for Glioblastoma, it remains a deadly disease with a poor prognosis. Every year, there are approximately 300,000 people diagnosed with GBM.[1] Sadly, more than 240,000 people die from this aggressive cancer globally annually.[2] The probability of being diagnosed with GBM is 60% higher in men as compared to women.[3] However, women who are diagnosed with GBM have higher survival rates compared to men (Figure 1A). Glioblastoma tends to occur most often in adults who are between 65 to 74 years old (Figure 1B).[4] Glioblastoma is separated into two different types, primary and secondary. Primary Glioblastoma is the most common and aggressive type, while secondary glioblastomas progress from low-grade diffuse astrocytoma or anaplastic astrocytoma and tend to manifest in younger patients. Thus, secondary glioblastomas have a lesser degree of necrosis and are preferentially located in the frontal lobe and less commonly found in the cerebellum, carrying a more favorable prognosis. The common symptoms of GBM include seizures, severe headaches, memory and language problems, sudden changes in personality and behavior, muscle weakness or paralysis, loss of sensation or numbness and tingling, constant fatigue, issues with coordination, speech, hearing, and vision problems.[5] This review article will explore some of the common causes and treatments for Glioblastoma.

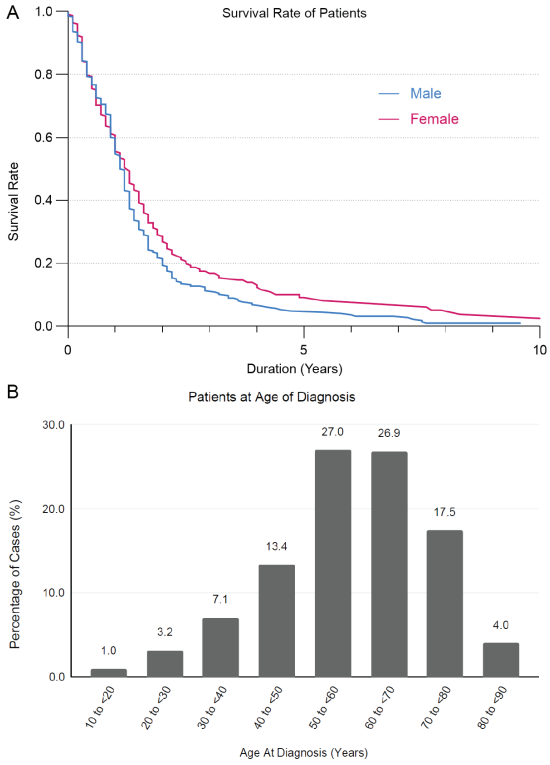

Figure 1: Statistics of GBM cases from the Cancer Genome Atlas (TCGA) project. (A) Survival rate of male and female patients over time. Female patients diagnosed with GBM have a higher survival rate. (B) Distribution of glioblastoma cases among patients of different ages. GBM tends to occur most often in adults between 65 to 74 years old. The results shown here are in whole or part based upon data generated by the TCGA Research Network: https://www.cancer.gov.tcga.

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In this review, we aim to provide readers with a unique perspective on the recent and newly discovered treatments, such as gene therapy and immunotherapy, apart from the conventional methods used to treat glioblastoma. Moreover, this review discusses primary research data to aid readers in better visualizing and comprehending the information used to explain the causes and treatments of glioblastoma. The majority of patients diagnosed with GBM have no family history of cancerous brain tumors. However, multiple studies have shown that some patients with a family history of cancerous brain tumors appear to be more susceptible to developing the same type of tumors as compared to those with no such family history.[6] Additionally, people who inherit certain rare genetic syndromes such as Turcot syndrome, Neurofibromatosis type 1, LiFraumeni syndrome, and Lynch syndrome have an increased risk for glioblastoma.

Discussion

Genetic Causes:

This section of the review explores the possible genetic causes of glioblastoma that commonly include *PTEN, NUP37, DNMT1, RAS* oncogenes, *TP53*, and *IDH1* genes. Analysis of gene mutations in GBM patients has shown that the *PTEN* and *TP53* genes are the top two most frequently mutated genes in both male and female patients diagnosed with GBM (Figure 2A, 2B).

i. TP53:

One cancer-critical gene responsible for GBM after mutation is the *TP53* gene, encoding the p53 protein (Figure 2A, 2B). The *TP53* gene is a tumor suppressor gene that arrests the cell cycle, apoptosis, and cellular senescence in response to DNA damage that cannot be repaired. *TP53* is one of the most commonly deregulated genes in cancer (deregulated in 50% of glioma cells),[7] resulting in GBM cell invasion, migration, proliferation, evasion of apoptosis, and cancer cell stemness. The p53-ARF-MDM2 pathway is deregulated in 84% of GBM patients and 94% of GBM cell lines. The most frequently deregulated component of the p53 pathway is a homozygous deletion of the CDKN2A/ARF locus, which happens in approximately 60% of GBM cases.[8]

ii. PTEN:

The PTEN gene, a tumor suppressor gene that inhibits the PI3K/Akt/mTOR axis, is mutated or deleted in approximately 40% of glioma cells and becomes inactivated.[9] *PTEN* gene deficiency promotes neurogenesis and gliogenesis, heightening the malignant progression of glioblastoma. PTEN dephosphorylates PIP3 (an enzyme that promotes cell growth and proliferation) back to PIP2, allowing downstream targets, including Akt and mTOR, to undergo deactivation. After suppression, mutation, or deletion of PTEN, levels of PI3Ks increase to promote the generation of PIP3 on the cell surface membrane to recruit and stimulate PH domains in PDK1 and Akt. After phosphorylation and deactivation of Akt and PDK1, the mTOR pathway is activated to promote increased cell growth and survival, metabolism, differentiation, mitosis, as well as the prevention of apoptosis. Anti-cancer agents such as tangeretin and icaritin are used to regulate PTEN signaling and minimize the progression of GBM. Silibinin is also another anti-cancer drug used to inactivate mTOR pathways, stimulating apoptosis in GBM.[10]

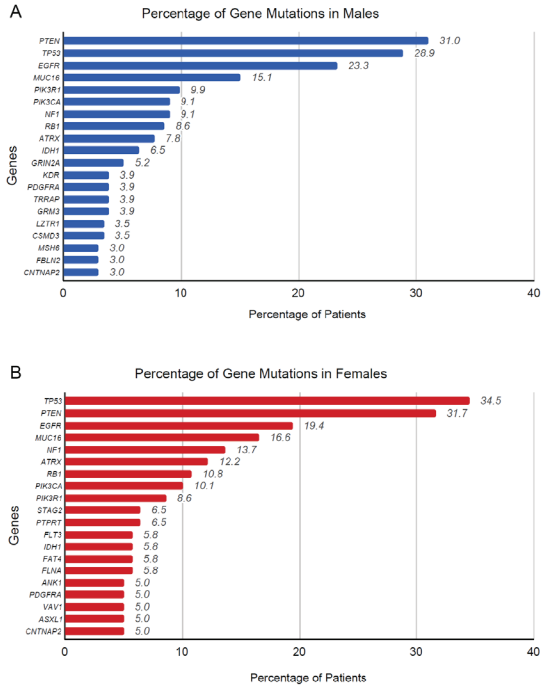

Figure 2: Statistics of Male and Female GBM cases from the Cancer Genome Atlas (TCGA) project. (A) Distribution of most frequently mutated genes in male patients. (B) Distribution of most frequently mutated genes in female patients. PTEN and TP53 are the top two most frequently mutated genes in both make and female GBM patients. The results shown here are in whole or part based upon data generated by the TCGA Research Network: https://www.cancer.gov/tcga.

iii. IDH1:

IDH1 is a gene on chromosome 2 that codes for the enzyme Isocitrate dehydrogenase 1. Upon undergoing a gain-of-function mutation, IDH1 converts alpha-ketoglutarate to the oncometabolite 2-hydroxyglutarate (2-HG),[11] causing genome-wide methylation changes in glioblastoma patients and eventual aberrant gene expression. This is thought to influence a range of cellular programs involved in epigenetic control and various processes leading to tumor development. The mutation of *IDH1* is considered to be a strong GBM prognostic factor. A high percentage of secondary glioblastomas and a very small percentage of primary glioblastomas harbor mutations in the *IDH1* gene. *IDH1* mutations are found in 70%-80% of grade II-III gliomas and 80%-90% of secondary GBMs, but are not commonly found in primary GBMs.[12] This shows that *IDH1* mutations are key factors that promote gliomagenesis by disrupting chromosomal topology and allowing aberrant regulatory interactions that induce oncogene expression. The *TWIST1* gene enhances GBM invasion along with mesenchymal changes, and the transcription factor *ZEB1* promotes the primary GBM tumor initiation, cell invasion, and chemoresistance. *ZEB1* is overexpressed in *IDH1/2* - mutant grades II-III glioma. *IDH1* mutation alters the normal morphology of glioma cells - glioma cells and nuclei become larger, and the

cell body protrusions are significantly longer. *IDH1* mutations are initial events that define major clinical and prognostic classes of gliomas.[13]

iv. NUP37 and DNMT1:

NUP37 (Nucleoporin 37) is proven to promote the proliferation and invasion of glioma cells through DNMT1-mediated methylation. DNA methylation, enabled by DNA methyltransferases (DNMTs), is a process that plays an instrumental role in regulating gene expression and controlling tissue differentiation.[14] DNMTs preserve DNA methylation patterns by attaching methyl groups to the 5-carbon positions of cytosines in CpG dinucleotides. DNMT1, a methyltransferase encoded by the *DNMT1* gene, maintains DNA methylation patterns, regulating gene expression. DNMT1 overexpression leads to the inhibition of DNA methylation, which has been observed in glioma tumors. NUP37 is overexpressed in glioma tissue samples. Through *in vitro* and *in vivo* functional experiments, NUP37 depletion effectively hindered the proliferation, invasion, and other cellular activities of the glioma cells. NUP37 depletion also increased apoptosis in glioma cell lines. Protein level analysis showed that DNMT1 was overexpressed in glioma tissue compared to normal brain tissue, and there was a silencing of tumor suppressor genes. There is a relationship between NUP37 and DNMT1. NUP37 promotes the expression of DNMT1 in glioma cells. Thus, in glioma tissues, there is a high expression of NUP37 and DNMT1, resulting in both the proliferation and invasion rate of glioma cells.[15]

v. RAS Oncogene:

Genetic mutations of cancer-critical genes also contribute to the uncontrollable mitotic division of glioma cells. When the *RAS* gene, a proto-oncogene that stimulates cell proliferation and division by transmitting growth signals from the cell surface to the nucleus, undergoes a gain-of-function mutation, the result is a hyperactive RAS protein, which is responsible for many cancers and disease progression. However, brain tumors, including Glioblastomas, are rarely associated with a mutation of the RAS gene.[16]

Environmental Causes:

This section reviews the environmental factors that are potentially involved in brain tumor carcinogenesis. The environmental factors include ionizing and non-ionizing radiation, exposure to carcinogens and glyphosates, as well as traumatic brain injury.

i. Radiation:

Exposure to ionizing radiation elicits a preferential activation of the DNA damage response pathway, as ionizing radiation from X-rays and gamma rays results in the production of free water radicals, which are very chemically reactive. These free radicals can interact with cellular DNA to produce double-stranded breaks, leading to chromosomal breaks and deletions, affecting cancer-critical genes. After exposure to ionizing radiation, glioblastoma-initiating cells have the ability to activate c-MET and NOTCH pathways.[17] Activated c-MET pathways promote cell growth, proliferation, and survival, as well as increasing the motility of glioma cells and their ability to invade surrounding tissue and metastasize. Activated NOTCH pathway upregulates the transcription of anti-apoptotic genes, promoting the survival rates of glioma cells. Some studies have shown that radiation did not cause apoptosis in the U343 cell line derived from glioblastoma multiforme, which makes it radioresistant.[18]

Radiofrequency radiation (RF) is a form of non-ionizing radiation that is believed to have insufficient energy to cause glioblastoma by directly damaging the DNA. However, a study conducted by Melnick *et al*. on glioma found lower survival rates in patients with glioblastoma associated with long-term use of wireless phones,[19] while other studies report that RF may cause oxidative damage by inducing an increase in lipid peroxidation and oxidative DNA damage formation in rat frontal lobes, and ultimately leading to glioblastoma.[20] To better conclude whether RF waves cause gliomagenesis, further research would need to be conducted.

ii. Carcinogens:

Exposure to environmental carcinogens is also another basis of neoplastic transformation, including GBMs.[21] There are two types of carcinogens. Genotoxic carcinogens bind directly to DNA, causing mutations in the genetic material. In smoke inhalation from air pollution, chemicals such as polycyclic aromatic hydrocarbons and toxic metals enter the lungs through respiration and accumulate in body tissues, binding to the DNA of cells to form adducts, causing DNA damage. Some chemical pollutants have lipophilic characteristics that allow them to cross the blood-brain barrier and accumulate in the brain parenchyma, triggering inflammation and leading to gliomagenesis. Non-genotoxic carcinogens such as phenobarbital, carbon tetrachloride, and diethylstilbestrol, found in industrial sources, pharmaceuticals, as well as consumer products, can affect fundamental processes regulated by or dependent on DNA and gene expression, including cell growth and differentiation.[22] They have proven to serve as tumor promoters or as inducers of inflammatory responses.

iii. Glyphosates in Pesticides:

We are often exposed to glyphosate through consuming contaminated food and water, occupational exposure, or domestic use. In soils, glyphosates are degraded by microorganisms into their major metabolite, aminomethylphosphonic acid (AMPA), which is proven to be carcinogenic by the World Health Organization's International Agency for Research on Cancer.[23] Glyphosates in pesticides produce effects that activate Akt and mitogen-activated protein kinases (MAPKs) signaling pathways by binding to and activating the epidermal growth factor receptor. These activated pathways are involved in the growth, survival, and metabolism of glioma cells, resulting in oxidative imbalance and inflammation. The DNA damage induced by glyphosate gives glioblastoma cells an advantage by increasing their proliferation and growth.[24]

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Traumatic Brain Injury:

There are limited case reports that have suggested an association between GBMs and traumatic brain injuries, but control and epidemiologic studies have been inconclusive. Some case studies have looked at service members diagnosed with GBM who have a history of blast exposure. However, the studied population is small, and further research is required to better understand the correlation of blast exposures.[25]

In this section, we will explore the current standard treatments such as chemotherapy, surgical resection, and radiation therapy.

Conventional Treatments:

i. Chemotherapy (Temozolomide):

Chemotherapy utilizes cytotoxic drugs that circulate throughout the bloodstream to neutralize the glioma cells. There are many types of chemotherapy drugs, such as procarbazine, lomustine, and vincristine, which can be used together during treatment. The most common chemotherapy drug for brain tumors like glioblastoma is a drug called temozolomide, which this review will focus on.[26] Temozolomide (TMZ) is an oral chemotherapeutic drug that induces DNA methylation and tumor cytotoxicity through cell cycle arrest. TMZ chemotherapy is often associated with a few adverse effects, including the risk of hematological complications, fatigue, and higher rates of infection.[27] TMZ is an imidazo-tetrazine lipophilic prodrug that can cross the blood-brain barrier. Thus, it can be administered orally and is activated at physiological pH through conversion to the metabolite 5-(3-methyl triazole-1-yl) imidazole-4-carboxamide (MTIC). MTIC is hydrolyzed to produce methyl diazonium ions, which are electrophilic methylated molecules that cause DNA damage to the glioma cells. TMZ also affects single strands of DNA at certain sites through methylation. Alkylation of the O6 site on guanine forms O6-methyl guanine adducts and leads to the insertion of thymine bases instead of cytosine. These unrepairable mutations cause single and double-stranded DNA breaks. This results in cell cycle arrest and G2/M phase and eventually apoptosis of glioma cells. However, more than half of GBM patients treated with TMZ do not respond well to the therapy. This is because GBM is highly heterogeneous and mutation-prone, causing GBM to develop resistance against TMZ. This phenomenon is highly driven by glioma stem cells, which play an important role in tumorigenesis and are accountable for a majority of tumor recurrences. MGMT, an endogenous DNA repair enzyme, is also speculated to help with TMZ resistance because of its role in counteracting DNA alkylation damage. It conducts mismatch repair and removes the methyl group in O6-methylguanine, neutralizing the drug-induced DNA damage. Thus, the overall efficacy of TMZ is being lowered.[28]

ii. Tumor Resection:

Tumor resection is often performed to reduce the symptoms of the glioma mass (protect neurological function) and prevent progression.[29] In tumor resection, the challenges come from the amount of cancerous tissue to be removed, as not all cancer cells show up on the MRI. Too much tissue removal impacts other brain functions. A few weeks after a maximal safe resection of the tumor, many patients would undergo radiation therapy and adjuvant temozolomide treatment. This invasive strategy increases survival for 6 months. However, microscopic total resection or complete resection of the tumor is impossible due to the lack of a clear margin. Hence, even when the tumor appears to have been eliminated, the median time to recurrence is around 9.5 months.[30]

iii. Radiation Therapy and Resistance:

Radiation therapy for glioblastoma uses strong X-rays, gamma rays, or protons to obliterate glioma cells in the brain. It results in direct and indirect DNA damage in glioma cells due to the water radiolysis that leads to the peroxide ions and radicals.[31] The high-energy beams are specifically directed at the tumor site to minimize exposure to surrounding normally functioning brain tissue cells. Even though radiotherapy is proven to be the most effective treatment for most primary tumors in the central nervous system, glioma cells have developed tolerance to radiation therapy and adapted to the radiotherapy-induced changes. In GBM stem cells, DNA repair mechanisms result in surviving cells that go on to reestablish the tumor. The effectiveness of radiation therapy also depends on the microenvironment of the tumor, including blood vessels, glioma stem cells, astrocytes, fibroblasts, neural precursor cells, immune cells, signaling molecules, and the extracellular matrix.[32]

Moving on, we will discuss new emerging glioblastoma treatments, including immunotherapy, gene therapy, ultrasound, and combination treatments.

Immunotherapy:

One of the new treatment methods that can complement conventional methods includes Immunotherapy, a class of treatment that manipulates the immune system to attack glioma cells with minimal adverse effects and reduces the risk of tumor recurrence (Figure 3A). Scientists have investigated the use of adeno-associated virus serotype 9 (AAV9) vectors to deliver a therapeutic single-chain antibody (scFv-PD-1) targeting PD-1, a protein that inhibits immune responses, into the tumor microenvironment (TME) of glioblastoma (Figure 3B). The experiment found that CT-2A glioma cells transduced with AAV-scFv-PD-1 successfully produced and secreted a functional antibody that binds to PD-1. Systemic administration of AAV9 can deliver the scFv-PD-1 construct to GBM cells and the TME (Figure 3C). Furthermore, the treatment with AAV9-scFv-PD-1 results in increased T-cell activation markers in the TME, implying a potential therapeutic effect by enhancing the immune system's ability to target the tumor (Figure 3D). The research conducted shows that AAV9 vectors can be used to successfully deliver therapeutic antibodies to glioma cells and potentially enhance immune-based treatments for GBM.[33]

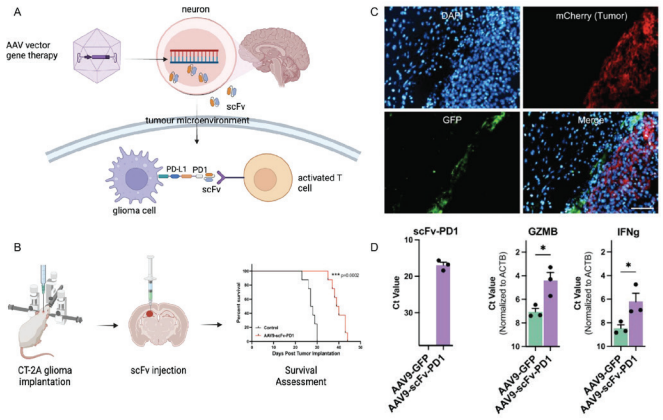

Figure 3: Illustration of Immunotherapy Treatment. (A) Illustration of the mechanism of immunotherapy. (B) Mice were intracranially inject with AAV9-scFv-PD1, Kaplan-Meier survival curves for mice treated with AAV9-scFv-PD-1 or AAV9-GFP with p=0.0002; (C) confirmation of GFB expression in GBM TME by immunofluorescence staining of the brain. (D) transcript expression levels of scFV-PD-1 and antitumoral immune response surrogate markers in GBM TME; *p<0.05; two-tailed, unpaired t-test. Treatment with AAV9-scFv-PD-1 results in increased T-cell activation markers in the TME, suggesting potential therapeutic effects by enhancing the immune system's ability to target the tumor. Figure (B-D) are taken from Maksoud *et al.* (2024) with author's permission. Illustrations are created in Biorender.

Gene Therapy:

Many barriers are hindering successful glioma treatment. The blood-brain barrier (BBB) is a selective semipermeable membrane between the blood and interstitium of the brain, composed of a monolayer of endothelial cells held together by restrictive tight junctions, forming a closed membrane boundary around all central nervous system (CNS) capillaries. It serves as a neuroprotective barrier that can block the passage of noxious agents but also the delivery of anti-tumor drugs, including gene therapy vehicles.[34] Another barrier includes the immunosuppressive TME, which consists of endothelial cells, neurons, astrocytes, resident immune cells, and non-cellular components. The TME facilitates communication between glioma cells and surrounding brain cells, as well as regulates processes such as biomass synthesis, cellular processes, and resistance to therapies that facilitate their survival. These barriers play an instrumental role in enabling the survival of glioma cells, even after therapy.[35] Thus, the passive targeting strategy is not sufficient to target invasive tumor cells, as the permeability and retention effects are weak near the infiltrating cancer cell tumor recurrence. To overcome this problem, scientists have innovated many different approaches to gene therapy aimed at targeting glioma cells.

i. Gene Delivery Strategies - Viral Vectors:

Replicating retroviruses are used to deliver suicide genes into tumor cells and integrate them into the host genome so that non-toxic drugs can be converted to cytotoxic substances, leading to GBM cell death. An example would be HSV-TK, Thymidine Kinase from the Herpes Simplex Virus Type 1, which converts the antiviral drug ganciclovir (GCV) into active GCV triphosphate that prevents DNA replication and cell division in tumor cells (see more in the *Suicide Gene Therapy* section). A replicated retrovirus can also be used as an integrated retrovirus when there is a recurrence of GBM. To illustrate, gene therapy agent TOCA 511 delivers the cytosine deaminase (CD) gene into tumor cells, which encodes for the CD enzyme that converts the prodrug 5-fluorocytosine (5-FC) to active antineoplastic 5-fluorouracil (5-FU), leading to tumor cell death.

Lentiviruses are more stable and less prone to insertion mutation compared to replicating retroviruses. It facilitates the transportation of pre-integration complexes through the nucleopore of the host cell. Lentiviral vector expresses shRNA to reduce the rate of transcription for the Bcl-2 and S-TRAIL enzymes to induce apoptosis in glioma cells. This leads to an increase in expression of activated caspase-3 and caspase-7, which further accelerates the apoptosis of the glioma cells.[36]

Adenoviruses are non-enveloped, icosahedral viruses. The end of the fiber complex of the virus, called the knob domain, binds to the coxsackie and adenovirus receptor on target host cells, mediating cell tropism. The adenoviral penton protein interacts with cell surface integrins, including INT αvβ3 and αvβ5, leading to endocytosis of the virus by the host cell. Conditionally replicative adenoviruses were also developed to selectively replicate within and kill tumor cells. This allows amplification of the input dose of the virus, high levels of expression of therapeutic transgenes, and rapid spreading of the therapeutic effect to other adjacent glioma cells. Oncolytic adenovirus can not only kill tumor cells by direct lysis after replication, but also stimulate an anti-tumor immune response. Replication-competent adenoviruses have proven to be safe for the treatment of patients.

Adeno-associated virus (AAV) for glioblastoma cannot replicate independently but must work together with helper viruses. Even though it does not integrate into the host cell genome, it is highly infectious to different tissue cells in vivo. It has the same structure as the adenovirus but contains a single-stranded linear DNA-deficient virus. It is best used through systematic injection compared to intracerebroventricular (ICV) injection or local injection because it can transduce most regions of the central nervous system and can suppress malignant GBM.

However, one challenge faced is the high expression of therapeutic genes in non-target cells away from the disease site, leading to high peripheral toxicity. There is also a large amount of AAV antibody in the blood, lowering the rate of survival of the AAV in the body.[37]

ii. Non-viral Vectors: Nanoparticles:

Drugs are combined with nanoparticles so that they can cross the BBB, allowing lesions to be detected for precise diagnosis. This is because nanoparticles carry a huge quantity of radioactive isotopes, allowing the imaging to be highly specific and sensitive, increasing the accuracy of the targeted therapy. To improve the quality of the MRI and CT scans, contrast agents that contain iron oxide nanoparticles or ferrites are used.[38]

Nanocarriers can also transport macromolecular drugs, including proteins and genes, to actively or passively target

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GBM cells with lower toxicity. They are highly customizable, allowing for conjugation of nucleic acids, homing peptides, or targeting ligands. The nanoparticles also have different sizes, hydrophobicity, and surface charge. Nanoparticles with a diameter smaller than 5nm are amphiphilic and can successfully cross the BBB through diffusion. Nano-formulated drugs are administered through convection-enhanced delivery (CED), and anti-GBM drugs can diffuse at precisely controlled infusion rates towards GBM tumors under a hydrostatic pressure gradient using microcatheters implanted inside the tumor. This increases the antitumor efficacy and lowers the toxicity levels of GBM treatments.[39]

iii. Suicide Gene Therapy – Conditional Cytotoxic Therapy:

Suicide gene therapy (SGT) is the most common type of gene therapy used to treat high-grade gliomas. SGT comprises a two-step mechanism. First, a viral vector (retrovirus or adenovirus) is used to deliver a gene that encodes an enzyme into the glioma cells. Subsequently, the prodrug is administered to be catalyzed into a toxic metabolite by the enzyme to initiate apoptosis. For this therapy to be successful, the prodrug must have a shape complementary to that of the enzyme, the activation of the prodrug must lead to cell death, the prodrug must be able to cross the blood brain barrier and there must be the bystander effect which helps to facilitate the killing of non-transduced cells due to the transfer of intermediate or final metabolites of the prodrug.[40]

Adenoviral vectors are proven to elicit an acute immune response, resulting in the secretion of proinflammatory cytokines. The disadvantage of vector-induced immune response is that it can limit transgene delivery, affecting treatment efficacy. The advantage is that it could decrease the immune system's tolerance for the glioma environment.

A research study concluded that the lentiviral delivery system was able to effectively introduce the herpes simplex virus thymidine kinase (HSV-tk) gene into glioma cells, which encoded the HSV-tk enzyme that converts a non-toxic prodrug, ganciclovir (GCV), into a toxic form. This initiates the killing of cancer cells. This treatment was found to have efficient bystander effects in glioblastoma cell lines.[41]

Researchers have also explored the use of non-viral vectors, specifically poly(oligo-D-arginine), which has shown low toxicity and has tumor growth suppression capabilities, to deliver the HSV-tk/GCV system across four glioma cell lines (C6, U87, F98, and 9L). The locomotor activity that was monitored in correlation to tumor control was maintained throughout the experiment, proving that the use of non-viral vectors is an efficient treatment that can ensure patients a stable life.[42]

iv. Tumor Suppressor Gene Therapy:

Tumor suppressor gene (TSG) therapy aims to restore the function of TSGs that have experienced loss-of-function mutations, resulting in the proliferation of glioma cells and contributing to the development of glioblastoma. Typically, the approach involves introducing the non-mutated copy of TSG using various virus vectors. The common target genes are *TP53*, *PTEN*, and *p16*.[43]

• TP53 Gene:

The most commonly used replication-deficient adenovirus is the type 5 adenovirus, Ad5CMV-p53, where the E1 region is substituted with cDNA of the wild-type p53 gene and is under control of a CMV promoter. This causes a downregulation of the growth of gliomas and suppression of angiogenesis in GBM. Ad5CMV-p53 is most efficient and effective when complemented with chemotherapy and radiation therapy. Moreover, systemic delivery of a nano-platform containing wild-type p53 sensitizes cancer stem cells and glioma cells to TMZ and increases apoptosis.[43]

• p16 Gene:

The overexpression of the p16 gene by recombinant replication-deficient adenovirus decreases the activity of matrix metalloproteinase-2 (MMP2) that facilitates glioma metastasis. Additionally, adenoviral delivery of the p16 gene further amplified radiation-induced cell killing by a non-apoptotic mechanism with abnormal nucleation in glioma cells. Researchers have proven that the restoration of the wild-type p16 gene successfully decreased the rate of glioma cell division and invasion. Adenoviral transfer of wild-type p16 cDNA and antisense urokinase-type plasminogen activator receptor (uPAR) led to a decrease in adhesion, metastasis, and proliferation of glioma cells. However, after the importation of the p16 gene into glioma cells and a cell-cycle arrest, there is a development of chemoresistance to some cytotoxic drugs.[43]

• PTEN Gene:

The PTEN gene is inactivated in 33% of all glioma cells, leading to the abnormal activation of phosphoinositide 3-kinases (PI3K) pathways, which increase glioma cell metabolism, growth, and survival. Adenoviral re-expression of PTEN in glioma cells causes dephosphorylation of PIP3 to PIP2 and suppresses AKT kinase activity, which leads to apoptosis. Glioma cells also displayed an anti-angiogenic response and decreased proliferation.[43]

v. BBB Disruptive Gene Therapy:

BBB disruptive gene therapy utilizes focused ultrasound (FUS), which activates gas-filled microbubbles that expand and contract to oscillate within the bloodstream. This elicits endothelial selective transfection without an aperture in the BBB. Researchers have also successfully developed a VEGFR2-targeted cationic microbubble (VCMB) gene delivery system complemented with FUS exposure to deliver herpes simplex virus thymidine kinase (HSV-TK) gene as well as ganciclovir (GCV) into glioma cells. pHSV-TK is a gene that activates GCV and causes glioma cell death. This technique resulted in a large rise in median survival in 9L gliosarcoma-bearing rats. However, it is crucial to note that targeted gene delivery becomes less precise and accurate as the ultrasound pressure increases. At higher pressures, the ultrasound can open the BBB, allowing plasmid DNA to be delivered not only to the endothelium but also to other brain cells.[44]

Wearable Devices:

To better treat GBM, a wearable device called Optune Gio delivers tumor-treating electric fields (TTFields) that are alternating and low-intensity to the scalp. This is administered via transducer arrays. The TTFields are an antimitotic treatment modality that affects the division cycle and organelle assembly of glioma cells. This device is an FDA-approved treatment option usually added to a patient's GBM treatment to complement temozolomide chemotherapy. Adult patients above 22 years old are eligible for this treatment. Treatment with TTFields is delivered through 4 transducer arrays with 9 insulated electrodes each placed on the shaved scalp and connected to a portable device that generates 200-kHz electric fields within the brain. A randomized clinical trial with patients diagnosed with GBM has consistently proven that those who received standard radiochemotherapy with the addition of TTFields to maintenance TMZ chemotherapy, compared to those with only maintenance TMZ, had a statistically significant improvement in progression-free survival and overall survival.[45] Approximately half of the patients using Optune Gio and TMZ chemotherapy were alive after 2 years, compared with only 31% of people using TMZ chemotherapy alone.[46] However, a higher incidence of localized skin toxic effects and anxiety, confusion, insomnia, and headaches was reported more frequently in patients treated with TTFields.

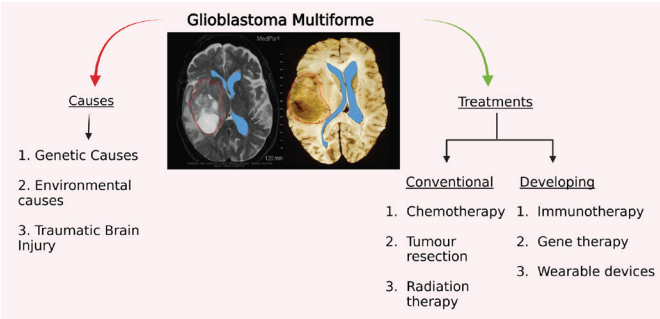

Figure 4: Summary of causes and treatments for Glioblastoma Multiforme discussed in this review. Recent developments in GBM treatments suggest that combination and personalized approaches for GBM patienta may give the best disease prognosis. Images of gliblastoma tumor obtained from two different patients from MedPix.[47]

■ Conclusion

In this review, we have discussed the causes and treatment strategies for Glioblastoma multiforme (Figure 4).[47] Patients with glioblastoma multiforme seek treatments for an improved quality of life as well as an extended lifespan. After tumor resection, which is a basic treatment method, neurological symptoms are usually improved. However, there are a small number of patients who face neurological deficits, epilepsy, as well as depression and anxiety as direct consequences of the surgical treatment.[48] When a combination of treatments that consist of surgery, chemotherapy, especially with the drug temozolomide, and radiation therapy is used, the median survival rate of the patients diagnosed with glioblastoma increases. However, due to the aggressive and infiltrative nature of GBM, recurrence is unfortunately common. Even though immunotherapy serves as a prospective treatment that offers a potential avenue for improved outcomes for glioblastoma patients, there are currently no immunotherapies that have received clinical approval for patient administration. Furthermore, immunotherapy is not only expensive but may also give rise to side effects and inflict damage to other organs in the body. Even though gene therapy has been proven to significantly extend survival rates, it may not be the best treatment for glioblastoma, as difficulties encountered range from imprecise injection placement and depth for tumor cells to poor survival of the retrovirus vectors in the brain and short diffusion distance.[49] This can lead to incomplete tumor eradication. Moreover, preclinical studies usually utilize xenogenic models with a short treatment duration. This almost completely differs from the treatment process of the more complex and long-standing human brain tumors. Additionally, there may be potential immune reactions towards the viral vectors, risking the safety of the patient. Further studies are still needed to obtain conclusive results. Thus, larger-scale phase III clinical trials need to be conducted to establish conclusive results in terms of the efficacy of gene therapy in malignant glioblastoma. More research can also be conducted on the aggressive nature of glioma cells as well as their microenvironment, so that more targeted treatment methods can be innovated to help patients suffering from glioblastoma.

Due to the aggressive and heterogeneous nature of GBM, a more targeted and personalized approach might be required for individual patients. Based on the known human genome and cancer atlas databases, we have an understanding of the genetic basis of causes of GBM, and this can form the basis of personalized medicine for each patient. Personalized or precision medicine will involve determining the genetic basis of the tumor and strategizing an approach to best eradicate the cancer. As an example, when a patient is diagnosed with GBM, a biopsy can be obtained to determine the histology of the tumor. Based on MRI, the tumor will be localized and graded, and if possible, a tumor resection. Surgical debulking is usually performed because high-grade GBM is extremely extensive without clear tumor margins. The tumor tissue can be analyzed for genetic mutations, and possible gene or immunotherapies can be suggested and personalized for the patient.

The initial diagnosis is usually devastating, and as patients are going through treatment, it is important to ensure that they have a stable support network through family and friends. Researchers have found that patients who have a strong support system recover faster and have a better quality of life.

Other novel therapies that have been explored by researchers but are outside the scope of our review include cytostatic hypothermia. Cryogenic freezing of brain tumor cells helped to safely halt brain tumor growth through the suppression of cell proliferation. This treatment method has been experimented on freely moving rodents successfully. Subzero temperatures indiscriminately ablate both the diseased and healthy tissue, offering little advantage over the current standard GBM therapies.[48]

For GBM patients, clinical trials can offer potentially life-saving new treatments that are not yet available to the general public. Even though there are unknown risks associated with new experimental drugs and treatments, doctors can en-

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courage open and informed discussions with patients to offer the chance to participate in drug trials. Patients who qualify for clinical trials can potentially gain a second chance at life.

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Author

Natalie Hwang is a junior college student studying at Hwa Chong Institution in Singapore. She is deeply passionate about neuroscience and the mechanisms of cancer. On a more specific level, Natalie is interested in unravelling the complexities of brain tumors and exploring innovative therapeutic treatments. She aims to pursue a career in medicine in the future.

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