F1000Reports MEDICINE

Published: 05 September 2012

New therapeutic approaches for malignant glioma: in search of the Rosetta stone

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F1000 Medicine Reports 2012, **4**:18 (doi:10.3410/M4-18)

Abstract

Malignant gliomas are heterogeneous, diffuse and highly infiltrating by nature. Despite wide surgical resection and improvements in radio- and chemotherapies, the prognosis of patients with glioblastoma multiforme remains extremely poor, with a median survival time of only 14.5 months from diagnosis to death. Particular challenges for glioblastoma multiforme therapy are posed by limitations in the extent of feasible surgical resections, distinct tumor heterogeneity, difficulties in drug delivery across the blood-brain barrier and low drug distribution within the tumor. Therefore, new paradigms permitting tumor-specific targeting and extensive intratumoral distribution must be developed to allow an efficient therapeutic delivery. This review highlights the latest advances in the treatment of glioblastoma multiforme and the recent developments that have resulted from the interchange between preclinical and clinical efforts. We also summarize and discuss novel therapies for malignant glioma, focusing on advances in the following main topics of glioblastoma multiforme therapy: immunotherapy, gene therapy, stem cell-based therapies and nanotechnology. We discuss strategies and outcomes of emerging therapeutic approaches in these fields, and the main challenges associated with the integration of discoveries that occur in the laboratory into clinical practice.

Introduction

Glioblastoma multiforme, the most common and aggressive primary malignant brain tumor, carries a dismal prognosis. Patients with glioblastoma multiforme have a survival rate of less than 10% at five years [1]. Particular challenges to treating glioblastoma multiforme are the inability of treatments to reach all tumor cells and its distinct cellular heterogeneity that result in rapid and aggressive relapse [2]. Surgical resection is usually inadequate for local control, and residual tumors often lead to recurrent disease. Although sensitive to high doses of radiation, glioblastoma multiforme treatment with radiotherapy is limited by normal tissue toxicity. Moreover, chemotherapy has had only modest effects on improving outcomes for glioma patients, mostly due to the protective and selective characteristics of the blood-brain barrier. Therefore, new therapeutic strategies are urgently needed [3,4].

Recent developments in tumor immunology, genetics, cell-signaling pathways, and cancer stem cells have significantly contributed to our understanding of gliomagenesis. Even though significant advances in basic research rendered promising results in the preclinical setting, many of these treatments have not yet made their way into the clinic. In this review, we will analyze the latest advances for the treatment of glioblastoma, presenting a critical review of their benefits, drawbacks and their potential use in a clinical setting.

Glioma immunotherapies

Glioblastoma multiformes secrete various immunosuppressive cytokines, such as prostaglandin-E2, transforming growth factor-β (TGF-β) and interleukin (IL)-10, that dampen the tumor-specific immune response [5]. Currently, there is renewed interest in developing therapies

that specifically target this problem. An attractive method is to utilize dendritic cells to generate host immune response against the tumor. Dendritic cells can be employed in various manners: 1) priming of dendritic cells with tumor antigens, followed by re-administration of these cells into the patient [6]; 2) mobilization of dendritic cells into brain tumors by using an FMS-like tyrosine kinase 3 ligand (Flt3L) [7]; 3) *ex-vivo* expansion of tumor-reactive cytotoxic T lymphocytes (CTLs) by peptide-pulsed dendritic cells [8]; and 4) targeting of overexpressed epitopes in glioblastoma multiforme, such as epidermal growth factor receptor variant III (EGFR vIII), by dendritic cell-based vaccines [9]. These strategies may serve as potential immunotherapies that target glioblastoma multiformes (Table 1).

EGFRvIII is a cell-surface receptor commonly mutated in malignant gliomas, whose overexpression leads to uncontrolled cell division. Rindopepimut, a 14-mer peptide vaccine against EGFRvIII is currently being tested in Phase III Clinical trial [10]. Moreover, EGFRvIII can also be targeted by radiolabeled monoclonal antibodies that are being tested in a Phase II clinical trial [11]. Due to their high specificity and potential generation of immune response against the tumor, immunotherapies are currently in the spotlight of glioblastoma multiforme therapies.

Novel gene therapeutic approaches in glioblastoma treatment

Gene therapy nowadays offers what appear to be infinite possibilities to target different populations of tumor cells (Table 2). It works primarily through (1) immune stimulatory approaches and induction of immunologic memory against the tumor; (2) conditional cytotoxic

Table 1. Novel glioma immunotherapeutic targeting strategies

Therapeutic approach	Mechanism	Target	Preclinical model	Clinical trial	Reference
To elicit host immune response	Immunization with autologous DCs	MHCII and T cell costimulatory molecules	X		[6,33]
To elicit host immune response	Cotransfection of Poly (I:C) and siRNA into DCs and glioma cells	MHCII and Th1 cell induction	X		[34]
Recruitment of DC to elicit immune repsonse	Differentiation of precursor cells into DC through STAT3-dependent mechanism	Flt3L targets precursor cells	X		[7]
Ex-vivo generation of tumor-antigen primed T cells	Peptide-pulsed DCs for ex-vivo expansion of CTLs	IL-2, IL-7, IL-15 targeting CD62L and CCR7	X		[8]
DC-based vaccine and peptide vaccine	Targeting EGFRvIII	EGFRvIII	X	X	[9,10]
Radiolabeled monoclonal antibodies	Targeting EGFRvIII	EGFRvIII		X	[11]
Plasmid-based vaccine	Induction of T-cell immunity against glioma cells	EphA2	X		[35]

Abbreviations: DC, dendritic cells; MHCII, major histocompatibility complex class II; siRNA, small interference RNA; Poly (I:C), polyriboinosinic polyribocytidylic acid; Th1, type 1 helper T cell; Flt3L, adenovirus expressing human FMS-like tyrosine kinase 3 ligand; STAT3, Signal transducer and activator of transcription 3; CTLs, Cytotoxic T Lymphocytes; IL-2, Interleukin-2; IL-7, Interleukin-7; IL-15, Interleukin-15; CD62L, L-selectin; CCR7, C-C chemokine receptor type 7; EGFRvIII, epidermal growth factor receptor variant III; EphA2, ephrin type-A receptor 2.

Table 2. Novel gene therapies

Therapeutic approach	Mechanism	Target	Preclinical model	Clinical trial	Reference
Antigen-based therapies	*In situ* production of mutated hIL-13 fused to PE via adenovirus vectors	IL-13 alfa 2R	X		[24]
Direct cytotoxic effect and induction of anti-glioma immune responses	Dual therapy: HC adenovirus expressing HC-Ad-TK + immunostimulatory HC-Ad-TetON-Flt3L	Flt3L	X	X	[12]
Decrease tumor invasion	Reduction of MMP-2 via siRNA	MMP-2	X		[36]
Reduce tumor cell proliferation, increase apoptosis	Blocking miR-21 function	miR-21	X		[37]
Repression of cancer stem cell population	Up-regulation of miR-128	miR-128	X		[38]
Increasing anti-glioma immune response	Inhibition of TGFβ2 through antisense oligonucleotides	TGFβ2		X	[14]

Abbreviations: hIL-13, human interleukin 13; PE, *Pseudomonas* exotoxin; IL-13 alfa 2R, interleukin 13 receptor alfa 2; HC-Ad-TK, high capacity (HC) adenoviruses expressing the herpes simplex tyrosine kinase; HC-Ad-TetON-Flt3L, immunostimulatory cytokine fms-like tyrosine kinase ligand 3; siRNA, small interference RNA; MMP2, matrix metalloproteinase-2; miR-21, micro-RNA 21; miR-128, micro-RNA 128; TGFβ2, transforming growth factor β 2.

approaches; and (3) RNA interference-based therapies, utilizing transcriptional inhibition as a therapeutic tool.

Gene therapy allows targeting of pathways that can induce synergy of both conditional cytotoxic and immune stimulatory approaches. A recent study, which is about to be tested in a clinical trial, developed a combination of newly engineered high-capacity adenoviral vectors that encode both HSV1-TK (conditionally cytotoxic herpes simplex virus type 1 thymidine kinase) and Flt3L. Flt3L, whose expression is under Tet-ON system control, recruits bone marrow-derived dendritic cells to the tumor site, triggering an anti-glioblastoma multiforme-specific immune response. HSV1-TK works through a conditional cytotoxic approach in which, upon virus (HSV1) infection, thymidine kinase (TK) is delivered to tumor cells. In the presence of TK and upon administration of a prodrug these tumor cells end up producing cytotoxic metabolites, which culminate in tumor death. The combination of HSV1-TK and Flt3L has been shown to kill proliferating tumor cells in a rat model, leading to an increased animal survival and the development of a long-term anti-glioma immunological memory [12].

Another recent successful approach to block important glioma pro-survival pathways has been transcriptional inhibition through RNA interference. One of the most promising RNA interference tools has been the use of antisense oligonucleotides. They inhibit gene expression at the translational level by binding to a specific RNA sequence. A phase III clinical trial to evaluate the efficacy of antisense transforming growth factor β II (TGFβ2) (in patients is also underway. It is believed that, by silencing the TGFβ2 isoform, it will be possible to reduce glioma growth and proliferation by indirectly activating the immune response [13,14]

Stem cells and stem cell modification

A number of *in vitro* and *in vivo* studies have demonstrated the unique migratory capacity of neural and mesenchymal stem cells to target glioma. In the setting of glioblastoma multiforme therapy, mesenchymal stem cells are attractive because it is relatively easy to isolate them from patients [15], while neural stem cells have shown more specific migratory potential towards glioblastoma multiforme.

Stem cell-based therapies rely mostly on expression of therapeutic genes and delivery of therapeutic agents (Table 3), and offer certain advantages over vector-based approaches. Modified stem cells can not only disperse into the tumor and reach more malignant cells but also allow for longer therapeutic gene expression. Such cells can be modified to express either direct cytotoxic molecules (such as tumor necrosis factor-related apoptosis inducing ligand, TRAIL) [16] or suicide genes that can convert pro-drugs into their active agents (cytosine deaminase or HSV-thymidine kinase). Following promising pre-clinical data, recently, a clinical trial was launched using human immortalized neural stem cells expressing a suicide CD gene (cytosine deaminase – HB1.F3.CD), followed by oral 5-fluocytosine. The introduction of the CD gene in a tumor induces the activation of the pro-drug 5-fluocytosine into 5-fluorouracil, resulting in intratumoral chemotherapy and, consequently, tumor shrinkage. (ClinicalTrails.gov Identifier: NCT 01172964) [17].

Alternatively, stem cells can function as *in situ* factories of therapeutic agents: such as immunomodulatory cytokines, antibodies (single-chain scFv–EGFRvIII) [18] or even oncolytic viruses [19]. Our lab has focused on the inherent tumor-tropic and adenovirus-replicating capabilities of such cells to target glioblastoma multiforme. We have shown that loading stem cells with conditionally replicative adenovirus (CRAds-pk7) does not significantly compromise their homing abilities, protects the payload from host immunosurveillance and, most importantly, prolongs animal survival better than direct adenovirus injection. Lastly, we noticed that neural stem cell-delivery approaches display superior therapeutic efficacy than mesenchymal stem cells in glioblastoma multiforme [20].

Overall, regardless of some pitfalls, carrier stem cells have proven to be a promising and an exciting new therapeutic approach for malignant gliomas.

Table 3. Stem cell-based carriers' therapies

Therapeutic approach	Mechanism	Target	Preclinical model	Clinical trial	Reference
Induction of apoptosis in glioma cells	NSC/MSC engineered to express therapeutic genes, such as TRAIL	Tumor cells	X		[16]
Selective killing glioma cells	Oncolytic virus delivery	Tumor cells	X		[39]
Selective killing glioma cells	Expression of suicide genes (CD) in NSCs – HB1.F3-CD	Tumor cells		X	[17]; ClinicalTrails.gov Identifier: NCT 01172964
Selective killing glioma cells	Delivery of therapeutic agents (scFv)	EGFRvIII	X	X	[18]

Abbreviations: NSC, neural stem cell; MSC, mesenchymal stem cell; TRAIL, TNF-related apoptosis-inducing ligand; CD, cytosine deaminase; scFv, single-chain variable fragment; EGFRvIII, epidermal growth factor receptor variant III.

Applications of nanotechnology in brain tumors

Nanomaterials offer several therapeutic strategies for the treatment of brain tumors [21,22]. They can either act as drug carrier systems or induce glioma cytotoxicity directly. Drug carriers consist of polymer or lipid-based systems that allow for intratumoral injection of chemotherapeutic drugs or help such agents to cross the blood-brain barrier [23]. Recently, biodegradable polymer-based nanoparticles and gold nanoparticles have been explored as vehicles to target brain tumors [24-27]. They are mainly delivered by direct intratumoral injection, *in situ* retention through receptor-mediated endocytosis, transcytosis or membrane permeabilization, and blood-brain barrier disruption.

Once in situ, nanoparticles need an external inducer to generate an oscillatory or rotating momentum to induce glioma cell toxicity. The source can be heat, light or even magnetic field. In fact, induction of nanoparticles via local hyperthermia is already under Phase II study for treatment of brain tumors. Our group has been focused on magnetic field induction of oscillatory microdiscs bound to IL-13Rα2 to kill glioma cells [28] (Table 4). Our *in vitro* results show that even a low-frequency magnetic field can achieve approximately 90% of cancer cell destruction.

In addition, nanotechnology can be combined with other therapies, such as stem cell-based carriers, offering new concepts for treatment of brain tumors [29,30]. Drug-loaded nanoparticles can be efficiently taken up by mesenchymal stem cells without affecting the cell viability [31,32]. Since they can be combined with a variety of drugs that are not able to cross the blood-brain barrier, drug-loaded nanoparticles greatly improved drug biodistribution and their therapeutic effect in brain tumor tissues. Today, they represent a new form of targeted tissue-specific delivery.

Conclusion

With the recent progresses made in understanding the molecular and genetic composition of malignant gliomas, promising new targets for therapy have emerged.

What does the future hold?

The integration of novel agents into existing treatment algorithms remains challenging. In recent years, basic and preclinical studies have revealed multiple new mechanisms of gliomagenesis and corresponding targets for treatment. Nevertheless, much from the translational research still needs to be explored to allow a better connection between basic science discoveries and clinical trials.

What questions remain?

Despite recent advances in glioblastoma multiforme treatment, there are still many open questions: (1) Determining the unique molecular and genetic profiles of tumors from individual patients; (2) Understanding the role of glioblastoma multiforme heterogeneity in therapeutic resistance; (3) Targeting glioma stem cells specifically without interfering with normal cell function or biologic stressors;

Table 4. Nanoparticle-based therapies

Therapeutic approach	Mechanism	Target	Preclinical model	Clinical trial	Reference
Tumor killing through local hyperthermia	SPIO generates heat under alternating magnetic field	Tumor cells through direct injection into the tumor		X	[40]
Tumor killing through spin-vortex magnetic discs under low-frequency alternating magnetic field	Mechanical oscillation of micro discs trigger apoptosis	Tumor cells	X		[41]
Use of semiconductor nanomaterials (photo catalyst)	Generation of cytotoxic reactive oxygen species under light exposure	Tumor cells	X		[28]
Deliver therapeutic drugs across BBB	Intravenous injection of PMLA platform conjugated w/AON therapeutic payload	Tumor cells	X		[24]
Deliver therapeutic drugs	Transferrin conjugated polimersome loaded w/doxorubicin	Tumor cells	X		[26]
Deliver therapeutic drugs	Gold nanoparticle drug conjugate (5nm core size)	EGFR	X		[25]
MRI Contrast reagent and drug delivery	Iron oxide nanoparticle	Tumor cells	X		[42]
Deliver therapeutic drugs	MSC loaded with drug-loaded nanoparticles	Tumor cells	X		[32]

Abbreviations: SPIO, superparamagnetic iron oxide nanoparticles; PMLA, polymeric nanobioconjugate drug based on the poly (β-L-malic acid); AON, antisense oligonucleotide; BBB, blood brain barrier; EGFR, epidermal growth factor receptor; MRI, magnetic resonance imaging; MSC, mesenchymal stem cells.

(4) Improving drug delivery methods across the blood-brain barrier and into the tumor.

An effective treatment of brain tumors requires certain obstacles to be overcome. Anti-cancer agents that have been shown to work well against glioma cells *in vitro* are not optimally effective *in vivo* because they do not reach the desired location in sufficient doses. Therefore, modifications need to be implemented to allow better penetrance throughout the CNS and blood-brain barrier. Although a complete oncobiological understanding can enhance the therapeutic efficacy of traditional anti-glioblastoma strategies, the neurobiological complexity of such a diffuse, limited-access and heterogeneous target structure requires profound studies and new drug delivery concepts. Nanoparticle-based drug delivery systems, *in situ* production of immunomodulatory and anti-angiogenic agents through gene therapy vectors, or cell-based delivery vehicles are presented as promising options.

Only a better understanding of glioma neurobiology and heterogeneity will allow the design of more efficient approaches that are capable of selectively targeting glioma tumor cells. This strategy will spare normal brain tissue, extending patient survival and improving quality of life.

Abbreviations

CD, cytosine deaminase; CTLs, cytotoxic T lymphocytes; EGFR, epidermal growth factor receptor; Flt3L, FMS-like tyrosine kinase 3 ligand; HSV, herpes simplex virus; IL, interleukin; TGF, transporting growth factor.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

We would like to thank Atique Ahmed for his advice and comments on the paper. This research was supported by the NCI (R01CA122930, R01CA138587), the NINDS (R01NS077388), the National Institute of Neurological Disorders and Stroke (U01NS069997) and the American Cancer Society (RSG-07-276-01-MGO).

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