

Malignant brain tumor: Current progresses in diagnosis, treatment and future strategies

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ABSTRACT

Malignant brain tumor remains as a mysterious disease in medical oncology in terms of its highly heterogeneous nature, complicity in early detection with limited treatment options. Glioma, being the most common form of malignant brain tumor has been evolved as one of the major threats to mankind across the globe. Once considered as a disease of the west, brain cancer in recent years has outstretched its claws across the eastern world including all developing countries. In India too, alarming number of fresh cases are being diagnosed annually from last few years, which necessitates urgent use of cutting age technologies along with novel formulation strategies to control the disease. Nanotherapeutic approaches at present hold promising potential to revolutionize the conventional drug delivery strategies through effective by-passing of blood-brain barrier with decreased off-target distribution. Further, nanocarrier platforms can be surface-engineered with specific moieties to offer tumor specific diagnosis, treatment and track monitoring. Such modified nanodrug carriers, however have largely been confined to *in vitro* laboratory studies with lack of sufficient *in vivo* data, which is the need of the hour for their rapid clinical translation. This review summarizes the present stages of diagnosis and treatment options for malignant brain tumor along with recent progresses made in this area over the past few years. Side by side a brief discussion is there regarding the practical hurdles faced by the nanotherapeutics for their successful clinical trials and large scale production with an eye towards future perspectives.

Keywords: Malignant brain tumor, diagnosis, treatment, advancements

Introduction

Malignant brain tumor, in recent times has been identified as one of the leading causes of mortality around the world ^[1]. With each passing year, an increasing number of brain cancer cases are being reported worldwide, which actually raised serious concern among medical professionals as well as pharmaceutical scientists ^[2]. At present, nearly 3 lakh fresh cases of malignant brain tumor are diagnosed annually. In 2020, an estimated 1,806,590 cases of cancer was diagnosed in the United States alone with nearly 6 lakh fatality ^[3]. Developing countries including India have now become the epicenter of the disease with uncontrolled rate of incidences across the country ^[4]. Among cancerous brain tumors, glioma represents the most common types in adults with highly heterogeneous nature in their clinical presentations. Glioblastoma is the fourth and most advanced stages of glioma with very limited treatment options and a survival rate of merely 6 months to 1 year ^[5]. Though advancements in the molecular biology including whole-genome and RNA sequencing, global epigenetic profiling etc. has helped a lot to unfold the core pathways of cancerous brain tumor, but such newer molecular techniques have indeed failed to make any significant improvements to bring tangential outcomes in cancer patients ^[6]. Moreover, absence of early-stage symptoms in any class of glioma delays

its timely diagnosis, which consequently leads to failure in treatments. As far as treatment approaches are concerned, surgery remains the first choice of treatment, but of course has a limited role; owing to the highly sensitive and complicated nature of the brain micro environment^[7]. But, once the cancer cells start migrating to other body parts (malignancy stage), then drug therapy remains the last hope. Further problem starts from there as majority of drugs through conventional form fail to reach at brain tissue with desired therapeutic concentration. Sub-therapeutic availability of drugs inside the brain tissue is primarily attributed to the presence of blood-brain barrier (BBB), the tight endothelial junction system. BBB owing to its architectural uniqueness stands as a major obstacle for majority of macromolecules to enter into the brain^[8]. Only the highly lipophilic and nanosize molecules can get access inside the brain, which thus makes the regular treatment strategies extremely challenging. Most of the cytotoxic drugs used for cancer therapy (in conventional formulation) have usually possess less BBB permeation capacity plus associated with non-specific distribution to almost all other vital organ/tissues, which further complicates the treatment outcomes with unavoidable severe healthy tissue toxicity. Early diagnosis and advanced treatments methods are thus the present need of the hour to tackle the progression of malignant brain tumors^[9].

In recent times, evolvement of cutting age technologies like magnetic resonance imaging (MRI), positron emission tomography (PET) etc. have revolutionalized the diagnosis of brain tumors to a great extent^[10]. Past years have also witnessed major advancements in the drug delivery research to target brain and central nervous system, the so called “no-go-zone” for conventional therapeutics. Many studies have already shown that brain tissue distribution profiles of anticancer drug(s) could be regulated by their trapping in submicron colloidal platforms (nanoparticulate carriers). Such advanced drug carrier platforms like polymeric nanoparticles, nanoliposomes, polymeric micelles, solid lipid nanoparticles, niosomes, dendrimers etc. possess the ability to improve the therapeutic window of the loaded cargo with increased bioavailability and lesser off-target distribution^[11]. Further, nanodrug carriers are also being designed for theranostics (therapy and diagnosis) purpose by surface engineering with specific tumor-targeting ligands for better diagnosis (track monitoring) and enhanced clinical effects. The engineered multifunctional nanocarriers thus possess the ability to specifically deliver the loaded cargo(s) at the brain tissue region with diminished non-specific drug wastage and fewer adverse effects^[12]. The present review basically summarizes the recent stages of diagnosis and treatment strategies for cancerous brain tumor along with progresses made in this area over the past few years. We will also throw some light on the challenges faced for successful application of novel nanotherapeutics at clinical stage with a brief discussion on future prospects.

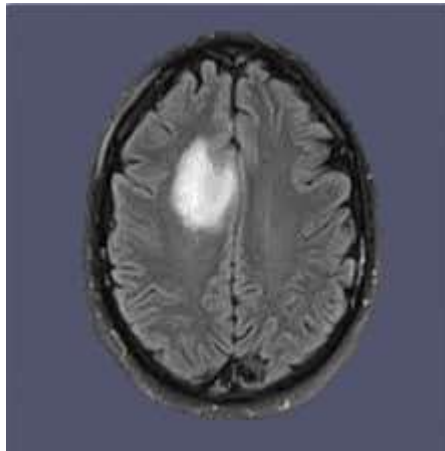
2. Diagnosis of tumors in the brain

2.1 MRI

MRI in recent times has been recognized as one of the most reliable technology to diagnose brain tumor. An MRI utilizes magnetic fields instead of X-rays to produce exact pictures of the body. To create a clearer image, a unique dye called contrast medium is generally used. This dye is injected into the vein of a patient or can be used in the form of a pill or liquid^[13]. Depending on the type of tumor suspected and the risk of it spreading to the brain, the MRI of selected tissue/area (brain, spinal cord, or both) can be taken. Gadolinium-enhanced intravenous (*i.v.*)

MRI is often used to improve the image of a brain tumor. This is when a patient has a routine MRI and then receives an *i.v.* injection of the contrast medium (gadolinium). A second MRI is then performed to obtain another collection of images using the dye. Further, the cellular structure of the brain can be well visualized using a specific MRI technique, preferably known as diffusion weighted imaging ^[14]. In this method, the amount of blood that reaches to the brain can also be estimated. Various MRIs used for tumor diagnosis includes the following

- Spinal MRI, which is used on or around the spine to diagnose a tumor.
- Functional MRI (fMRI), that offers information on particular regions of the brain responsible for muscle activity and expression. During the fMRI, a patient is generally asked to do certain pre-planned tasks that trigger changes in the brain which can be seen on the fMRI picture.
- Magnetic resonance spectroscopy (MRS) is a type of MRI test that offers data on the chemical composition of brain. Further, it also helps to predict the difference between dead tissues (caused by post-treatments) and fresh tumor cells in the brain.



(MRI image of a brain tumor, *image source:pubmed*)

2.2 Biopsy

Biopsy has been used as an inevitable technique to detect the nature of cells in the tumor and involves removal of a sample of the pathological tissue and subsequent analysis through a microscope ^[15]. The cells collected during the biopsy may indicate which type of brain lesion (abscess, tumor) is there and whether it is benign (not cancerous) or cancerous (malignant). Finding the particular type of cell is extremely helpful for the physicians to prescribe the required treatment plan. Main aim of a brain biopsy is to identify any abnormality seen on an MRI or CT scan by collecting a sample of cells from the suspected location ^[16]. Biopsies are generally of two types such as open biopsies and stereotactic needle biopsies. In the open biopsy, a cut is made in the skull and a few fragments of tissue from the brain surface are extracted. The collected tissue is then tested under a microscope for its specificity and presence of malignancy property, if there. Contrary to that, in needle biopsy a needle is utilized to penetrate into the brain tumor or lesions. A stereotactic frame is used to direct the needle through the brain during this procedure ^[17]. At first, a small burr hole is drilled in the skull. The biopsy needle is then moved through the brain to check any irregular lesion or tumor using the image guidance system. Several tiny fragments of tissue are gathered and processed for testing ^[18]. After the operation, the needle is removed and a

thin titanium plate is inserted over the burr opening area with subsequent closure of the skin opening by sewing, glue, or staples. Higher antibiotic doses are also recommended to accelerate healing.



(Biopsy of a brain tumor, *image source: pubmed*)

2.3 Computed tomography (CT) scan

CT scan utilizes X-rays to render precise cross-section visuals of the brain. CT scans, unlike standard X-rays provide accurate images of soft tissues in the brain. Though after the evolvments of MRI, CT scans are being less preferred as diagnostic tools for brain cancers, however, it is still effective in some situations ^[19]. They are the easy options in case of unavailability of MRI (costly) and in people who are obese or having a fear of enclosed spaces. CT scans also illustrate more detail of the bone structure surrounding the tumor area. In CT angiography, a contrast fluid is inserted through *i.v.* route, while the patient remains under CT scanner ^[20]. The scan provides clear images of the blood vessels in the brain that becomes helpful for physicians to schedule surgery. In some cases, CT angiography may provide clearer information of the blood vessels in and around the tumor than MR angiography.

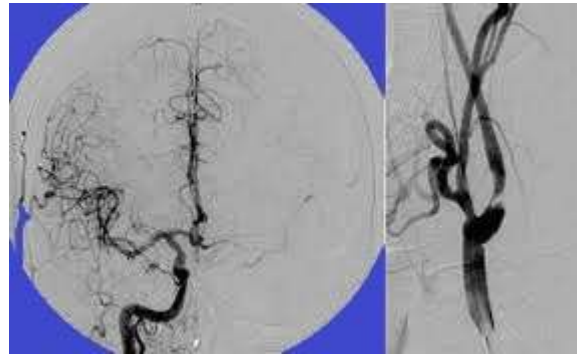
2.4 Positron emission tomography (PET)

PET scan is an imaging procedure that is most advanced detection tool for brain tumors. The scan uses radioactive molecules to trace the exact location and size of the tumors in the brain ^[21]. These tracers are designed to attach with glucose compounds (sugar moiety); the primary food of the brain. Responsive regions of the brain (having tumor) can use glucose at a greater rate than passive areas. In this technique, an *i.v.* catheter is inserted into the shoulder of the patient. Through this, a special dye containing radioactive tracers is released into the veins ^[22]. Tracers are concentrated in the regions of elevated blood supply (tumor region with uncontrolled cell proliferation). The pictures in brain PET scans depict multi-colored images of the brain with colors varying from dark blue to deep red. Areas of intense brain function occur in warmer shades, such as yellow and red ^[23]. A tumor is visualized as a darker spots on the PET scan. In people with Alzheimer's and other types of dementia, larger-than-normal darker area is spotted on the scan, which suggests non-functional or damaged area in the brain.

2.5 Cerebral angiography:

Cerebral angiography is an X-ray diagnostic test, which produces a brain angiogram or an image to locate blockages or any other abnormalities in the blood vessels inside the brain tissue ^[24]. In this method too, a contrast medium is injected into the blood to draw a clear picture of the blood vessels in order to identify any blockages or abnormalities. In this procedure, a catheter is

implanted and pasted into the carotid artery. A contrast color is then allowed to flow through the catheter and through the artery. The whole operation normally takes one to three hours to obtain the final image^[25].



(Cerebral angiography image, source: Pubmed)

2.6 Lumbar puncture

Certain forms of brain tumors can spread from the brain to the Cerebrospinal fluid (CSF), which thus can be utilized as the basis for testing of malignancies in brain tumors. The lumbar area of the lower back is selected for puncturing. A needle is then placed between two lumbar bones (vertebrae) to extract a sample of CSF during a lumbar puncture^[25-26].

3. Treatment strategies and advancements

3.1 Surgery

Surgery is the first line of treatment option for any tumors in the brain, which involves elimination of the undesirable mass and a few nearby healthy tissues. Removing the tumor is necessary for its identification and genetic analysis, and also to make multiple brain tumor therapies easier for better clinical outcomes. Use of cortical visualization, improved imaging, and fluorescent dyes all together have contributed a lot in furthering research towards brain tumor surgery^[27]. Further, computer-assisted techniques such as image guided surgery assist the surgeons in precisely mapping out the position of tumors. In this method, before starting of surgery, a fluorescent dye known as 5 aminolevulinic acid is orally administered to the patient, which is subsequently absorbed by the proliferating cells. The stained cells can be then visualized under a special microscope which enables physicians to extract as much of the tumor as possible. Further, when a tumor is located near the speech centre of the brain, it is becoming more normal to perform part of the procedure while the patient is awake. Then, using special electrical stimulation methods, the location of the part of the brain that regulates speech is determined^[28]. This method helps to avoid undesirable damage to the surrounding tissue while removing the tumor. However, surgery can not be the treatment choice always, since sometimes the tumor is too far away for the surgeon to reach or is too close to a sensitive area. If the tumor is inoperable, other treatment options might be suggested including a biopsy or partial tumor removal.

3.2 Chemotherapy

Chemotherapy is the use of medications to kill tumor cells, usually by preventing tumor cells from dividing, proliferating and migrating. A chemotherapy protocol usually consists of a pre-scheduled number of cycles administered over a predetermined time period. In chemotherapy regimen, usually a patient receives one drug at a time or may receive a combination of two drugs at a time^[29].

As previously stated, chemotherapy for a brain tumor is usually administered after surgery or after radiation therapy, especially when recurrence of tumor is reported after initial treatment. Few medications have been found more effective at crossing the BBB^[30]. Few recently adopted chemotherapy strategies include:

- Gliadel wafers for administration of carmustine. (These wafers are mounted in the region of the tumor that was removed during surgery).
- Low-dose temozolomide (Temodar) supplement (monthly) after radiation treatment for 6 months to a year.
- Three drugs, namely lomustine (Gleostine), procarbazine (Matulane), and vincristine (Vincasar) have been recommended after radiation therapy to extend the lives of patients with grade III oligodendroglioma.

When on active care, patients are checked with a brain MRI every 2 to 3 months. The interval between MRI scans is then increased based on the grade of tumor. Patients are often subjected to routine MRIs to monitor their health after surgery. If the tumor proliferates after surgery, than advanced treatment option is tried^[31].

Chemotherapy side effects vary depending on the person and the dosage used, but they may include fatigue, infection risk, nausea and vomiting, hair loss, loss of appetite, and diarrhea. These symptoms normally disappear after the completion of chemo-cycles. However, certain medications, on rare occasions can cause hearing loss or damage to kidneys. To protect kidneys from further damage, patients are recommended extra fluid through intra-venous route^[32].

3.3 Radiation therapy

The use of high-energy X-rays to kill tumor cells is preferably known as radiation therapy. It can be used to delay the progression of a brain tumor. It is usually recommended after surgery and can be combined with chemotherapy^[33]. External-beam radiation therapy, which delivers radiation from a machine outside the body, is the most prevalent form of radiation treatment. Brachytherapy is another form of radiation therapy that uses implants to provide radiation^[34]. A radiation therapy regimen consists of predetermined numbers of procedures administered over a fixed time span. External-beam radiation therapy can be directed at a brain tumor in the following ways:

- Conventional radiation therapy, in which anatomic markers and X-rays are used to assess the treatment site. The amount of radiation given is determined by the grade of the tumor.
- Intensity modulated radiation therapy (IMRT), a kind of 3D-CRT that can target a tumor more precisely. It can provide higher doses of radiation to the tumor while delivering fewer to healthy tissue around it. The radiation beams are divided into smaller beams in IMRT, and the strength of each of these smaller beams can be adjusted to desired level

- Proton therapy is a form of external-beam radiation therapy in which protons are used instead of X-rays. Protons have the ability to kill tumor cells at high energies. Thus proton beam therapy is commonly used for tumors where less radiation is needed due to their complicated location.
- Stereotactic radio surgery is the application of a single, heavy dose of radiation to the tumor rather than on healthy tissues. It is most effective for cancers that are limited to a single region of the brain and for some non-cancerous tumors. Stereotactic radio surgery equipment comes in a variety of types.

Stereotactic radio surgery is applied in fractions of small doses over multiple days or weeks. This procedure is used to treat cancers that are similar to delicate tissues such as the optic nerves or brain system ^[35]. The radiation oncologist may use all of the above radiation techniques, depending on the size and location of the tumor. In certain cases, a mixture of different strategies might be the most effective option to get higher clinical benefit ^[36].

Radiation therapy can cause nausea, mild skin reactions, hair loss, disturbed stomach, and neurologic symptoms including short term memory loss. The majority of side effects subside quickly after therapy is stopped. Furthermore, radiation therapy is limited to adult patients mostly and often not approved for children below age of five. Long-term side effects of radiation therapy also include memory and hormone disturbances, as well as neurological problems such as difficulties in remembering and completing complex activities.

3.4 Targeted therapy

Targeted therapy is a technique that uses unique tumor markers (proteins/receptors/genes) that are over-expressed during tumor development. This method of therapy inhibits tumor cell development and spread while limiting damage to healthy cells ^[37].

The targets of all tumors are not the same, and certain tumors can have more than one target. Patients are allowed for different tests to determine the genes, proteins, and other factors in the tumor to find the most effective treatment. This allows physicians to provide the most appropriate care the patient wherever possible ^[38]. Furthermore, scientific trials are continuing to learn more about particular molecular targets and experimental therapies aimed at them. Following are two widely used forms of targeted treatment for cancerous brain tumor:

- Bevacizumab (Avastin, Mvasi) is an anti-angiogenesis drug that is used to treat glioblastoma multiforme after failure of conventional chemotherapy. This anti-angiogenesis treatment primarily aims to interrupt angiogenesis or to “starve” the tumor cells.
- Larotrectinib (Vitrakvi) is another form of targeted therapy that does not target a single type of tumor but instead focuses on a particular genetic modification known as an NTRK fusion. This form of genetic alteration has been discovered in a variety of tumors, including several brain tumors. It is approved as a therapy for certain brain tumors that is difficult to be removed surgically and might have progressed after other therapies ^[39].

Immunotherapy:

Immunotherapy offers the opportunity to allow the patient's own immune system a chance to help eliminate the cancer. Immunotherapy's strength is that it efficiently treats relatively small tumors

in experimental animal models. For some patients, immunotherapy has worked for them while not showing long-term toxicity^[40].

Conclusions and Future Prospects

The overall survival rate of the brain tumor patients with advanced stage is still very poor. Although these statistics are daunting, recent advances in the understanding of brain tumor biology have fuelled a new sense of optimism that more effective and less toxic treatments can be developed. It is a fact that surgery, radiation therapy or conventional chemotherapy in combination are not sufficient to control the progression of brain cancers and thus targeted therapy is the only option, which needs to be fully explored to bring some fruitful changes in future days. However, Despite of eye catching increase in volume of brain tumor related research, targeted therapy yet has not been able to show significant advantages in terms of efficacy and safety over conventional treatments. In general, targeted therapy is tried after failure of regular chemo- regimen. Many studies on targeted therapy are still limited to *in vitro* testing on brain tumor cell lines to depict the superiority over their conventional counter parts, which practically cannot be fully relied up on to draw decisive conclusion. Pre-clinical reports of targeted therapy on laboratory animals are also limited with serious dearth of *in vitro-in vivo* correlation data. Furthermore, many novel drug carriers like nanoparticulate formulations are yet to see day light in many developing countries. The prime factor is the high cost of such formulations as compared to their conventional form, which puts a pressure on pharmaceutical companies for its large scale manufacturing. High cost factor, lack of reliable *in vitro-in vivo* data restricts application of targeted therapy at larger clinical perspective. Many novel drug formulations intended for brain cancer therapy are still stocked at various stages of clinical trials and thus are unable to clear the regulatory hurdles for marketing. Future research on targeted therapy should have multiple focuses on different aspects for their tangential outcome. First of all, exact mechanisms and targets of the therapy need to be further explored through in depth application of molecular biology. Gene detection technique should be emphasized more to provide individualized treatment to brain cancer patients. Secondly, success of targeted therapy for malignant brain tumors should include application of targeted therapy in pre-operative neoadjuvant therapy/postoperative adjuvant therapy and in combination with other types of established chemotherapies to fully explore their efficacy. Appropriate dosage of various targeted nanodrug formulations needs to be optimized at clinical stages to improve the patient tolerance and also to reduce undesirable side effects. Many leading research laboratories at various central/state university level are often remain reluctant to share their findings to others, especially industry personnel. It should be remembered that a successful collaboration with continuous exchange of dialogues and research ideas/innovations between the researchers of academic and industry is the only option to bring tangible outcomes in targeted therapy for malignant brain tumors. While nanocarrier based therapeutics is slowly expanding its wings across various dimensions of neuro-oncology with hopeful opportunities, challenges still remain. Targeted therapy along with immunotherapy has slowly been transformed from their preclinical stages to clinical trials during the last decade. Continuous research on experimental animal models, *in vitro-in vivo* correlation studies, increased clinical testing of the optimized targeted nanocarriers, financial support from national funding agencies along with successful collaborative approach all together are the present needs of the hour to bring pivotal treatment breakthroughs in coming years.

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Author contribution:

Gurudutta Pattnaik^{3†} has equal authorship right with the first author.

Conflicts of Interest

The authors of the manuscript have no conflict of interest to declare.

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