Viral and Non-Viral Gene Therapy for Glioblastoma: New Insights into the Treatment of Malignant Brain Tumors

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Abstract

Glioblastoma (GBM) is the most common and malignant type of primary brain tumor, due to its high heterogeneity and aggressive brain invasion patterns. Despite recent advances in imaging and surgical techniques, which allowed a more accurate diagnosis and enhanced tumoral resection while maintaining or even improving neurologic function, current therapeutic options for GBM lack effective long-term impact on disease control and patient survival remains around the year mark. Therefore, the development of new therapeutic strategies that provide highly specific tumor cell killing and effective/complete eradication of cancer is critical to improve the life expectancy of GBM patients.

Driven by the tremendous advances in molecular biology, gene therapy has emerged as a promising strategy that uses nucleic acid delivery for the treatment of different forms of cancer, including GBM. In this regard, numerous/a wide range of therapeutic approaches involving versatile delivery vehicles, including non-replicating and oncolytic viruses, lipid- and polymer-based nanoparticles and stem cells, have been developed and tested for therapeutic potential. In this report, we review the principles behind such approaches and underscore their application to GBM treatment, by summarizing recent results obtained both in vitro and pre-clinical studies including the authors’, as well as those reported in patients. A brief discussion on the major achievements and the trends identified by these studies is intended to provide new prospects for future developments and further improvements of the existing approaches towards successful treatment of malignant brain tumors.

Keywords: Glioblastoma (GBM); Gene therapy; Non-replicating viruses; Oncolytic viruses; Liposomes; Polymer-based nanoparticles; Stem cells

Abreviations: 5-FC: 5-Fluorocytosine; 5-FU: 5-Fluorouracil; Akt: Protein Kinase B; BBB: Blood-Brain Barrier; Bcl-2: B-cell Lymphoma 2; BCNU: Bis-Chloroethyl nitrosourea; CD: Cytosine Deaminase; CED: Convection-Enhanced Delivery; CPP: Cell-Penetrating Peptide; CTX: Chlorotoxin; EGFR: Epidermal Growth Factor Receptor; EHCO: (1-Aminoethyl) Iminobis [N-(Oleicylcysteinylhistinyl-1-Myristoyl) Propionamide]; FDA: Food And Drug Administration; GBM: Glioblastoma; GCV: Ganciclovir; GCV-Tp: Ganciclovir Triphosphate; GFP: Green Fluorescent Protein; HIF-1α: Hypoxia-Inducible Factor 1α; HSV-Tk: Herpes Simplex Virus Type 1 Thymidine Kinase; IDH1: Isocitrate Dehydrogenase (IDH1); IFN-β: Interferon-μ; IL-2: Interleukin 2; IL-12: Interleukin 12; MA: Myristic Acid; MGMT: O-6-Methylguanine-DNA Methyltransferase; MRI: Magnetic Resonance Imaging; Mscs: Mesenchymal Stem Cells; Mtor: Mammalian Target Of Rapamycin; NF1: Neurofibromin; NF-Kb: Nuclear Factor Kβ; NK: Natural Killer; Nscs: Neural Stem Cells; Ovs: Oncolytic Viruses; PDCD4: Programmed Cell Death 4; PDGF: Platelet-Derived Growth Factor; PE: Pseudomonas Exotoxin; PEG: Polyethylene Glycol; PEI: Polyethylenimine; PFS: Progression-Free Survival; PI3K: Phosphoinositide 3-Kinase; PTEN: Phosphatase And Tensin Homolog; PUs: Polyurethane; RB: Retinoblastoma; Rcs: Replication-Competent Retroviruses; Rcs: Replication-Competent Viruses; Rdvs: Replication-Defective Viruses; RTK: Receptor Tyrosine Kinase; Scs: Stem Cells; Shiras: Short Hairpin Rnas; Sirnas: Small Interference Rnas; SLN: Solid Lipid Nanoparticles; SNALP: Stable Nucleic Acid Lipid Particle; S-TRAIL: Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand; TCGA: The Cancer Genome Atlas; TGF-B2: Transforming Growth Factor-Beta 2; TZM: Temozolomide; VEGF: Vascular Endothelial Growth Factor; VEGF-A: Vascular Endothelial Growth Factor A

Introduction

Glioblastoma (GBM), the most common and malignant type of a class of tumors arising from glia or glial precursors - glioma - is one of the deadliest forms of human cancer, with a median patient survival of 12 to 15 months after diagnosis [1,2]. It is a highly heterogeneous and aggressive type of tumor, characterized by hallmark features of uncontrolled cellular proliferation, diffuse infiltration throughout the brain parenchyma, extensive angiogenesis, resistance to apoptosis and development of necrosis [1]. Over the last decade, a concerted effort has been made to identify and characterize all the genomic and signaling abnormalities driving GBM tumorigenesis. In this regard, important findings from integrated genomic analysis of The Cancer Genome Atlas (TCGA) data [3,4] and targeted proteomic analysis [4] revealed not only the existence of molecular subtypes of GBM (classical, mesenchymal, proneural and neural), driven by different genetic alterations, but also of common pathways mutated in this disease. The majority of GBM tumors harbor abnormalities in p53, retinoblastoma (RB) and receptor tyrosine kinase (RTK) pathways, suggesting that such abnormalities constitute a core feature of GBM pathogenesis [3]. Chromosome 7 amplification and chromosome 10 deletion are also common chromosomal aberrations found in GBM, whereas alterations in the genes coding for the protein neurofibromin...
and the isocitrate dehydrogenase (IDH1) are associated with specific GBM subtypes (mesenchymal and proneural, respectively). As stated by Dunn and colleagues, the findings from the abovementioned studies depict GBM as a heterogeneous collection of distinct diseases with multiple dependencies both within and across each particular subtype [5].

Due to the highly infiltrative nature of GBM, cancer cells from the tumor core usually spread into the surrounding brain tissue. Complete surgical resection is, therefore, nearly impossible, although partial tumor removal decreases the symptoms associated with the presence of an intracranial mass, and allows tissue collecting for histopathological and genomic characterization.

**Standard Treatment for Newly Diagnosed Patients and Alternative Therapeutic Approaches**

The standard post-surgery treatment for GBM consists of concomitant administration of the alkylating drug temozolomide (TMZ) with fractionated radiotherapy, followed by up to six cycles of adjuvant TMZ [6]. Although this treatment regimen has shown to provide a slight increase in overall patient survival (14.6 months), when compared to that of radiotherapy per se (12.1 months) [7], tumor resistance to this drug usually occurs, due to the action of different DNA repair mechanisms that restore the structural integrity of the methylated DNA bases [5,8-10], and hence, GBM relapse is nearly universal [7]. However, as opposed to the newly diagnosed GBM, currently there is no standard treatment for recurrent GBM [11].

Therapeutic modalities that increase tumoral delivery of chemotherapeutic agents have been exploited for the treatment of GBM. The post-surgical implantation of biodegradable carmustine wafers, an approach that was approved by the US Food and Drug Administration (FDA) for the local treatment of patients with newly diagnosed and relapsed GBM [11,12], was shown to provide a small patient survival benefit (2 months) when applied either per se [13] or in combination with TMZ and radiation followed by rotational chemotherapy [14]. Nevertheless, such therapeutic approach has inherent limitations, including the delay in wound healing and the increased risk of edema [15], which restrict its widespread application. The local delivery of a therapeutic agent through a stereotactically inserted catheter, via pressure gradient-assisted diffusion (also known as convection-enhanced delivery, CED) [16], has also shown to lead to enhanced anti-tumoral activity for various agents, including anti-neoplastic drugs and toxins [17,18], although its clinical application has not resulted in significant patient survival benefit [11].

Cytostatic drugs, such as cisretinoic acid, thalidomide and tamoxifen, anti-angiogenics and growth-factor inhibitors have also been tested for the treatment of GBM [19]. These compounds act by interfering with intracellular signaling pathways and the tumor microenvironment, thus preventing its growth and spread, rather than promoting direct tumor cell death. Although clinical studies involving anti-angiogenesis monotherapy for the treatment of GBM failed to demonstrate sufficient anti-tumor activity to be used in single-agent regimens [20,21], their therapeutic effect may be more effective when combined with cytotoxic therapy [22]. Phase II clinical trials involving administration of bevacizumab, a humanized monoclonal antibody that inhibits the highly expressed vascular endothelial growth factor A (VEGF-A), in combination with chemotherapy, revealed an increase in GBM patient progression-free survival (PFS) over those treated with the drug [23,24]. Furthermore, clinical trials involving targeted therapy to growth factor (RTK)-driven pathways, including the P13K/Akt/mTOR pathway [25,26], resulted in modest non-durable responses, which appear to be highly dependent on the functional status of the tumor suppressor PTEN [27].

**Gene Therapy Approaches for the Treatment of GBM**

Gene therapy aims at delivering therapeutically important nucleic acids to either replace a defective gene or to express/modulate a specific gene in a target cell/tissue. The majority of gene therapy clinical trials have been addressed to cancer [28], among which fifty-seven were specifically, designed for the treatment of high grade gliomas, including GBM, as reported in the Wiley database (January 2013). The generated gene therapy approaches for GBM involved delivery of different therapeutic nucleic acids, either per se or mediated by vehicles, including viruses, lipid- and polymer-based nanoparticles and stem cells. Table 1 displays examples of clinical trials involving the application of these delivery systems to high grade gliomas.

The delivery of unconjugated nucleic acids has been tested for the treatment of high grade gliomas [39]. The most promising results have been obtained with a phosphorothioate-modified antisense oligonucleotide targeting the mRNA of the transforming growth factor-beta 2 (TGF-β2) (trabedersen (AP12009), Antisense Pharma), a protein that is highly overexpressed in high grade gliomas and induces tumor cell proliferation, angiogenesis, invasion and metastasis [40]. Phase I/II clinical trials involving the CED of this agent in high grade gliomas.

| Vector | Therapeutic gene and clinical setting | Reference(s) |
|--------|---------------------------------------|--------------|
| naked nucleic acids | transforming growth factor β antisense inhibitor, phase III | [29] |
| Viruses | replication-defective (adenoviruses and retroviruses) | HSV-1k, phases II, III | [30-33] |
| replication-competent (retroviruses) | bacterial CD, phase I/II | A |
| Oncolytic (herpes simplex G207) | phase 1b | [34] |
| Oncolytic (adenoviruses ONIX-015) | phase 1 | [35] |
| Lipid-based nanoparticles | cationic liposomes | interferon-β, phase I/II | [36] |
| | cationic liposomes | HSV-1k, phase I/II | [37] |
| | cationic liposomes | interleukin-12 carried by RD Semliki Forest virus, phase I/II | [38] |
| Stem cells | neuronal stem cells | bacterial CD, phase I | B |

**Table 1:** Representative gene therapy clinical trials involving viral and non-viral vectors towards high grade gliomas.
gliomas patients revealed that this approach is safe, well tolerated and significantly increases patient survival, when compared to that observed for patients treated with standard chemotherapy [29]. Importantly, phase III clinical trials are currently ongoing for the treatment of high grade glioma patients.

The development of vectors that can efficiently and specifically deliver nucleic acids that sensitize tumor cells or lead to tumor cell death promoted the generation of effective gene therapy approaches for the treatment of cancer. As viruses remain the most efficient vectors in transducing tumor cells, it is not surprising that the majority of gene therapy trials for high grade gliomas have involved these carriers [41]. Both replication-defective (RDVs) and replication-competent (RCVs) viruses have been employed in clinic trials for the treatment of GBM.

**Replication-Defective and Replication-Competent Viruses**

RDVs lack the genes that are involved in the viral replication and, consequently, cannot produce new virions and induce target cell death. RDVs have been, therefore, engineered to incorporate conditional or direct cytotoxic transgenes in their genome [42].

The most widely used conditional cytotoxic transgene is the Herpes simplex virus type 1 thymidine kinase (HSV-tk), which codes for the enzyme thymidine kinase that converts the prodrug ganciclovir into the metabolite deoxyguanosine monophosphate. This metabolite is further phosphorylated by cellular kinases to the corresponding nucleoside triphosphate, a highly toxic nucleotide analog that incorporates into DNA strands causing early chain termination [43]. This enzyme/prodrug combination has the capacity to promote cell death, not only in the recipient cells but also in the neighboring cells, by the so-called “bystander effect”. Such effect may occur via different mechanisms, including the passage of phosphorylated ganciclovir metabolites from transduced to untransduced cells through intercellular gap junctions [44] or apoptotic vesicles [45], thus compensating for the low efficacy of vectors in transferring genes into the tumor cells. Despite the encouraging results from pre-clinical studies, numerous clinical trials for GBM involving non-replicating adenoviral/retroviral-mediated HSV-tk delivery demonstrated, at best, a modest increase in patient survival, when compared to that observed with standard radiotherapy and chemotherapy [30-33]. The limited efficacy of this therapeutic approach may be explained by the difficulties for ganciclovir to cross the blood-brain barrier (BBB) and reach the tumor, resulting in inefficient local viral dispersion and low transduction of tumor cells [46].

Among the direct cytotoxic transgenes, those encoding human toxin-based chimeric proteins have shown potential to be used in the treatment of several human diseases, including cancer [47]. In this regard, an interesting therapeutic approach for GBM took advantage of the transduction efficiency of non-replicating adenoviral vectors to deliver a transgene encoding the highly toxic Pseudomonas exotoxin (PE), which inhibits protein translation in the target cell leading to cell death [48]. The authors constructed doxycycline-dependent adenoviral vectors encoding the PE fused with a mutated human interleukin-13 (IL-13), in order to target the chimeric protein to a variant of the IL-13 receptor (IL13Rα2), which is overexpressed in GBM. In pre-clinical experiments with human xenografts, intratumoral delivery of the adenoviral construct resulted in significant antitumoral activity and increased animal survival, in comparison to that observed for intratumoral administration of protein formulations [48]. Indeed, earlier phase I-III trials involving the protein formulations failed to provide significant survival benefits due to short half-life and poor local distribution [49,50], which indicates that the adenoviral administration may confer an important advantage towards a therapeutic effect. Future clinical trials should clarify whether this approach is clinically safe and will provide significant therapeutic benefit.

RDVs were also shown to efficiently deliver non-cytotoxic genes, including siRNAs and shRNAs to glioma cells. Saydam and colleagues developed a HSV-construct expressing anti-epidermal growth factor receptor (anti-EGFR) siRNAs, which was shown to promote efficient knockdown of EGFR and significant decrease in proliferation of human GBM cells, both in culture and upon subcutaneous implantation of the transduced cells in athymic mice [51]. Similarly, Kock et al. developed lentivirally-based constructs that encode an anti-B-cell lymphoma 2 (anti-Bcl-2) shRNA to downregulate Bcl-2, and the secreted tumor necrosis factor-related apoptosis-inducing ligand (S-TRAIL) to induce apoptosis in glioma cells [52]. Upon transduction of cultured glioma cells, increased caspase 3/7 activation and apoptosis were observed in cells transduced with viruses encoding anti-Bcl-2 and S-TRAIL, when compared to that observed for cells transduced with viruses coding for anti-Bcl-2 or S-TRAIL per se. Moreover, following intracranial implantation of lentivirally-transduced cells, reduced tumor growth was detected in animals injected with cells expressing the anti-Bcl-2 shRNA and S-TRAIL, when compared to that observed in animals injected with cells expressing only S-TRAIL [52].

The limited efficacy of RDVs reported in several human clinical trials towards cancer, including GBM, prompted investigators to use RCVs as an alternative system. This type of carriers is capable of replicating in infected cancer cells and forming progeny that can spread throughout the tumor mass [41]. Among the RCVs, those exhibiting the capacity to lyses infected cells, which are also known as oncolytic viruses (OVs), do not need to carry transgenes to cause cytocytotoxicity.

Several types of OVs have been investigated as oncolytic agents for therapeutic application in GBM. One of the most widely studied is the conditionally replicating HSV G207, a mutated HSV that expresses the HSV-tk gene and is capable of infecting rapidly dividing cells, which allows the combination (of virus) with pro-drugs like ganciclovir to further increase the oncolytic effect [53]. A phase 1b trial demonstrated that multi-dose administration of G207 into GBM, before or after tumor resection, is clinically safe and provides limited antitumoral activity [34]. A different recombinant HSV engineered to target the human epidermal growth factor receptor 2 (HER2), frequently overexpressed in GBMs, was also shown to be safe and decrease tumor growth upon intratumoral administration in an animal model of GBM [54].

Viruses with capacity to infect both dividing and non-dividing cells, including adenoviruses, have also been tested in pre-clinical models and clinic trials for high grade gliomas. Pre-clinical studies in human malignant glioma xenografts involving ONYX-015, a modified oncolytic adenovirus capable of replication in p53-defective tumor cells [55] demonstrated that intratumoral viral delivery resulted in cell lysis and impaired tumor growth, although the response was independent of the p53 status. A phase 1 clinical trial for recurrent high grade glioma revealed that the viral administration into the tumor cavity after surgical resection is safe and does not cause significant inflammatory response. In addition, increased survival was observed in patients receiving the highest doses of ONYX-015 [35].

In contrast to replication-competent HSVs and adenoviruses, replication-competent retroviruses (RCRs) do not possess cytolitic activity and depend on the incorporation of cytoxic genes into the vector genome to cause tumor cell death [56]. In this regard, an
interesting approach that has been successfully tested in pre-clinical studies involves a RCR vector engineered to efficiently deliver a modified cytosine deaminase (CD)-coding gene to glioma cells. The bacterial CD catalyzes the conversion of the pro-drug 5-fluorocytosine (5-FC) to the anticancer agent 5-fluorouracil (5-FU) within the tumor cells. Tai and colleagues reported a significant survival benefit in glioma-bearing animals injected with RCRs and further treated with single or multiple cycles of 5-FC, when compared to that observed for animals treated with saline vehicle control [57]. Importantly, phase I/II testing is currently undergoing in recurrent high-grade glioma patients (http://clinicaltrials.gov/show/NCT01156584).

Despite the potential of oncolytic virotherapy, the incapacity to reach distant tumor pockets after local delivery limits its efficacy, which may be related to several factors, including large viral particle size and cell-to-cell barriers [58]. In this regard, the antitumor activity of OV's has been reported to be significantly reduced by immune degradation as a result of tumor infiltration of natural killer (NK) cells, macrophages and microgila [46].

Over the last few years, viral vectors have also been employed in ex vivo approaches towards the generation of antigen-specific lymphocyte T cells for adoptive GBM immunotherapy. For this purpose, autologous T cells are genetically modified, via virally-mediated gene delivery, to express receptors targeting tumor-specific antigens, which are designated chimeric antigen receptors (CARs) [59]. Therapeutic approaches involving T cells expressing CARs for glioma-specific antigens, including IL-13RA2, erythropoietin-producing hepatocellular carcinoma A2 (EphA2) and a truncated form of the EGFR (EGFRvIII) have shown potent antitumor activity in animal models [60-62], which should be confirmed in future clinical trials.

**Lipid-based Nanoparticles**

Synthetic non-viral vectors present several advantages over viral carriers, including their reduced immunogenicity and toxicity, as well as physicochemical versatility, which allows modification of their surface for targeting to specific tissues or cells [63,64].

Lipid-based carriers have been successfully applied for the delivery of therapeutic nucleic acids to high grade gliomas in pre-clinical models [65,66]. One of the most popular approaches involves the intratumoral liposome-mediated delivery of genes coding for immunostimulatory proteins, such as interferon-β (IFN-β), in order to stimulate an antitumoral immune response [67-69]. In this regard, studies from Yoshida and colleagues demonstrated that local administration of cationic liposomes containing the IFN-β gene in glioma-bearing mice induces NK and T-cell activation, resulting in a significant increase of animal survival [67,68]. Following these studies, phase I-II clinical trials involving five patients with recurrent malignant glioma [36] have shown that injection of complexes of cationic liposomes with the human IFN-β gene into the tumor cavity was well tolerated by all patients and a considerable decrease in tumor volume (50%) was observed in two of them. However, due to the reduced number of patients involved in this study, no significant conclusions could be drawn. Subsequent clinical trials involving a larger number of patients should clarify whether an “immunogene” therapy approach will be clinically beneficial for the treatment of GBM. Cationic liposomes encapsulating the HSV-tk gene (combined with systemic ganciclovir) or a replication-incompetent Semliki Forest virus vector carrying the human interleukin-12 (IL-12) gene have also shown some promise in clinical trials for GBM [37,38].

Cationic liposomes were also used for delivery of siRNAs to glioma/GBM cells. Liposome-mediated delivery of anti-O-6-methylguanine-DNA methyltransferase (anti-MGMT) siRNAs enhanced the cytotoxicity of TMZ in cultured GBM cells and in both subcutaneous and intracranial GBM mouse models (when administered intratumorally) [66]. In a different study, liposome-mediated delivery of anti-H-ferritin siRNAs into subcutaneously implanted mouse gliomas was also shown to enhance the cytotoxic effect of the alkylating agent bis-chloroethyl nitrosourea (BCNU) [70].

Modified liposomes have also been successfully applied for the delivery of therapeutic genes to glioma cells. Saw and colleagues generated polyplexes by complexing nuclear factor κB (NF-kB) decoy oligonucleotides with a cell-penetrating peptide (CPP) composed of nine arginines (R9), which were then entrapped within R9-modified anionic liposomes, yielding a liposomal gene carrier with R9 peptides both on the surface and in the core of the particles [71]. The resulting nanocarriers were shown to be efficiently internalized by cultured U87 GBM and to promote oligonucleotide-mediated blocking of NF-kB transcription activity, which increased the cytotoxicity of the drug paclitaxel [71]. In our laboratory, we have recently developed stable nucleic acid lipid particles (SNALPs), which were targeted towards brain tumor cells by covalent coupling of the peptide chlorotoxin (CTX) to the liposomal surface [72]. Our studies demonstrated that targeted liposomes were able to efficiently and specifically deliver encapsulated anti-miR-21 oligonucleotides to cultured U87 GBM cells (Figures 1A-1D), which resulted in suppression of miR-21 expression, increased levels of the tumor suppressors PTEN and PDGFR (Figure 1E), caspase 3/7 activation and increased cytotoxicity of the tyrosine kinase inhibitor sunitinib (Figure 1F). Importantly, in vivo studies revealed that CTX enhances particle internalization into established intracranial tumors (Figure 1G).

Advances in lipid chemistry also allowed the development of another type of lipid-based nanocarriers for delivering nucleic acids to glioma cells. In an interesting study by Jin and colleagues, cationic solid lipid nanoparticles (SLN) were generated by a modified emulsification-solvent evaporation protocol [73] and conjugated to pegylated anti-MET siRNAs. The authors have shown that incubation of cultured U87 GBM cells with these carriers resulted in a decrease in both MET expression and cell proliferation [73]. Importantly, in an orthotopic GBM xenograft tumor model, intravenous administration of the developed SLN nanoparticles led to specific intra-tumoral delivery of the siRNAs, which resulted in decreased tumor cell proliferation and tumorigenesis [73].

**Polymer-Based Nanoparticles**

Polymer-based nanoparticles have been extensively used in animal models of cancer, including GBM. Zhan et al. developed a polyethylene glycol-polyethyleneimine (PEG-PEI) gene carrier targeted to cancer cells by coupling a cyclic RGD sequence (cyclic arginine-glycine-aspartic acid-D-tyrosine-lysine) [74]. This approach took advantage of the ability of the RGD peptide to bind to integrin αvβ3, a protein involved in invasion and angiogenesis that is overexpressed in GBM at the brain tumor border and vasculature. When complexed with a plasmid coding for TRAIL and combined with PEG-conjugated paclitaxel-loaded polyactic acid micelles, the resulting targeted nanoparticles were found to prolong survival in human intracranial GBM xenografts [74]. Similarly, Li et al. [75] conjugated PEI to the hydrophobic molecule myristic acid (MA) to deliver TRAIL-coding plasmids to glioblastoma cells. In addition to the capacity to cross the BBB, the generated nanoparticles were shown to increase the survival of nude GBM-bearing mice, when compared to that of animals injected with nanoparticles lacking MA or a saline solution [75].
Polyurethanes (PU), which are conventionally used in tissue engineering and gene delivery due to their biocompatibility and physicochemical properties [76,77], have also been used for the delivery of therapeutic genes to GBM. Using a combination of cationic PU with shortbranch PEI (PU-PEI) as a delivery vehicle, Yang and colleagues reported efficient delivery of miR-145 to CD133+ GBM cells.

Figure 1: SNALP internalization in U87 human GBM cells and mouse astrocytes, PTEN/PDCD4 expression, tumor cell proliferation and biodistribution of systemically-administered SNALP-formulated FAM-labeled oligonucleotides. For evaluation of SNALP internalization, (A, B) U87 cells and (C, D) mouse astrocytes were incubated with rhodamine-labeled CTX-coupled (CTX) or nontargeted (NT) liposomes encapsulating FAM-labeled anti-miR-21 or scrambled oligonucleotides, as determined by Western blot. Results are presented as PTEN and PDCD4 expression levels relative to control. * p<0.05 to cells incubated with a similar amount of CTX-coupled liposomes encapsulating scrambled oligonucleotides. For evaluation of tumor cell proliferation, U87 cells were incubated with CTX-coupled liposomes encapsulating anti-miR-21 or scrambled oligonucleotides for 4 hours, washed with PBS and further incubated for 24 hours with fresh medium. Cells were subsequently exposed to 15 μM of sunitinib for 24 hours, rinsed with PBS, after which cell viability was evaluated by the Alamar Blue assay. Scrambled/anti-miR-21 1 μM + S15: cells transfected with scrambled or anti-miR-21 oligonucleotides and further incubated with 15 μM sunitinib. * p<0.05 compared to cells incubated with SNALP-formulated scrambled oligonucleotides and further treated with 15 μM sunitinib. (F) Flow cytometry analysis (fluorescence intensity plots) of tumor and brain homogenates from animals injected intravenously with CTX-coupled and NT liposomes encapsulating FAM-labeled siRNAs or saline solution (PBS). # p<0.05 compared to animals injected with a similar amount of NT SNALP-formulated siRNAs. Adapted from Costa et al. [68].
cells, which resulted in a considerable decrease in their tumorigenic potential and facilitated differentiation into CD133-negative cells [77]. When administered intratumorally, in combination with radiotherapy and TMZ, in an orthotopic GBM-CD133+ xenograft mouse model, nanoparticle-formulated miR145 significantly reduced tumorigenesis and improved animal survival, as compared to that observed in animals treated with PU-PEI per se [77].

Targeted delivery systems based on the multifunctional carrier (1-aminoethyl)imidobis[(N-oleiclysteinylhistiyl-1-aminoethyl)propionamide] (EHCO), a polymeric surfactant with pH-sensitive amphiphilicity, demonstrated capacity to efficiently deliver siRNAs to target cells and promote endosomal release [78,79]. In this regard, Wang and colleagues developed a tumor-targeted delivery system by coupling the PEG-conjugated chitosan and PEI, which was functionalized with the tumor-targeting peptide CTX, to improve tumor specificity and enhance cellular internalization [81]. The developed targeted nanovector was shown to specifically deliver anti-green fluorescent protein (anti-GFP) siRNAs to culture GFP-expressing C6 rat glioma cells and facilitate endosomal release, which resulted in efficient knockdown of GFP. Importantly, this carrier was reported to enhance magnetic resonance imaging (MRI) contrast in vitro, which, in combination with its capacity to target tumor cells, reveals potential to be used in the MRI monitoring of glioma treatment in vivo [81].

Despite the encouraging results obtained in pre-clinical studies, polymer-based nanocarriers have yet to be tested in clinical trials for GBM, to fully demonstrate their potential as a relevant tool towards application in a therapeutic context.

Neural and Mesenchymal Stem Cells

Stem cells (SCs) have been extensively used as vehicles for the delivery of therapeutic genes to brain tumors due to their remarkable capacity to target tumor cells, when injected either in loco [82] or intra-arterially [83,84]. Although the molecular basis of tumor tropism of SCs is not yet fully understood, several in vitro studies provided evidence that tumor-secreted cytokines and growth factors, including the vascular endothelial and platelet-derived growth factors (VEGF and PDGF, respectively) act as chemoattractants that promote SC migration towards tumor cells [85,86]. Two types of SCs from distinct origins – neural and mesenchymal - have been preferentially used in pre-clinical high grade glioma studies. Neural stem cells (NSCs) can be obtained from adult brains, fetal brains and embryonic SCs [87], while bone marrow and adipose tissue are excellent sources of mesenchymal stem cells (MSCs) [88,89].

Several in vivo studies demonstrated that SC-mediated gene delivery is able to induce tumor cell death and increase animal survival. Modified MSCs expressing immunostimulatory molecules, such as interleukin-2 (IL-2) and IFN-β, were shown to promote an antitumoral immune response and extend the survival of glioma-bearing rodents when administered intra-arterially [83,84]. Similarly, intratumoral MSC-based TRAIL gene delivery combined with the lipoxygenase inhibitor MK886 showed a greater therapeutic efficacy in glioma xenografted mice, when compared to that observed with single-agent treatment [90]. A phase 1 clinical trial involving the post-resection local injection of genetically-modified NSCs expressing the bacterial CD combined with oral 5-FC is currently being tested in humans for the treatment of recurrent grade glioma (http://clinicaltrials.gov/ct2/show/NCT01172964).

SCs have also been suggested as potential cell-based carriers for delivery of OVs, due to their exceptional migratory capacity and ability to act as immunosuppressors, which allows the therapeutic viruses to evade the host immune system [58,91]. Sonabend and colleagues demonstrated that MSCs were very effective in delivering oncolytic adenoviruses to glioma-bearing mice, even when administered at sites distant from the tumor mass [92]. Similarly, positive results have been reported using viral-loaded NSCs to treat intracranial tumor-bearing mice [93].

Nevertheless, a few concerns are associated with the clinical application of NSCs. The genetic modification of SCs to express therapeutic genes may result in insertion of the gene into a critical locus, dysregulating the normal cell function and inducing cancer cell phenotype [94,95]. Indeed, transformed NSCs have been suggested to be involved in the development of brain tumors [96]. Another limitation of SCs as nucleic acid delivery systems concerns the number of cells that can be obtained from each patient and the time involved in cell preparation to be successfully applied in the patient [95].

Conclusions and Future Perspectives

Considerable progress has been made over the last few years on the development and application of gene therapy approaches using a variety of delivery systems for treatment of GBM, as evidenced by the large number of pre-clinical and clinical trials reported for this disease. The demonstrated efficacy of such systems to mediate delivery of different types of nucleic acid molecules to target cells and the achieved therapeutic activity in anti-tumor strategies, both in animals models and patients, makes them highly promising in successful human gene therapies. Future clinical trials will allow to firmly determining whether the generated strategies will provide a significant therapeutic advantage, as complementary or alternative approaches, to the current treatment options.

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