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Magnetic nanoparticles: an emerging technology for malignant brain tumor imaging and therapy

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Abstract

Magnetic nanoparticles (MNPs) represent a promising nanomaterial for the targeted therapy and imaging of malignant brain tumors. Conjugation of peptides or antibodies to the surface of MNPs allows direct targeting of the tumor cell surface and potential disruption of active signaling pathways present in tumor cells. Delivery of nanoparticles to malignant brain tumors represents a formidable challenge due to the presence of the blood–brain barrier and infiltrating cancer cells in the normal brain. Newer strategies permit better delivery of MNPs systemically and by direct convection-enhanced delivery to the brain. Completion of a human clinical trial involving direct injection of MNPs into recurrent malignant brain tumors for thermotherapy has established their feasibility, safety and efficacy in patients. Future translational studies are in progress to understand the promising impact of MNPs in the treatment of malignant brain tumors.

Keywords

convection-enhanced delivery; EGFR; GBM; glioblastoma; iron oxide nanoparticles; magnetic nanoparticles; malignant brain tumors; MRI; thermotherapy

The emerging field of cancer nanotechnology has stirred interest in the use of nanomaterial platforms for malignant brain tumor therapy. Typically, nanoparticles (NPs) with at least one dimension less than 100 nm have been utilized preclinically and in human clinical trials [1,2]. With current engineering advancements, NPs with varying size, shape, composition and surface chemistry are being synthesized and investigated. The development of multifunctional nanoplatforms for brain tumor therapy that can provide the benefit of simultaneous imaging and therapy holds great promise in the diagnosis and treatment of malignant brain tumors. Some of the most commonly used types of NPs that are being explored in the treatment of malignant brain tumors include polymeric particles, micelles [3], nanoshells [4], quantum dots [5] and magnetic iron oxide NPs (IONPs) [6].

Magnetic NPs (MNPs) have garnered attention in the past two decades and are being explored for applications in tumor therapy and imaging [7]. Besides their use as MRI contrast agents, MNPs are being investigated for their utility in targeting tumor cells with therapeutic biomolecules, such as anticancer drugs, antibodies and siRNA [8–11]. The favorable toxicity profile of iron oxide-based MNPs, along with their ability to cross the

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blood–brain barrier (BBB), makes them an attractive nanoplatform for malignant brain tumor therapy. The unique magnetic properties of MNPs can also be exploited for generating local hyperthermia with safely applied magnetic fields for thermotherapy [12] and magnetic targeting of malignant brain tumors [13].

MNPs are most commonly comprised of a core-shell morphology with an iron oxide core (usually magnetite [Fe_3O_4] or maghemite [Fe_2O_3]), coated with a biocompatible material (e.g., polysaccharide, synthetic polymer, lipid, protein or small silane linker) (Figure 1) [14]. The applications of MNPs for imaging and therapy require a special surface coating that is nontoxic and biocompatible, which can provide targeted delivery with particle localization in a specific area [15]. A surface coating that is neutral or negative in charge may be optimal for targeting malignant brain tumors [16]. The final hydrodynamic size of MNPs plays an important role in the delivery of MNPs as larger NPs (>100 nm) may be difficult to target malignant brain tumors.

Multifunctional MNPs & malignant brain tumor challenges

The most difficult to treat malignant brain tumor is glioblastoma (GBM). GBM is the most common primary brain tumor in adults and is one of the most devastating cancers, and despite over four decades of technological advances in imaging, surgery, and adjuvant therapies such as radiation and chemotherapy, less than 5% of GBM patients are alive 5 years after diagnosis [17–20].

There are multiple reasons for the inability to properly treat GBM tumors. They are extremely aggressive in a locally invasive fashion [21]. Infiltrating tumor cells extend beyond the area of the main tumor mass and are difficult to detect by MRI and during surgery [22]. The goal of surgical resection is to remove the gadolinium contrast-enhancing portion of the tumor, given the potential morbidity of removing adjacent nonenhancing normal neural tissue [23,24]. However, all resections of GBMs leave behind noncontrast-enhancing infiltrative tumor cells that reside away from the primary tumor. These invasive tumor cells are responsible for recurrence and, ultimately, the demise of patients with GBM, despite radiation therapy and chemotherapy. Recently, a subpopulation of GBM cells, known as glioblastoma stem cells (GSCs), have been shown to be integral to tumor development and perpetuation [25–28]. GSCs have been shown to be more resistant to chemotherapy and radiation than the bulk of tumor cells [29,30]. GSCs are highly tumorigenic and become enriched in post-therapeutic recurrences following radiation in animal models, probably permitting recurrent tumor formation and accounting for failure of conventional therapies [29]. New therapeutic strategies need to be developed for improved long-term management of GBM, including GSCs. Since these tumors rarely metastasize away from the CNS and the majority recur in a local fashion, local tumor control must be achieved to significantly extend the survival of patients with GBM.

The Cancer Genome Atlas project has recently chronicled the most common genetic alterations in GBMs [31,32]. The EGF receptor (EGFR) represents the most common alteration present in GBM tumors and is a candidate for targeting of GBM cells for imaging and therapeutic purposes. EGFR amplification or mutation occurs in almost half of all GBMs (45%). The majority of GBM tumors (54%) overexpress the wild-type EGFR protein and 31% overexpress both the wild-type EGFR and the EGFRvIII mutant [33,34]. EGFR phosphorylation and activation results in a series of downstream signaling events that mediate GBM cellular proliferation, motility, adhesion, invasion and resistance to chemoradiation, as well as inhibition of apoptosis [35–40]. The EGFRvIII mutant is a constitutively active EGFR that does not require ligand activation and is present in 20–30% of GBMs.

Multifunctional MNPs that allow for targeted imaging and therapy concurrently hold great potential for GBM management. Strategies for local tumor control include use of EGFR antibody-conjugated MNPs for targeting brain cancer cells (Figures 2 & 3) and their overactive signaling pathway, and thermotherapy in combination with radiation therapy in preclinical and human studies. Some of the major applications of MNPs for malignant brain tumor management are described below.

MNPs for MRI contrast enhancement

MNPs have attracted clinical interest due to their unique magnetic properties that enable their detection by MRI [41,42]. In comparison to other nanomaterials, the accumulation and retention of MNPs in tumors can be directly imaged by MRI. Iron oxide-based MNP formulations are US FDA-approved, are in clinical use for abdominal imaging [43], and their applications in brain tumors continue to be explored. Iron oxide-based MNPs have shown great potential as T_1 or T_2 contrast agents in MRI imaging [44,45], with superparamagnetic iron oxide-based NPs as the most commonly investigated type of MRI contrast agents [46]. Ultrasmall superparamagnetic iron oxide-based NPs (USPIOs), which have a hydrodynamic diameter ranging from 10 to 50 nm, have been considered as a new type of MRI contrast agent for more than two decades [47]. USPIOs can be better visualized in T2-weighted MRI sequences (T2 contrast agents) as a hypointense (dark) signal (negative contrast enhancement). Alternatively, paramagnetic NPs are visible with T1-weighted MRI sequences (T1 contrast agents) as a hyperintense (bright) signal (positive contrast enhancement) [48–50].

Compared with the traditional gadolinium (Gd)-based MRI contrast agents, USPIOs are an important alternative for imaging of malignant brain tumors. USPIOs are slower to be eliminated from the circulation [51], compared with Gd-based contrast agents, which are rapidly cleared from the circulation via the kidneys [52,53]. USPIOs are also taken up by tumor cells as well as by reactive phagocytic cells (e.g., microglia) found in brain tumors. The USPIOs can reside within brain tumors much longer than Gd-based agents [54]. While Gd-based contrast agents need to be readministered, single systemic administration of USPIOs can be imaged from 24 to 72 h post-administration [55]. USPIOs have an enhanced capacity to highlight infiltrating tumor margins due to their uptake by phagocytic cells located at the tumor periphery [54]. Moreover, preliminary studies have revealed no major toxicity of USPIOs in patients with renal dysfunction, who are generally at increased risk for Gd-induced nephrogenic systemic fibrosis, primarily due to renal elimination of Gd-based agents [55–57].

In preliminary clinical trials with GBM patients, USPIOs have revealed more intense MRI contrast enhancement compared with Gd-based contrast agents [55]. MNPs also hold promise in detection of a therapeutic response in brain tumor patients after undergoing adjuvant treatments. USPIOs can provide more precise measurements of cerebral perfusion parameters such as cerebral blood flow, cerebral blood volume and mean transit time in brain tumor areas because of their ability to remain intravascular for longer periods of time than Gd. Assessment of these vascular parameters can lead to determination of a brain tumor response to therapy, radiation necrosis or tumor recurrence [54,57].

MNPs for optical delineation of brain tumors

MNPs also hold great promise in enabling intraoperative brain tumor delineation via optical imaging. Owing to the infiltrative nature of GBMs, achieving complete tumor resection is a major challenge. Modern imaging techniques, such as intraoperative MRI and neuro-navigation, have shown a positive influence in the extent of tumor resection and prolonged survival [58–60]. Recently, fluorescence-guided surgery after oral administration of 5-

aminolevunilic acid has resulted in more complete resection of malignant gliomas [17,61]. In an attempt to further improve intraoperative identification and resection of infiltrating brain tumors such as GBMs, optical imaging techniques using fluorescent and visible dyes are being investigated [62–64] and have shown improvement in the extent of tumor resection [65,66].

Fluorescent and optical dyes can be combined with MNPs to generate multimodal nanoplatforms for improved tumor delineation and resection. For example, iron oxide-based MNPs conjugated with the near-infrared fluorescing molecule, Cy5.5, have been developed and tested in preclinical rodent models [67–69]. This multimodal nanoprobe has the dual advantage of malignant brain tumor detection with both MRI and intraoperative optical devices for visualization of brain tumors preoperatively and intraoperatively.

MNPs for brain tumor-targeted imaging & therapy

Uptake of MNPs by malignant brain tumor cells has been demonstrated in culture and *in vivo* in the past (Figure 3) [70,71]. The influence of surface functionalization has recently been shown to enhance the internalization of MNPs in cancer cells [72]. Functional modifications of MNPs, involving surface binding of molecules specific for malignant brain tumors, has been increasingly used in order to more specifically target MNPs [42]. Targeted MNPs can be concentrated in tumors, providing sensitive tumor imaging as well as targeted therapy of tumors [73,74]. Antibodies, peptides (including toxins), cytokines and chemotherapeutic agents have been reported as possible MNP-targeting options [75]. We have recently utilized a GBM-specific antibody bioconjugated to iron oxide-based MNPs for the targeted imaging and therapy of GBM. Amphiphilic triblock copolymer IONPs were conjugated with a purified antibody that selectively binds to the EGFR deletion mutant, EGFRvIII, which is solely expressed by GBM tumors [1]. MRI contrast enhancement of EGFRvIII-expressing GBM cells occurred after treatment with the EGFRvIII-IONPs. Treatment of patient-derived GBM neurospheres containing GSCs with the EGFRvIII-IONPs resulted in tumor cell apoptosis. GBM cell treatment also resulted in disruption of EGFR cell signaling and decreased phosphorylation of the EGFR tyrosine kinase. A significant increase in overall animal survival resulted after local intratumoral convection-enhanced delivery (CED). Conjugation of MNPs with peptides that target receptors on the tumor cell surface can enable internalization of the NP–peptide conjugate via receptor-mediated endocytosis. Examples of two peptides for targeting NPs to GBM cells include chlorotoxin and F3. Chlorotoxin is derived from scorpion venom and specifically binds to matrix metalloproteinase-2, which is overexpressed on the surface of GBM cells and other cancer cells [76,77]. matrix metalloproteinase-2 accounts for degradation of the extracellular matrix during tumor invasion and therefore chlorotoxin results in inhibition of GBM cell invasion [78,79]. Chlorotoxin conjugated to MNPs can act as an MRI contrast agent as well as an intra operative optical dye [67,68,80]. F3 is a small peptide that specifically binds to nucleolin overexpressed on proliferating endothelial cells of tumor cells and the associated vasculature [81]. Multifunctional NPs conjugated with F3 peptides have been used to deliver encapsulated MRI contrast enhancers and photosensitizers to malignant brain tumors implanted in rats. These F3-coated IONPs can provide significant MRI contrast enhancement of intracranial rat-implanted tumors, compared with non-coated F3 NPs, when administered intravenously [82].

Conjugation with chemotherapeutic drugs is an alternative method that has been used for targeting of MNP-based MRI contrast agents to brain tumors. Polyethylene glycol-coated IONPs have been conjugated with the chemotherapeutic agent methotrexate for tumor targeting [83]. Such drug-loaded MNPs can result in targeted tumor therapy, as well as facilitating monitoring of the delivered drug load via MRI imaging [84]. These

multifunctional NPs have increased uptake by tumor cells, resulting in increased accumulation and cytotoxicity of tumor cells [85].

MNPs for stem cell tracking

The remarkable feature of MNPs to act as MRI contrast agents has also been used in tracking stem cell tropism to malignant brain tumors *in vivo*. It is documented that intracranially administered neural stem cells have tropism for GBM tumors [86]. This striking characteristic makes neural stem cells attractive for tumor-targeting gene therapy [87,88]. Mesenchymal stem cells have the potential to become an alternative of neural stem cells for GBM-targeting gene therapy [89]. Mesenchymal stem cells that have been labeled with IONPs and can be visualized using MRI enable the determination of stem-cell migration into the growing tumor [90,91]. Labeling of hematopoietic stem cells with IONPs has also been investigated. Migration of magnetically labeled hematopoietic stem cells to sites of active tumor angiogenesis can be tracked using MRI [92].

MNPs for hyperthermia

Hyperthermia is one of the most promising applications of MNPs and can be utilized for thermotherapy of tumors. Moderate temperature elevations in the range of 41–46°C can induce various effects both at the cellular and tissue levels. Cells undergo heat stress, resulting in protein denaturation, protein folding, aggregation and DNA cross-linking [93]. Other cellular effects of moderate hyperthermia include induction of apoptosis and heat-shock protein expression. At the tissue level, effects include changes in pH and perfusion and oxygenation of the tumor microenvironment [94–97]. The combination of adjuvant treatments such as radiation [98] and chemotherapy [99] with hyperthermia can provide an increased anti-tumor effect.

MNP-based hyperthermia involves selective distribution of NPs throughout the tumor with application of an alternating magnetic field (AMF) of appropriate field amplitude and frequency to heat up the particles. A predictable and sufficient amount of heat known as the specific absorption rate is produced. The MNPs utilize several different mechanisms to convert the magnetic energy into heat energy. Some of the major heat-generating mechanisms include: Néel and Brownian relaxations, and hysteresis loss. Heat generation through Néel relaxation is due to rapidly occurring changes in the direction of magnetic moments relative to crystal lattice. Brownian relaxation results from the physical rotation of MNPs within the medium in which they are placed. Both internal (Néel) and external (Brownian) sources of friction lead to a phase lag between the applied magnetic field and the direction of magnetic moment, which tends to produce thermal losses [100].

For thermotherapy of tumors, MNPs with high heating capacity (greater saturation magnetization, optimal anisotropy and larger size) are required [100–104]. MNPs made up of a number of different magnetic materials including iron oxide, and metal NPs including manganese, iron, cobalt, nickel, zinc, magnesium and their oxides have also been studied [105–115]. Some of the wellknown iron oxide-based hyper thermic agents include magnetites and maghemites. Magnetite is the material most often used in biomedical applications. Yet another category is based on ferrites, such as cobalt ferrites ($CoFe_2O_4$), manganese ferrites ($MnFe_2O_4$), nickel ferrites ($NiFe_2O_4$), lithium ferrites, mixed ferrites of nickel– zinc–copper and cobalt–nickel ferrites [109–117]. There are also ferromagnetic NPs that are iron based and have greater magnetic properties than IONPs [101]. These iron-based NPs produce greater hyperthermia effects at much lower concentrations than IONPs. FeNPs are comprised of an Fe core surrounded by an iron oxide layer to permit stability. Nevertheless, owing to their lack of toxicity, excellent biocompatibility and their capacity to

be metabolized [118–120], iron oxide-based MNPs are actively being studied for thermotherapy of brain tumors.

MNP-based hyperthermia has been evaluated for feasibility in animal models and in human patients with malignant brain tumors. Dextran- or aminosilane-coated IONPs have been used for thermotherapy in a rodent GBM model [12] and in a human clinical trial in patients with recurrent GBM [2,98]. Intratumoral injection of aminosilane-coated IONPs (core size 12 nm) and application of an AMF (100 kHz) in several sessions before and after adjuvant fractionated radiation therapy significantly improved the survival of patients with recurrent GBM. A high concentration of IONPs (>100 mg/ml) was required to achieve effective thermotherapy, with a median peak temperature within the tumor of 51.2°C. This Phase II clinical trial successfully demon strated safety and efficacy of thermotherapy of malignant brain tumors with MNPs in humans.

Postmortem studies have been performed with GBM patients treated with thermotherapy using MNPs [121]. In brain autopsies, the MNPs were dispersed or distributed as aggregates at sites of intracranial injection. The MNPs were phagocytosed by macrophages in the brain and were also found in GBM cells.

Malignant brain tumor delivery of MNPs

A major challenge for the use of MNPs in the targeted imaging and therapy of malignant brain tumors is effective delivery. Systemic delivery is limited by the BBB, nonspecific uptake of MNPs, nontargeted distribution and systemic toxicity. The deposition and nonspecific uptake of MNPs in the reticuloendothelial system (RES) in the liver, spleen and circulating macrophages after systemic intravenous administration can interfere with the delivery of NPs to brain tumors [122,123]. Nonspecific uptake of MNPs and the BBB can both interfere with the targeted delivery of NPs and jeopardize the goal of MR imaging and therapy of malignant brain tumors. Attachment of tumor-homing peptides to MNPs has recently been shown to permit better targeting of malignant brain tumors systemically in a rodent model. The concept of magnetic targeting of malignant brain tumors has also been demonstrated in preclinical rodent models [13,124] as a method to enhance the systemic delivery of IONPs to brain tumors. CED has been used to bypass the BBB and the RES and seems to be the most promising delivery approach for MNPs to brain tumors.

Systemic delivery—The use of MNPs for systemic intravenous delivery to brain tumors is being actively studied [125]. Malignant brain tumor vascular characteristics are different from those of the intact brain [126,127]. Structural abnormalities of tumor vascular architecture, such as open endothelial gaps and extensive erratic angiogenesis, account for leakiness and increased permeability of tumor vasculature [128,129]. The enhanced permeability retention (EPR) effect is used to describe the selective extravasation of macromolecules, into the tumor interstitium through the hyperpermeable tumor vasculature [130]. Attachment of specific tumor-homing peptides to MNPs can also be used to target the brain tumor vasculature for enhanced penetration of the NPs into the vascular and extravascular tumor tissue in a rodent brain tumor model [82,125].

Magnetic targeting—The magnetic properties of MNPs can be used for enhancing their delivery to tumors beyond the extent of the EPR effect. Magnetic responsiveness of the core of MNPs allows them to be guided by an external magnetic field. Interaction of locally administered MNPs with an applied external magnetic field can increase retention of MNPs at the tumor site [131,132]. The magnetic targeting approach has been described in a rodent GBM model [13,124]. MNPs intravenously injected under the application of an external magnetic field resulted in selective accumulation and retention at the brain tumor site by

MRI in comparison to control animals, which did not undergo magnetic targeting. In a recently published study, IONPs coated with a polycationic polyethyleneimine have been used for brain tumor magnetic targeting and intra-arterial drug delivery. The polyethyleneimine-modified IONPs showed high cellular uptake as well as selective magnetic targeting and capture in GBM tumors after intracarotid administration [133].

In an effort to enhance the delivery and deposition of MNPs into malignant brain tumors, combined use of magnetic targeting with focused ultrasound (FUS) has been explored. FUS with microbubble formation can result in increased permeability of the BBB [134]. FUS represents a noninvasive technique that can selectively disrupt the BBB and increase the EPR effect locally within the brain [135,136]. FUS and magnetic targeting have been used synergistically to enhance the delivery and the deposition of chemotherapy (epirubicin)-loaded MNPs into tumor-bearing animals. Epirubicin delivery and brain tumor accumulation were significantly enhanced by the combined FUS/magnetic targeting approach of epirubicin-MNPs [137]. Moreover, the deposition of epirubicin-MNPs through this combined technique can be monitored and quantified *in vivo* by MRI, due to the intrinsic magnetic properties of MNPs that enable them to be used as MRI contrast agents [138].

Convection-enhanced delivery—A novel approach for efficient drug delivery into brain tumors is CED. CED has been designed to infuse agents directly into the brain parenchyma, bypassing the BBB and avoiding nonspecific uptake [139]. CED involves continuous delivery of an agent with a specific infusion rate and volume through one or more infusion catheters that have been stereotactically placed directly within and around brain tumors. A pump is connected to each infusion catheter in order to ensure a positive pressure gradient during delivery. The positive pressure gradient during infusion establishes fluid convection, which supplements simple diffusion. Simple diffusion alone governs local intracerebral delivery achieved by stereotactically guided injections. The additive effect of convection to simple diffusion through CED enhances the distribution of small and large molecules into the brain and increases the locoregional concentration of the infused compound [140]. Agent surface properties (cationic charge), large hydrodynamic size (>100 nm), catheter positioning and high interstitial tumor pressures can compromise agent distribution [16,140–144].

CED of therapeutic agents has been used in multiple human GBM clinical trials to determine efficacy [145,146]. Imaging CED in the brain for agent distribution and tumor targeting is the single largest impediment to this delivery strategy. Visualizing the distribution of infused agents is necessary to ensure accurate delivery into target sites and provides feedback on catheter placement and control of agent delivery [147,148]. Ineffective drug distribution is one major criticism of CED, as it may compromise malignant brain tumor targeting and therapy [143].

Owing to the small size of MNPs, CED is an optimal method for targeting brain tumors (Figure 4). Penetration of MNPs through the extracellular matrix in the brain is possible due to the larger effective pore size (50 nm) [141,149]. CED of dextrancoated maghemite MNPs has recently been depicted by MRI in a normal rat brain model [150]. Direct imaging of MNPs by MRI can permit distribution studies of NPs in the brain after CED. This study has also shown that the distribution area of MNPs into the rat brain obtained by CED is significantly affected by surface coating of MNPs as well as infusate viscosity. CED of dextran-coated MNPs resulted in greater distribution volumes in the rodent brain in comparison to pristine MNPs. The use of biocompatible polymers, such as dextran, may reduce the interaction of MNPs with the extracellular matrix and permit greater distribution in the brain.

We have studied the CED of polymer-coated IONPs conjugated to an EGFRvIII antibody, which is overexpressed exclusively in a subset of GBM tumors [1]. In our study, we assessed the distribution of MNPs after CED into EGFRvIII-expressing human GBM xenografts implanted in rodents (Figure 5). MRI was performed in order to monitor the therapeutic targeting as well as the distribution of conjugated IONPs after CED. Distribution of the EGFRvIII antibody-conjugated IONPs within the GBM xenografts was initially observed and the dispersion of the NPs intratumorally and peritumorally in the brain occurred for several days after CED. In conclusion, our study demonstrates the feasibility of CED for effective delivery of MNPs within brain tumors as well the infiltrating cancer cells beyond the tumor mass responsible for tumor recurrence.

Expert commentary

GBM remains the most difficult to treat cancer due to its resistance to standard therapies in addition to its invasive growth into the normal brain. Current treatments involve surgical resection of the main tumor mass. In all cases, infiltrating cancer cells are left behind. Adjuvant chemoradiation is given to patients to attempt treatment of these residual tumor cells, which in the majority of patients is unsuccessful leading to tumor regrowth and ultimately causes their demise. Multiple different experimental agents attempt to target brain cancer cells at their surface (e.g., EGFR) and intra cellularly where different cell signaling pathways are active [17]. The genomic signature of GBM is now known and has provided important information on the biology of GBM, including different tumor subtypes that may exist [32]. The GSC hypothesis may account for the resistance of tumor cells to standard therapies and also explain the regrowth of tumors. Treatment strategies need to target both the bulk of GBM tumor cells and the subpopulations of GSCs that are known to exist [28].

Delivery of agents to malignant brain tumors poses a different challenge for the effective targeting of tumor cells. The existence of the BBB makes it difficult for most therapeutic agents to reach the brain, let alone the brain tumor mass. Furthermore, confirmation of access of therapeutic agents to brain tumors by imaging is lacking and confounds the ability to detect efficacy of therapeutic agents.

Multifunctional MNPs are a promising nanoplatform for the imaging and treatment of malignant brain tumors. As discussed, their inherent ability to be directly imaged provides direct evidence of their delivery to malignant brain tumors. The uptake of MNPs by tumor cells and phagocytic cells permits MRI contrast enhancement of malignant brain tumors after systemic delivery. Prolonged circulation times of MNPs after a single administration may provide better MRI contrast of malignant brain tumors than currently used Gd-based contrast agents. Better assessment of cerebral perfusion parameters can lead to determination of a brain tumor response to therapy, radiation necrosis or tumor recurrence [54,57].

Conjugation of GBM-specific antibodies or peptides [68,82] to MNPs can selectively target tumor cells or the tumor vasculature [125] and promote an anti-tumor effect by apoptosis [1]. Targeting of patient-derived GSCs has also been shown *in vitro* with antibody-conjugated MNPs [1]. Understanding the intra cellular effects of MNPs after the targeting of the tumor cell surface (e.g., EGFR) and downstream intracellular signaling pathways remains important in describing the actual mechanisms of tumor cell death with MNPs.

Hyperthermia generation by application of an AMF to MNPs may be the most exciting and promising functionality of MNPs. The ability to heat up MNPs by application of an AMF that is safe to normal cells provides the basis for a new treatment strategy in the fight against malignant brain tumors such as GBM. Patients can undergo stereotactic injection of MNPs within their brain tumor and receive multiple sessions of AMF treatments since the MNPs

can stay in the brain for weeks after implantation (Figure 6). A recent Phase II human clinical trial completed in Germany serves as an important step in understanding the safety and efficacy of thermotherapy of malignant brain tumors [2]. This Phase II study relied on a high dose (>100 mg/ml) of aminosilane-coated iron oxide-based MNPs for thermotherapy of recurrent GBM tumors in patients. These patients could not undergo any MRI scanning after intracranial MNP implantation and had to have CT scans in follow-up due to the imaging artifact and potential safety issues associated with the large amount of MNPs used. Future work is needed to utilize MNPs of small size with greater specific absorption rate values that can be implanted at lower concentrations so that patients can undergo follow-up imaging by MRI to determine accurate thermo therapy treatment efficacy and distribution of the MNPs in the brain.

Systemic administration of MNPs remains an attractive method to deliver MNPs to malignant brain tumors for therapeutic purposes. Multiple treatments can be administered without the need for surgery, which is associated with direct intracerebral delivery. The RES and BBB remain formidable challenges to MNP access to malignant brain tumors after systemic administration. Recent work has shown that MNPs targeted to GBM cells and the tumor vaculature by conjugation of specific peptides can have a significant anti-tumor effect [125]. Iron oxide-based MNPs conjugated to a tumor-homing peptide and a proapoptotic peptide have been shown to delay tumor development in treatment-resistant tumors implanted in rodents after multiple systemic administrations.

Despite limited success with systemic delivery of MNPs, the ability to concentrate MNPs in brain tumors remains a challenge that has not been overcome. CED provides a MNP delivery method that can avoid nonspecific uptake of NPs, bypass the BBB, minimize systemic toxicity and provide for the effective targeted therapy of infiltrating malignant glioma cells with MRI guidance. The use of bioconjugated MNPs may permit the advancement of CED due to their hydrodynamic size, surface properties, sensitive imaging qualities on standard MRI (T_2-weighted imaging) and their anti-tumor effects.

Five-year view

Within the next 5 years, the incorporation of various nanotechnology platforms will probably advance the management of malignant brain tumors. The multifunctional clinical nature of nanotechnology will provide for the targeting, imaging and therapy of infiltrating brain tumor cells associated with the most difficult-to-treat malignant brain tumor known as GBM. Brain cancer stem cells, which escape surgical treatment and are responsible for tumor recurrence and therapy resistance, will also be targeted. MNPs will be used to disrupt brain cancer cell pathways that are overactive and contribute to the resistance of malignant brain tumors to various therapies. Surface-coated MNPs conjugated to tumor-homing peptides or brain tumor-specific anti bodies will be delivered systemically or locally to brain tumors by CED for therapeutic targeting of malignant brain tumors. The ability to image MNPs by MRI will provide precise information regarding therapeutic agent delivery and brain tumor targeting, as well as the ability for therapeutic follow-up monitoring. Surgical resection will still be required to remove the main malignant brain tumor mass and alleviate the mass effect on the surrounding brain. Intraoperative visualization of MNPs within malignant brain tumors and at the brain tumor margin will be possible with fluorophore conjugation to MNPs and the use of fluorescent-guided brain tumor resection. Local hyperthermia treatment of malignant brain tumors will also be possible with the same NPs with the use of AMFs that are safe for normal, noncancerous cells. The persistence of MNPs within malignant brain tumors after their delivery will permit the use of multiple AMF sessions in patients. MNP targeting of malignant brain tumors will be used in combination

with standard radiation and chemotherapies for enhancing the overall survival of patients and clinical benefit.

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Key issues

- Brain cancer known as glioblastoma (GBM) remains the most difficult to treat malignant brain tumor due to its resistance to standard therapies and its invasive growth into the normal brain.
- Treatment strategies need to target both the bulk of GBM tumor cells and the subpopulation of GBM stem cells that are known to exist and are responsible for tumor resistance to standard therapies and tumor recurrence.
- Delivery of agents to malignant brain tumors poses a significant challenge for the effective targeting of tumor cells due to the existence of the blood–brain barrier and nonspecific uptake in the body.
- Multifunctional magnetic nanoparticles (MNPs) are a promising nanoplatform for the imaging and treatment of malignant brain tumors.
- The promising biomedical applications of MNPs for the imaging and treatment of malignant brain tumors revolve around their unique magnetic properties, biocompatibility and uptake by cancer cells.
- The uptake of MNPs by tumor cells and phagocytic cells permit MRI contrast enhancement of malignant brain tumors after systemic delivery.
- The ability to conjugate tumor-specific antibodies and peptides to MNPs enhance their ability to target brain cancer cells and brain cancer stem cells while sparing normal surrounding cells in the brain.
- The ability to heat up MNPs by application of an alternating magnetic field that is safe to normal cells provides the basis for thermotherapy of malignant brain tumors.
- The magnetic responsiveness of the core of MNPs allows them to be guided by an external magnetic field for magnetic targeting of malignant brain tumors.
- Convection-enhanced delivery provides a MNP delivery method that can avoid nonspecific uptake of nanoparticles, bypass the blood–brain barrier, minimize systemic toxicity and provide for the effective targeted therapy of infiltrating malignant glioma cells with MRI guidance.

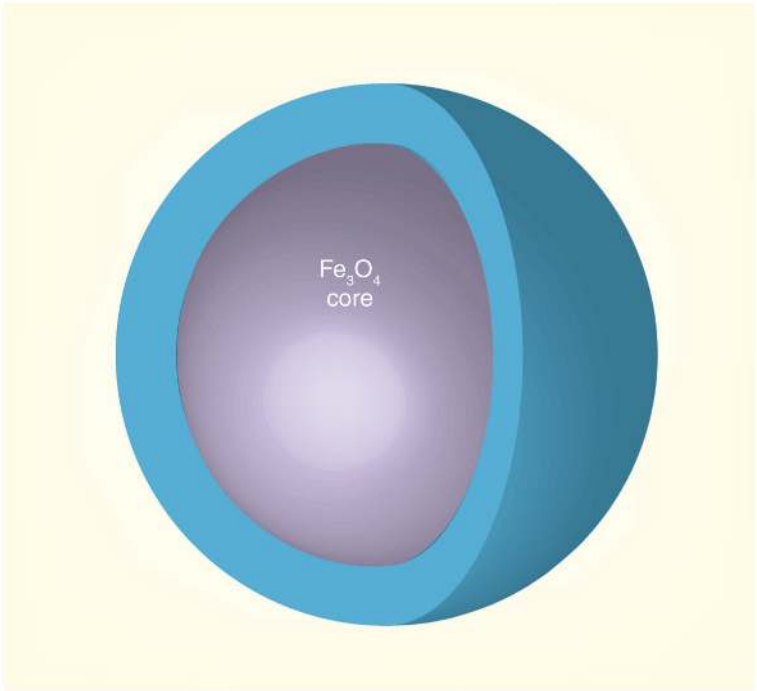

Figure 1. Illustration of a magnetic iron oxide nanoparticle

A typical magnetic nanoparticle is depicted by a core-shell morphology with an iron oxide core (usually magnetite Fe_3O_4) coated with a biocompatible material (e.g., polysaccharide, synthetic polymer, lipid, protein or small silane linker). The coating can be easily modified to make the particles tumor specific while also imparting stability in physiologic settings.

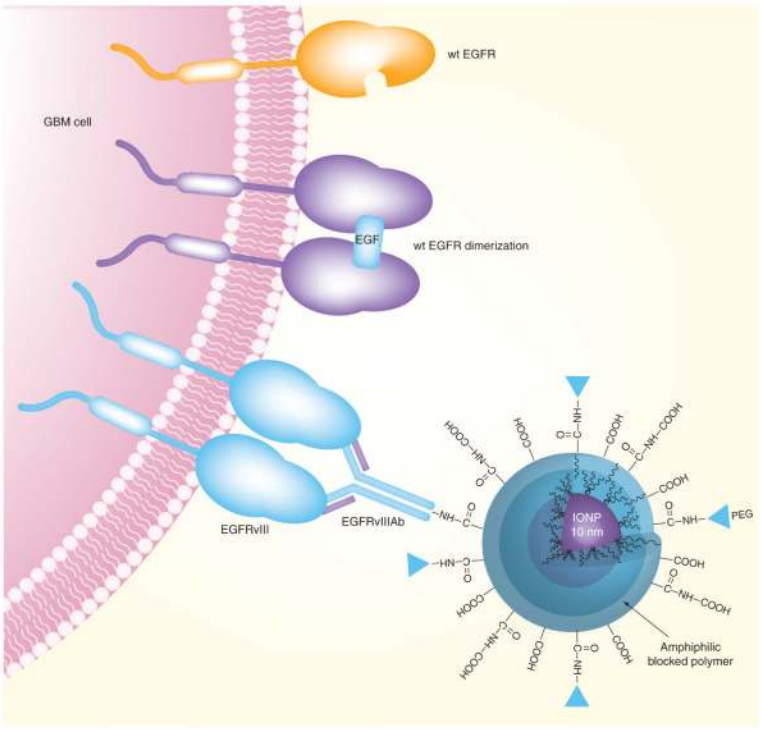

Figure 2. Illustration of an EGF receptor vIII-expressing glioblastoma cell bound by an EGF receptor vIII antibody-conjugated magnetic nanoparticle construct

The wt EGFR dimerizes upon ligand binding. The truncated EGFRvIII deletion mutant, which does not require a ligand for activation, is bound by an EGFRvIII antibody-conjugated magnetic nanoparticle conjugate (EGFRvIIIAb-iron oxide nanoparticle). The EGFRvIIIAb-iron oxide nanoparticle is comprised of a 10-nm iron oxide core surrounded by an amphiphilic triblock copolymer, which is covalently conjugated to the EGFRvIIIAb.
Ab: Antibody; EGFR: EGF receptor; GBM: Glioblastoma; IONP: Iron oxide nanoparticle; wt: Wild-type.

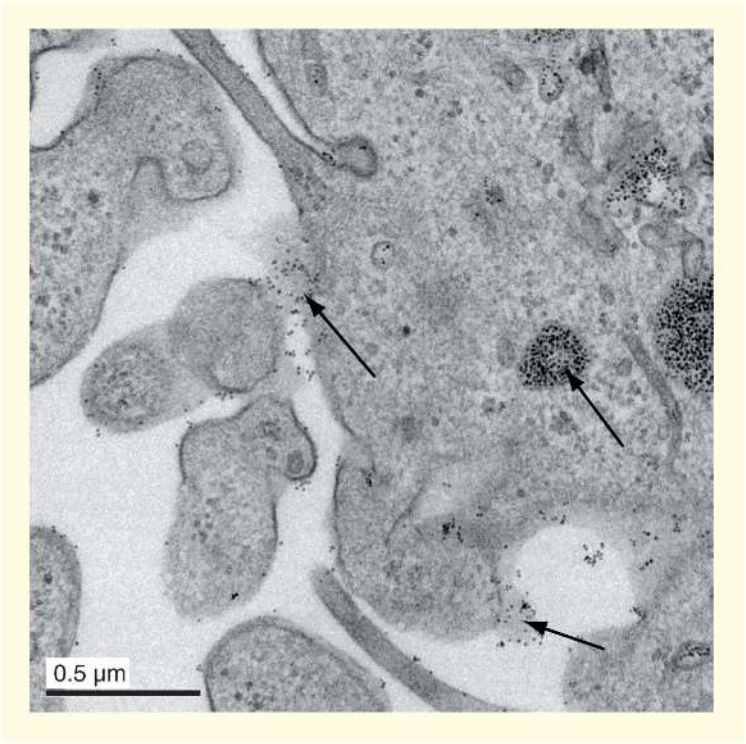

Figure 3. Transmission electron microscopy of an EGF receptor vIII-expressing glioblastoma cell bound by magnetic nanoparticles

Transmission electron microscopy confirms glioblastoma cell binding and internalization of the magnetic nanoparticles (shown by black arrows; magnification 10,000×).
Reproduced from [1].

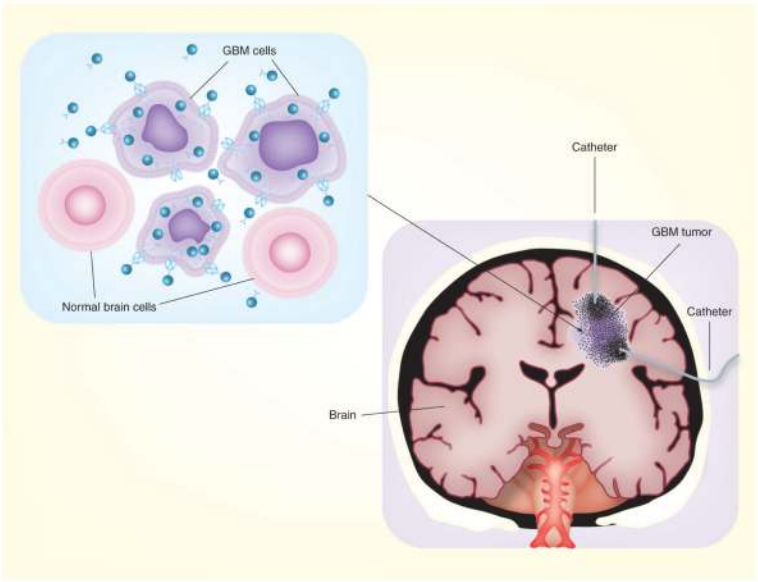

Figure 4. Targeting of glioblastoma tumor and infiltrating cancer cells by magnetic nanoparticle contrast-enhanced delivery

Coronal depiction of the brain with placement of two intratumoral catheters and magnetic nanoparticle convection-enhanced delivery. The glioblastoma tumor and its infiltrating margins are shown. Intra- and peri-tumoral magnetic nanoparticle distribution is shown after convection-enhanced delivery. A magnified view of EGF receptor-expressing GBM cells adjacent to normal brain cells is shown at the tumor margin. Targeting of the EGF receptor-expressing GBM cells with antibody-conjugated magnetic nanoparticles is shown.
GBM: Glioblastoma.

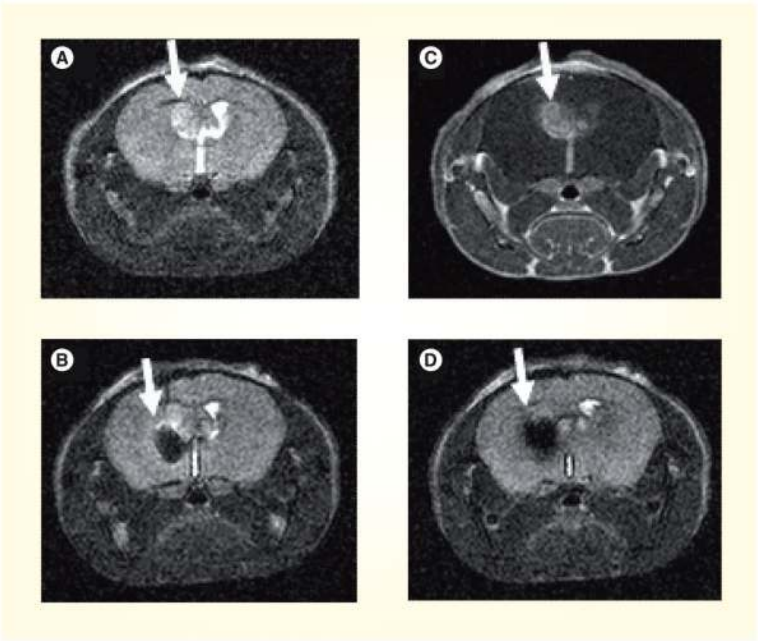

Figure 5. Contrast-enhanced delivery of EGFRvIIIAb-iron oxide nanoparticles in a mouse glioblastoma model

(A) T_2 -weighted MRI showing a tumor xenograft with bright signal 7 days post-tumor implantation (arrow); **(B)** Tumor shown (arrow) by contrast enhancement after injection of the gadolinium contrast agent (Gd-DTPA); **(C)** MRI signal drop (arrow) after convection-enhanced delivery of EGFRvIIIAb-iron oxide nanoparticles; **(D)** EGFRvIIIAb-iron oxide nanoparticle dispersion and T_2 signal drop (arrow) on MRI 4 days after convection-enhanced delivery.

Reproduced from [1].

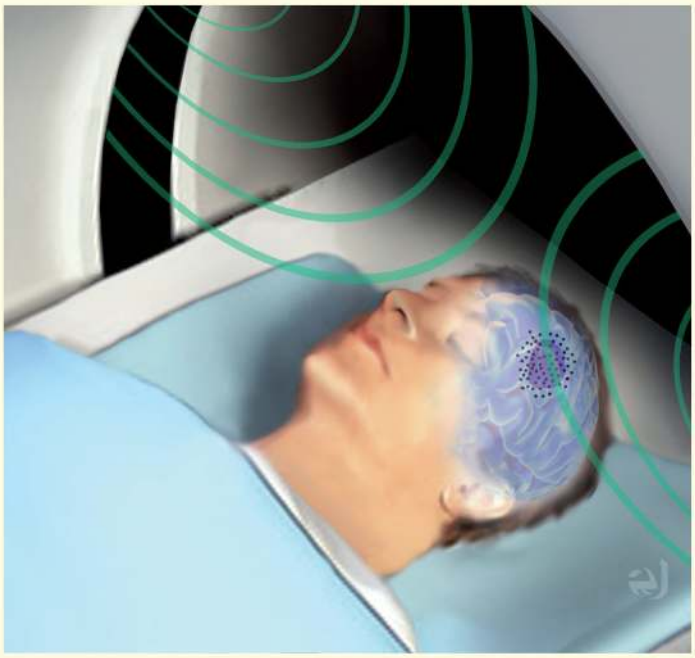

Figure 6. Intratumoral thermotherapy of a malignant brain tumor with magnetic nanoparticles

A patient who has undergone intratumoral implantation of magnetic nanoparticles is depicted undergoing an alternating magnetic field session for treatment of his malignant brain tumor by thermotherapy.