

Nanotechnology for treatment of glioblastoma multiforme

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ABSTRACT

Glioblastoma multiforme (GBM), a grade IV astrocytoma as defined by the World Health Organization (WHO) criteria, is the most common primary central nervous system tumor in adults. After treatment with the current standard of care consisting of surgical resection, concurrent temozolomide (TMZ), and radiation, the median survival is only 15 months. The limited and less-effective treatment options for these highly aggressive GBMs call for the development of new techniques and the improvement of existing technologies. Nanotechnology has shown promise in treating this disease, and some nanomaterials have demonstrated the ability to cross the blood–brain barrier (BBB) and remain in GBM tissues. Although the retention of nanoparticles (NPs) in GBM tissue is necessary to elicit an antitumor response, the delivery of the NP needs to be enhanced. Current research in nanotechnology is directed at increasing the active targeting of GBM tissue not only for the aid of chemotherapeutic drug delivery but also for imaging studies. This review is aimed at describing advancements in increasing nanotechnology specificity to GBM tissue.

Key words: glioblastoma multiforme, nanotechnology

INTRODUCTION

Glioblastoma multiforme (GBM), a grade IV astrocytoma as defined by the World Health Organization (WHO), is the most common brain tumors in adults, accounting for 54% of all gliomas and 16% of primary brain tumors.^[1,2] The standard of care for GBM, as defined by the Stupp protocol, is maximal safe surgical resection followed by oral consumption of temozolomide (TMZ), which is normally administered concurrently with radiation. Even with such aggressive treatments, the median survival of an GBM is only 15 months after its initial diagnosis.^[3,4] With such a short median survival, it is essential that we uncover novel techniques to treat patients with GBM. Current research shows that the genetic profile of GBM is leading to resistance to TMZ and radiation, but a major battle in the treatment of GBM is drug delivery across the blood–brain barrier (BBB). There is hope for patients with GBM because nanotechnology has

been able to demonstrate the ability to cross the BBB.

A number of nanomaterials, including liposomes, nanoemulsion, polymeric micelles, and iron oxide nanoparticles (IONP) have been investigated as carriers for therapeutic agents for the treatment of GBMs. These materials demonstrated a favorable effect, known as the enhanced permeability and retention (EPR) effect, via positive targeting that allows the retention of nanomaterials in tumor tissues. To enhance the EPR effect, active targeting is being applied to nanotechnology to increase the delivery to the tissue. At present, convection-enhanced delivery (CED) is applied to increase the uptake of nanomaterials into brain tumor tissues. Nanomaterials are also used with siRNA to suppress the gene function that makes GBM highly aggressive. More importantly, these nanomaterials can be used to deliver chemotherapeutic agents specifically to the tumor tissues without causing systemic toxicity.

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BBB PENETRATION THROUGH EPR EFFECT

The inability to cross the BBB in known standard chemotherapy options is the major obstacle in achieving remission after surgical resection followed by chemotherapy that is often combined with radiation. The BBB is formed by a single layer of endothelial cells that are bound by tight junctions and is the main defense of keeping exogenous substances out of the brain while maintaining homeostasis.^[5,6] The high angiogenic nature of an GBM allows for the formation of “leaky” vessels. It has been shown that Claudin-1, a key component of tight junctions in endothelial cells, is downregulated in GBM vasculature compared to normal blood vessels in the brain parenchyma, leading to a more permeable BBB.^[7] In addition to the loss of a key structural component of endothelial tight junctions, vascular endothelial growth factor (VEGF) has been shown to increase the BBB permeability in addition to stimulating angiogenesis in response to hypoxia.^[8,9] Along with increased angiogenesis, the disruption of the normal brain parenchyma by an infiltrative GBM disrupts the lymphatic system. Combining these two weaknesses of GBMs in defense, drugs are able to penetrate and retain in the tumor tissue; this phenomenon is termed the enhanced permeability and retention (EPR) effect.^[10] The EPR effect allows nanotechnology to have an advantage over all other chemotherapeutic agents, that is, its ability to gain access to the GBM tissue. Even though nanotechnology is able to cross the BBB, active targeting to tumor tissue needs to be improved.

Polymeric nanoparticles and CED

Paclitaxel (PTX), a chemotherapeutic agent, has been encapsulated in a nanoparticle (NP) composed of poly(lactide-co-glycolide) (PLGA). The encapsulation allows the toxic chemotherapeutic agent sequestered only within the NP until it gets into the tumor tissue by the EPR effect, which reduces systemic toxicity. Although the EPR effect is highly beneficial to nanotechnology, it is a passive process and only small amount of NPs can enter the GBM tissue. The polymeric NPs that do not enter tumor tissues will be deposited in the reticuloendothelial tissue of the liver, kidney, and spleen.^[11,12] To enhance the delivery of this polymeric NP loaded with PTX into the brain parenchyma, CED, a method to maintain a pressure gradient during interstitial infusions, was used. CED has been shown to significantly enhance the delivery of small and large molecules within the brain.^[13] PLGA NPs have been delivered using CED on an intracranial rat model with U87MG xenograph and resulted in a longer median survival as compared to treatments with free PTX or PTX-loaded PGLA NPs delivered without using CED.^[14]

Polymeric micelles and CED

Polymeric micelle NPs are assembled with polymers that have both hydrophilic and hydrophobic characteristics, with the hydrophobic portion forming the micelle core. Conjugation of a chemotherapeutic drug to the hydrophobic core can sequester the toxic component until it is retained in the tumor tissue, which is accomplished by conjugating the chemotherapeutic drug to polyethylene glycol (PEG). Inoue *et al.* conjugated doxorubicin (DOX) to aspartic acid residue of poly(ethylene glycol)-*b*-poly(aspartic acid) block copolymer and then used CED to deliver the polymeric micelle into the brain parenchyma of an L9 intracranial syngeneic rat tumor model. When compared to CED liposomal or free DOX, mice treated with the DOX polymeric micelle showed a longer median survival, indicating its potential for an improved treatment for brain tumors and GBMs.^[15]

Liposomal nanoparticles and active tumor targeting

The liposomal delivery system for chemotherapies is an advancement that may lead to an increased delivery of chemotherapeutic agents to tumor tissues and a greater retention within tumor tissue. Liposomes are formed spontaneously when phospholipids are added to an aqueous solution. In the phospholipid bilayer, amphiphilic or hydrophobic compounds can be incorporated, rather than encapsulated, in the aqueous core.^[16] Modification of the liposome with PEG allows the attachment of functional groups, including ligands, specifically targeting tumor tissues and preventing the NP to be found by the reticuloendothelial system, thus termed a “stealth” liposome.^[17]

Increasing the dose of chemotherapeutic agents and enhancing specific targeting of tumor cells is the primary focus in treatment of GBMs. In conventional chemotherapeutic delivery, high doses of chemotherapeutic agents are needed to gain access to the brain parenchyma, which is limited by systemic toxicity. Liposomal NPs may be the solution to these problems with conjugation of ligands that can actively target molecules overexpressed in GBM tissue. In one case, IL-13 was conjugated to the liposome-containing DOX. IL-13 was chosen because it has been shown that high-grade astrocytoma contains a large portion of IL-13R α 2 as compared to normal cortex.^[18] This liposomal NP was able to actively target GBM tumor cells without being expelled from the tumor cell by the P-glycoprotein, a mechanism of chemotherapeutic resistance.^[19]

In another study, Yang *et al.* has reported liposomal NP as a carrier for DOX that is conjugated to atherosclerotic plaque-specific peptide-1 (AP-1), allowing the NP to

bind to the IL-4 receptor and be taken into the cell by endocytosis.^[21] Here AP-1 can target the IL-4 receptor, a receptor found overexpressed on brain tumor cell lines compared to normal cortical cells. It has also been shown that conjugation of endotoxin to the surface of the liposomal NP increases the delivery and enhances tumor killing in GBM tissue.^[22,23]

Conjugation of a surface ligand is not the only method to increase liposomal NP drug delivery into GBM tissue. When CED has been used with liposomal NP, the BBB can be circumvented and drug delivery can be increased by delivering the chemotherapeutic agent directly to the brain parenchyma under positive pressure.^[13] Noble *et al.* showed that camptothecin (CPT)-11/irinotecan, a CPT-derived topoisomerase I inhibitor, can be encapsulated in the liposome and have increased therapeutic effect when using CED method. By using CED of the CPT-11/irinotecan liposomal NP, a higher chemotherapeutic dose was delivered to the brain parenchyma with lower systemic toxicity compared to CPT-11/irinotecan liposomal NP that was not delivered by CED. This finding shows strong promise for a future direction in not only liposomal NP delivery to GBM but also the usefulness of CED in drug delivery of the liposomal NPs.^[20]

NANOTECHNOLOGY FOR TREATING GBM CANCER STEM CELLS

Histopathologically, GBM can be distinguished from low-grade gliomas by area of necrosis, microvascular hyperplasia, and pseudopalisades of cells migrating away from the area of necrosis.^[25] These pseudopalisades are located at the cortex–tumor junction, and migrate away from hypoxic and necrotic core. The histologic presentation of microvascular hyperplasia and necrosis may be essential in understanding the infiltrative nature of GBMs.

It has been shown recently that cancer stem cells (CSCs) are able to initiate phenotypically human glioblastoma or medulloblastoma in an intracranial xenograph mice model.^[26] With the evidence that GBMs can be initiated from CSCs, it is essential that the location of these cells to be determined in order to fully understand the involvement of CSCs in GBM and how to effectively target these specific cells. Unfortunately, the location of CSCs has yet to be fully elucidated. Whether glioblastoma CSCs reside in perivascular or hypoxic niches within the tumor is currently a topic of controversy. Calabrese *et al.* showed that CD133+/Nestin+ cells are located in perivascular niches and thus hypothesized that growth factors secreted by the endothelium are responsible for maintaining the population of CSCs.^[27,28] On the other hand, Heddlston *et al.* showed that a hypoxic microenvironment causes the upregulation

of hypoxia-inducible factor 1-alpha (HIF-1 α) and hypoxia-inducible factor 2-alpha (HIF-2 α). Both of these factors play a role in angiogenesis, but HIF-2 α has been shown to maintain the CSC phenotype and even cause the conversion of non-CSC to a CSC phenotype. The ability of HIF-2 α to initiate angiogenesis and maintain CSC populations may explain the conflicting data of GBM CSCs being located in perivascular niches, as it was the hypoxic environment that the CSCs resided that drove the angiogenesis from HIF-2 α .^[29,30]

As HIF factors are involved in angiogenesis and maintaining CSC populations, it is crucial to find a treatment that is able to downregulate HIF. One method of targeting the expression of HIF is to control the production of reactive oxygen species (ROS). During periods of hypoxia, ROS concentration rises intracellular, overcoming the glutathione levels that are able to reduce them to nontoxic substrates. The increased ROS are able to stabilize HIF, allowing for the transcription of VEGF and angiogenesis.^[31-34] Bevacizumab, a humanized monoclonal antibody that binds to and inhibits VEGF, combined with irinotecan, a topoisomerase I inhibitor, is currently approved for the treatment of recurrent GBMs and is designed to abolish VEGF-driven angiogenesis.^[35,36] Although targeting VEGF is useful in the treatment of GBM, a NP that could stop the stabilization of HIF could be promising. It has been shown that the pretreatment of intracerebral-glioblastoma-bearing mice with Tempol, an ROS scavenger, has synergistically suppressed tumor growth and increased survival rate with TMZ chemotherapy.^[37]

By using a nanoemulsion delivery system, chemotherapeutic agents can gain access to GBM tumor tissues by the EPR effect and have the ability to function as an ROS scavenger, reducing the concentration of ROS in the cytosol and decreasing the stabilization of HIF. A nanoemulsion delivery system of CPT with ROS scavenger abilities has been developed to target GBM tumor tissues. CPT, an inhibitor of DNA topoisomerase I, has poor water solubility and is, therefore, not a suitable candidate for intravenous (IV) injection. More water-soluble CPT derivatives, irinotecan and topotecan, have been developed to overcome this hydrophobic interaction but nanotechnology is able to use the hydrophobic nature of CPT as an advantage.^[38] A CPT prodrug was developed using a tetraethylene glycol (TEG) spacer linked to CPT and α -lipoic acid (ALA). The CPT-TEG-ALA prodrug molecule is enzymatically degraded by oxidation, acting as an ROS scavenger, to release CPT in its active form within the GBM. To create a stable NP, CPT-TEG-ALA is combined with α -tocopherol, vitamin E, an additional ROS scavenger, creating even more control of ROS production and preventing HIF production. By decreasing

HIF through ROS scavenging, GBM CSC populations and angiogenesis can potentially be significantly decreased, resulting in a less-aggressive GBM.^[39,40]

REDUCING TMZ AND RADIOTHERAPY RESISTANCE IN GBM BY NANOTECHNOLOGY

In the recent years, genetic marks CPT have not only offered more insight into the nature of GBMs but also served as an indicator for therapeutic response to chemotherapy and radiation after surgical resection. Isocitrate dehydrogenase 1 gene, (*IDH1*) has recently been shown to differentiate a primary GBM from a secondary GBM, developing from a low-grade glioma. The importance of the ability of *IDH1* to identify a secondary GBM is so significant that the median overall survival increase from 1.1 years in wild-type *IDH1* to 3.8 years with the presence of mutated *IDH1*.^[41,42]

With TMZ being the most effective chemotherapeutic agent in the treatment of GBMs, it is crucial to understand how the resistance to TMZ develops. O⁶-Alkylguanine-DNA alkyltransferase (MGMT) is a DNA repair gene that removes adducts formed at the O⁶-position of guanine or O⁴-position of thymine, the mechanism of action of TMZ.^[43] Methylation of CpG islands in the MGMT promoter region allows for silencing of the gene, stopping the production of the enzyme that is responsible for the tumor cell to repair DNA damage. MGMT has been shown to be an effective marker for the responsiveness to TMZ and other alkylating agents, showing an increase in survival and a slower progression to disease.^[44,45] Collectively, these two genes when combined have a better prediction of glioblastoma survival than *IDH1* or MGMT independently, with *IDH1*mt/MGMTmet having the longest survival rate and *IDH1*wt/MGMTunmet with the lowest survival rate.^[46]

CSCs have shown to elevate the levels of MGMT expression. The ability of CSCs to survive in hypoxic regions makes it virtually impossible for traditional chemotherapies to reach the cells that are crucial for tumor survival and progression.^[29,30,47,48] With increased drug delivery to GBM tissue because of the EPR effect, liposomal NPs have been shown to reduce TMZ resistance. When using PEG, the functional groups covalently attached to the liposome can be used to specifically target specific markers on GBM CSCs. Kim *et al.* is currently using a cationic liposome to associate an antibody to the transferrin receptor, termed sIC nanocomplex. While the transferrin receptor allows for the BBB crossing and entrance into CSCs, the liposome acts as a nanocarrier for chemotherapeutic agents, siRNA, and so on. At present, sIC-TMZ and sIC-p53 have been constructed and have shown promising results. The sIC-

TMZ nanocomplex is more efficient in killing GBM cancer cells than free TMZ. Interestingly, MGMT methylation is directly correlated with TMZ resistance and the sIC-p53 nanocomplex has been shown to downregulate MGMT expression.^[49,50]

IONPs are inorganic NPs that are being used to deliver therapeutic agents to tumor tissues in patients with GBM but the iron oxide core can be a useful imaging agent. IONPs are able to function as a contrast agent in MRI, particularly for T2-weighted images. In addition, the iron oxide core is biodegradable and can be reused/recycled by cells using normal biochemical pathways for iron metabolism.^[51]

Keivet *et al.* used an IONP that provides T2 contrast in MRI while also delivering siRNA against apurinic endonuclease 1 (Ape1), an enzyme that is crucial in base excision repair (BER) pathway. The NP consists of a super-paramagnetic iron oxide core coated with a copolymer of chitosan, PEG, and polyetheleneimine (PEI). With the aid of this NP, siRNA is able to avoid degradation and enter GBM tumor tissues, which results in successfully knock down of the expression of Ape1 and increased radiosensitivity in GBM cells and tumors.^[52]

IONPs are not limited to the delivery of siRNA but can be used to make chemotherapeutic agents have a prolonged half-life in the blood circulation and increase tumor targeting. Gemcitabine (GEM), a chemotherapeutic agent, causes DNA damage that cannot be repaired by MGMT, a DNA repair enzyme that is the cause of TMZ resistance in GBM.^[53] To deliver GEM, the nanocarrier of an IONP is immobilized by GEM, chlorotoxin (CTX), and hyaluronic acid.^[54] The CTX has been shown to target GBM tumor cells and also inhibit the infiltrative nature of GMB, which is the main reason why a complete surgical resection is impossible.^[55,56]

GEM is not the only chemotherapeutic agent that is being incorporated into IONPs. PTX and fluorescein has been loaded into a PEG-coated magnetic IONP conjugated with cyclodextrin and CTX (IONP-PTX-CTX-FL) and used to treat methylated and unmethylated MGMT GBM cell lines *in vitro*. The results showed that the IONP-PTX-CTX-FL NP could selectively target GBM cell lines and was effective in killing MGMT-resistant GBM tumor cells.^[57]

CONCLUSION

GBM is a highly aggressive glioma that largely remains a mystery to the scientific community. Even after the standards of care are delivered, the mean survival of patients with GBM is 15 months after initial diagnosis. It

is crucial that novel ideas are needed until breakthroughs are being made that can increase the life expectancy of patients with GBM.^[1,3,4]

Extensive research is ongoing and the aggressive nature of GBM is being understood. Weaknesses of GBM are also being uncovered. For instance, the rapid expansion of GBM tissue, as evidenced from the necrosis and microvascular proliferation, creates a “leaky” BBB that allows NPs to cross. Owing to limited and damaged lymphatic systems in the brain parenchyma, NPs are retained in the GBM tumor tissue, which is termed the EPR effect.^[10] The ability of nanotechnology to reach the brain parenchyma is a major advantage and gives promise to this field of research in GBM treatment.

The EPR effect is a passive uptake route and does not lead to complete deposit of NPs in GBM tissues. To limit the uptake into reticuloendothelial organs, such as liver, kidney, and spleen, CED is being used to allow increased uptake of NPs into GBM tumor tissues by injecting the particle under pressure into the brain parenchyma. CED does show promise into increasing the uptake of NPs into GBM tissues.

As the specificity of NPs to GBM tissue increases, the benefit of nanotechnology will become increasingly apparent. Nanotechnology is a very broad term that has particles in the form of liposomes, polymeric NPs, polymeric micelles, and nanoemulsion. Each of these NPs provides unique transporting mechanisms for chemotherapeutic agents and siRNA. In addition, IONPs have imaging capabilities that can be useful in delineating tumor tissue from normal parenchyma. The diverse nature of NPs and their abilities to cross the BBB make them essential in the future of GBM research and drug development.

Conflict of Interest

None declared.

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