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Effective treatment of glioblastoma requires crossing the blood–brain barrier and targeting tumors including cancer stem cells: The promise of nanomedicine

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Abstract

Glioblastoma multiforme (GBM) is the most aggressive and lethal type of brain tumor. Both therapeutic resistance and restricted permeation of drugs across the blood–brain barrier (BBB) play a major role in the poor prognosis of GBM patients. Accumulated evidence suggests that in many human cancers, including GBM, therapeutic resistance can be attributed to a small fraction of cancer cells known as cancer stem cells (CSCs). CSCs have been shown to have stem cell-like properties that enable them to evade traditional cytotoxic therapies, and so new CSC-directed anti-cancer therapies are needed. Nanoparticles have been designed to selectively deliver payloads to relevant target cells in the body, and there is considerable interest in the use of nanoparticles for CSC-directed anti-cancer therapies. Recent advances in the field of nanomedicine offer new possibilities for overcoming CSC-mediated therapeutic resistance and thus significantly improving management of GBM. In this review, we will examine the current nanomedicine approaches for targeting CSCs and their therapeutic implications. The inhibitory effect of various nanoparticle-based drug delivery system towards CSCs in GBM tumors is the primary focus of this review.

Keywords

Targeted nanomedicine; Glioblastoma multiforme; Cancer stem cells; Blood–brain barrier; Therapeutic resistance; Chemosensitization

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Conflict of interest

Drs. Chang, Pirollo, and Kim are three of the inventors of the described technology, for which several patents owned by Georgetown University have been issued. The patents have been licensed to SynerGene Therapeutics, Inc. for commercial development. Dr. Chang owns equity interests in SynerGene Therapeutics, Inc. and serves as a non-paid scientific consultant to SynerGene Therapeutics, Inc. Dr. Harford serves as salaried President & CEO of SynerGene Therapeutics and own stock in same.

Transparency document

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1. Introduction

High-grade malignant glioma, glioblastoma multiforme (GBM), is the most aggressive and lethal form of brain tumor characterized by extensive infiltration into the surrounding brain parenchyma [1]. High rates of recurrence, overall resistance to therapy, devastating neurological deterioration, and dismal survival rates make GBM one of the most dreaded cancers [2]. Under the standard treatment regimen, which includes resection followed by radiotherapy and chemotherapy [typically temozolomide (TMZ)], GBM patients can expect a median survival of 14.6 months. Less than 5% of patients live longer than 5 years [3]. This poor prognosis is due primarily to: A) the highly aggressive and infiltrative nature of GBM tumors resulting in incomplete resection; B) limited delivery of therapeutics across the blood–brain barrier (BBB); and C) therapeutic resistance which results in tumor recurrence [4]. Concurrent treatment with the alkylating agent TMZ and radiation has demonstrated only limited efficacy in newly diagnosed GBM patients with an extension of median survival by just 2.5 months when compared to radiation therapy alone [5]. Furthermore, even in patients with strong initial responses, the majority of these patients later develop recurrences wherein TMZ treatment is largely ineffective [6]. Thus, there is a critical need for means to overcome this drug resistance and improve the efficacy of current GBM therapies.

2. Chemoresistance of GBM CSCs

The cancer stem cell (CSC) hypothesis suggests that tumors are initiated from, and maintained by, a small fraction of tumor cells that have stem cell-like properties including expression of stem cell markers, long term self-renewal, and the ability to reproduce the original parent tumor when grown as *in vivo* xenografts [7]. Stem cell-like properties afford resistance to cytotoxic therapies and allow CSCs to continue to differentiate into rapidly proliferating progenitor-like and more differentiated tumor cells (non-CSCs). This cellular hierarchy has been identified in GBM tumors as well as many human cancers including leukemia, breast, colon, lung, and prostate [8].

It has been suggested that CSCs might be the postulated mediator of therapeutic resistance because their cellular properties give them the ability to be refractory to current treatment strategies [9,10]. In GBM tumors, CSCs tend to be more resistant to radiotherapy and chemotherapy than non-CSCs [11,12]. Recent studies have shown CSCs to have intrinsic resistance to chemotherapy [10,13]. For example, the increased transcription of anti-apoptotic genes and efflux transporters was reported in CD133-positive GBM CSCs [14,15]. They also possess increased abilities to repair DNA damage and to promote angiogenesis [16,17]. Therefore, although anti-cancer therapies could effectively debulk the tumor mass, surviving CSCs can reinitiate tumor formation [18]. Such recurrences are often metastatic and more resistant to therapies. Unfortunately, the majority of currently existing cancer therapies – including hormonal, radiation, and chemotherapy – may not efficiently eliminate CSCs. Thus, given their critical role in tumor initiation, maintenance, and recurrence, a great deal of effort is currently focused on the therapeutic targeting of CSCs as a new strategy in drug design for cancer treatment and the prevention of recurrence [10–12,19]. However, a

formidable challenge in CSC-specific therapies involves the development of effective means for specifically delivering the therapeutics to the CSCs.

3. Emerging role of nanomedicine in anti-cancer therapies

Owing to rapid advances in protein engineering and materials sciences, considerable progress has been made recently in the field of nanomedicine to develop novel nanoscale strategies for cancer diagnosis and treatment [20]. Many different types of delivery systems have been developed including liposome, polymers, and inorganic nanoparticles (NPs). Approximately 150 drugs are in development for cancer treatment based on nanotechnology [21]. Some of them are currently undergoing clinical trials and over 20 therapeutic nanomedicines have been approved for clinical use [22]. Compared to conventional drug formulations, carefully designed nano-formulations can offer significant advantages such as increased drug solubility, extended retention time and stability in the body, selective targeting, and reduced side effects while delivering treatments that are more potent [21]. Another advantage is facilitation of drug delivery across biological barriers, such as BBB which limits access to brain tumors [4]. Additionally, NPs can also facilitate a combination of diagnostics with therapeutics (theranostics) for cancer [23]. Various nanomedicine strategies are being applied to enhance the therapeutic response in drug-resistant tumors including strategies for anti-CSC therapy by directly targeting them for elimination.

4. Targeted drug delivery using nanoparticles: passive vs active

Delivering effective quantities of drug into the right target cells through clinically feasible methods represents a major challenge for the successful development of cancer nanomedicines. Various biological barriers (such as mucosal and BBB) significantly hamper *in vivo* delivery of drugs to the tumor site [24]. To reach tumor by systemic administration, NPs have to travel through the blood stream, extravasate through the vessels, diffuse through the extracellular matrix, penetrate the tumor cell membranes, and be released into the cytoplasm. Thus, a series of biological barriers stands between systemically administered NPs and their target site inside the cells [25]. Nanodelivery platforms designed to overcome such barriers and can be divided into two classes.

4.1. Passive targeting

As a result of rapid and defective angiogenesis, blood vessels in tumors may have a leaky endothelium failing in its normal barrier function thus allowing entry of macromolecules (up to 400 nm in size) [26]. When administered intravenously, NPs passively extravasate into tumor tissue through the leaky vasculature, accumulate in the tumor bed due to dysfunctional lymphatic drainage, and release therapeutic payloads into the vicinity of tumor cells. This process is known as the enhanced permeability and retention (EPR) effect [27]. All nanomedicines that are currently approved for clinical use in the treatment of solid tumors rely on the EPR effect [20]. However, the passive targeting strategies suffer from several limitations because the EPR effect depends entirely on the diffusion of drugs, and it is difficult to control diffusion and some drugs cannot diffuse efficiently. Moreover, the permeability of tumor vessels may not be the same throughout a single tumor, and certain tumors may not even exhibit the EPR effect at all [28]. In the case of brain tumors, the EPR

effect is very unlikely to be efficient due to a dense brain matrix impeding diffusion and the elevated interstitial fluid pressure [1].

4.2. Active targeting

To overcome the above limitations, active targeting strategies have incorporated onto NPs affinity molecules such as antibodies, peptides, or aptamers that bind to antigens or receptors on the target cells to enhance the therapeutic efficacy by increasing cellular uptake and accumulation [20,29]. Generally, actively targeted NPs comprise a targeting moiety and a cargo-carrying moiety (Fig. 1). Payloads can be packaged inside the cargo moiety, and the surface of these NPs can be engineered to incorporate the targeting moiety. After binding of the NPs to certain receptors, the NPs can be internalized *via* receptor-mediated endocytosis thereby increasing cellular uptake of their payload. Some receptors rapidly recycle to the cell surface to allow repeated cycles of uptake. Another important consideration for targeted NPs is to engineer the targeting moiety to enable payload ‘piggybacking’ without disrupting the normal receptor-binding properties [25]. Recent identification of putative markers of CSCs has led to a quest for methods to deliver therapeutic agents to CSCs in various types of cancer using these markers. While the relevance of putative CSC surface markers remains controversial [11,30], a few studies have reported targeting of CSCs based on the distinct stem cell surface antigens [2,31–33].

5. Crossing the BBB: a challenge for GBM therapy

The BBB is a highly selective diffusion barrier that protects the brain from toxins and other compounds from blood [4]. However, a consequence of this protection is that entry of therapeutic molecules from blood to brain is also impeded [34]. To address this issue, conventional approaches utilized injection of therapeutics directly into the brain through stereotactic surgery or employed passive targeting based on the EPR effect. In GBM, a disruption of BBB by the primary tumor sites is often observed [35]. This altered BBB allows passive accumulation of chemotherapeutic drugs in the vicinity of the disruption [36]. However, the degree of BBB disruption varies depending on the region of the GBM tumors and may be negligible for certain tumors. Because BBB is intact near the growing edge of the infiltrative tumor area where the invasive tumor cells may reside [37], the infiltrating tumor cells cannot be reached efficiently by passive targeting [38,39]. Because isolated CSCs have been identified in the infiltrative peritumoral parenchyma of GBM [40], it is necessary to have a more effective means to transport therapeutic molecules across the BBB.

Strategies have been proposed for CNS drug delivery involving mechanical or chemical disruptions of the BBB by MRI-guided focused ultrasound [41], convection enhanced diffusion [42], microdialysis catheter [43], hyperosmotic agents [44], hydrophilic surfactants such as polysorbate 80 [45,46], or chemical modulators of blood vessels [47]. Clearly, a general disruption of the BBB to allow therapeutic agents to enter the brain would compromise the normal protective role of the BBB. Moreover, these approaches to disrupt the BBB have resulted in minimal therapeutic improvements generally speaking since the drugs and/or particles would still need to penetrate the brain parenchyma and effectively reach their target cell populations [4].

Nanomedicines relying on receptor-mediated transcytosis might facilitate more efficient entry of therapeutic molecules across the BBB with deeper tumor penetration [48]. Various receptor and/or transporter systems already exist on the BBB including glucose transporters, insulin receptors, and transferrin receptors (TfR) normally used for iron delivery to the brain *via* its ligand, diferric transferrin. Antibodies or ligands that bind to TfR [6,48], glutathione receptor [49], and insulin receptor [50] have been reported to facilitate transcytosis of NPs *via* binding to these receptors on endothelial cells [51]. In a study using dendrimer NPs conjugated with transferrin (Tf), increased accumulation of tamoxifen in glioma cells were observed in an *in vitro* BBB model [52]. In our previous study, we demonstrated that the systemically administered TfR-binding NPs (designated as scL) are able to cross the BBB *via* transcytosis and efficiently deliver the payloads to intracranial tumors (Fig. 2) [48]. In our NPs, an anti-TfR single-chain antibody fragment (TfRscFv) was used as a targeting moiety. After binding to TfR on cerebral endothelial cells, scL actively traverses the BBB *via* receptor-mediated transcytosis. The TfRscFv has advantages in human use over the Tf molecule itself or a full-length monoclonal antibody (MAb) against TfR, namely: A) the small size (~28 kDa) of the TfRscFv can maintain the nanosize of the complex; B) the recombinant nature of TfRscFv has practical advantages for large-scale production, which will ultimately be required for commercialization or the proposed therapy; C) its recombinant nature (as opposed to a blood product like Tf) also presents no issues related to potential contamination with blood borne pathogens; and D) because TfRscFv lacks the Fc region of the MAb, non-antigen-specific binding through Fc receptors is eliminated.

6. Anti-CSC nanomedicine in GBM

Although there are numerous studies reporting the potential of nanomedicines in GBM therapy, only a few address the targeting of CSCs. One example of a CSC-directed therapy involves cationic polyurethane-short branch polyethylenimine (PU-PEI) NPs that delivers plasmid DNA encoding a tumor-suppressive microRNA145 (miR145) [53]. This study showed radiosensitization and chemosensitization of CSC-derived brain tumors and prolongation of animal survival after local intracranial injection of PU-PEI-miR145 NPs. Although this NP lacks a CSC-targeting moiety, reduction of CSC-like properties was observed because miR145 down-modulated the expression of Oct4 and Sox2 genes related stemness. However, the absence of active CSC-targeting and the requirement for intracranial administration will likely be hurdles for this delivery system in translation to the clinic.

A nanomedicine has been described employing a CD133 MAb recognizing the CSC surface marker CD133 as a targeting moiety [2]. In this proof of principal study, photothermal therapy using single-walled carbon nanotubes (SWNTs) conjugated with anti-CD133 MABs (CDSWNTs) demonstrated a selective lysis of CD133-positive GBM CSCs, while CD133-negative GBM cells remained intact *in vitro*. In the same study, inhibition of tumor growth was also observed after near-infrared laser irradiation of subcutaneous xenograft tumors derived from CDSWNTs-laden, CD133-positive GBM CSCs. This study demonstrated the concept of targeted elimination of CSCs using a CSC-targeting nanomedicine. It is noteworthy that this particular study used an ectopic GBM tumor model and did not assess systemic delivery of the nanomedicine.

It is plausible that the depletion of CSCs may not be sufficient to eliminate tumors if the remaining differentiated tumor cells are still capable of sustaining growth in a tumor mass [8,54]. Furthermore, recent studies have shown that GBM non-CSCs may have the plasticity to dedifferentiate into GBM CSCs in response to microenvironment stresses such as hypoxia or radiation [55,56]. Therefore, a more effective therapeutic approach would be to target both CSC and non-CSC populations using a common target [e.g., TfR or epidermal growth factor receptor (EGFR)].

Being associated with tumorigenesis and aggressive phenotypes, both wild-type EGFR and the EGFRvIII deletion mutant have been major targets for GBM therapy. However, clinical trials of EGFR- and EGFRvIII-targeted therapies yielded unsatisfactory results [57]. EGFR is overexpressed in the majority of GBM tumors including some of the CSC populations. In a recent study, a CSC-targeted therapy was tested that utilizes cetuximab that binds both EGFR and the EGFRvIII deletion mutant. Cetuximab was conjugated to iron-oxide nanoparticles (IONPs), and cetuximab-IONPs were infused by intratumoral convection-enhanced delivery in an orthotopic rodent GBM model [58]. Extended survival was observed in animals treated with cetuximab-IONPs compared with cetuximab-treated animals, demonstrating *in vivo* efficacy of the anti-EGF nanomedicine in GBM tumors. However, the fate of CSCs and the molecular basis for the observed therapeutic benefits are still unclear in this study.

Another approach has used either the ligand [diferric transferrin (Tf)] or antibodies against TfR, since various types of cancer cells display elevated TfR expression [59]. Once ligand (or antibody) is bound to the TfR, the receptor-ligand (or receptor-antibody) complex is internalized *via* receptor-mediated endocytosis. Importantly, we have observed that TfRs are overexpressed on both CSCs and non-CSCs in various types of cancer including GBM [8]. Thus, the TfR is an attractive target for both anti-CSC and anti-cancer therapy in GBM more broadly.

One example is Tf-conjugated lipopolyplex NPs (Tf-NPs). *In vitro* treatment of CSC-enriched GBM tumor-spheres with Tf-NPs delivering miR1 resulted in significant inhibition of cell migration and expressions of EGFR and MET [60]. However, systemic treatment with Tf-NP-miR1 was not tested to assess *in vivo* targeting of GBM CSCs.

Employing an anti-TfR single-chain antibody fragment as a targeting moiety, we have tested whether TfR-binding scL NPs can target both tumor cells and CSCs. *In vitro* and *in vivo* targeting studies demonstrated that scL NPs can cross the BBB *via* transcytosis and efficiently deliver payloads into both CSC and non-CSC populations in GBM (Fig. 2) [8,48]. This scL nanodelivery platform is currently in multiple clinical trials. A systemically administered tumor- and CSC-targeted nanocomplex (in a product called SGT-53) is capable of delivering the exogenous wild-type tumor suppressor gene p53 across the BBB and results in sensitization of highly TMZ-resistant GBM tumors to TMZ and improved survival in a rodent model [48]. Furthermore, SGT-53 treatment in combination with TMZ resulted in extensive apoptosis of CD133-positive CSCs [48]. In the follow up study, we also demonstrated that SGT-53 could inhibit development of TMZ resistance in TMZ-sensitive GBM [6]. These results suggest that combining CSC-targeting SGT-53 with conventional

TMZ treatment could limit the development of, and overcome TMZ resistance, thereby prolonging TMZ's anti-tumor effect to yield what could be a much more effective therapy for GBM. Continuing advances in the therapeutic delivery methods raise hope that new innovative nanomedicines might eventually provide effective novel therapies against GBM. SGT-53 is now being tested in a clinical trial for recurrent glioblastoma in combination with TMZ (Clinical-Trials.gov Identifier: NCT02340156).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Schematic representation of targeted scL nanocomplex. scL is comprised of a targeting and cargo moiety. Various payloads can be packaged inside a cargo moiety made of cationic liposome shell. TfRscFv was incorporated on the surface of scL as a cancer cell- and CSC-targeting moiety to form nano-sized complexes. A representative electron microscopic image of scL complex is shown. The scale bar represents 100 nm.

Fig. 2.

Schematic representation of active targeting of CSCs and non-CSC tumor cells using scL nanocomplex. In the blood stream, scL can either actively transcytose endothelial cells mediated by TfR or passively extravasate through the disrupted BBB allowing the EPR effect. Once crossing the BBB, scL binds to TfR on the surface of non-CSC tumor cells and CSCs, but not normal brain cells. After receptor binding, scL-receptor complex readily internalizes *via* receptor-mediated endocytosis.