The combination of nanoparticles and RNAi technology to combat glioblastoma with patient specificity
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Background

Glioblastoma multiforme (GBM) is a WHO grade IV glioma\(^1\). It is the most common and aggressive primary brain tumor in adults and accounts for 50 to 60% of astrocytic tumours\(^2\). GBM can be molecularly classified into IDH-wildtype (primary) and IDH-mutant (secondary)[iii]. IDH-wildtypes (90%) clinically present as a de novo tumour with a worse prognosis than IDH-mutant (10%), which develop from a lower-grade astrocytoma\(^3\). The standard of care treatment for GBM includes any combinations of surgical resection, radiation, and chemotherapy with a median overall survival of 9.9 to 15 months for primary GBM and 24 to 31 months for secondary GBM\(^3\). It is known that conditions such as Turcot’s syndrome, Li-Fraumeni syndrome, and Lynch syndrome increase the risk of GBM incidence, though the lack of molecular knowledge translates into scarce treatment options\(^4,5,6\). Three sub-types of adult GBM have been identified based on their molecular and genetic profiles: classical, mesenchymal, and proneural\(^7\). Each are characterized by the unique overexpression or loss of major proteins and genes, which account for different responses to treatments by patients. Due to the aggressive nature of GBM, the main limitations to treatment include the delivery of macromolecular drugs, which is impeded by the blood-brain barrier (BBB), as well as the rapid tolerance to chemotherapeutic drugs, like temozolomide (TMZ). Additionally, GBM is highly infiltrative such that even complete resection of the tumour (98% of the volume) often leads to its re-emergence. For these reasons, GBM has a high morbidity rate and treatments have been able to prolong survival only in short-term.

Among the various clinical trials being developed, the potential of RNA interference (RNAi) and its promising results opens a new route for the advancement of a new therapeutic approach in treating GBM. RNAi is a post-translational process that inhibits gene expression or translation by using host machinery to degrade mRNAs. One of its mechanisms involves small-interfering RNA (siRNA) that specifically targets and degrade complementary mRNA via the RISC/Argonaut machinery, thus inhibiting subsequent translation\(^8\).

Research problem

On one hand, many clinical trials that treat GBM focus only on a factor such as a single drug, oncogene or tumor suppressor gene. However, the molecular profile of GBM is unique for each individual and the trials often fail because of the broad range of responses from patients. On the other hand, RNAi technology has advanced immensely over the last decade, but there is a lack of RNAi technology testing in humans that must be overcome before RNAi can be looked at as a viable way to treat GBM. Presently, there exists impediments to human RNAi therapy such as renal filtering when it is administered into the bloodstream and siRNA degradation by endogenous enzymes once it enters cells\(^8\). As such, this research proposal aims to offer a new clinical treatment of GBM, adjuvant to standard of care therapy, that inhibits tumour growth and prevents its subsequent re-emergence based on a patient-tailored approach.

Specific aims

1. Use GBM biomarkers to tailor siRNA therapy to the patient’s molecular profile.
2. Develop a reliable way to administer siRNA in GBM using nanoparticles (NPs)\(^9\).

Hypothesis

We believe that a siRNA-conjugated nanoparticle delivery system will improve the progression-free survival (PFS) of a patient by a significant amount. The efficiency of GBM treatments will be improved due to the specificity and non-invasiveness of the technique.

Methodology

First, using the tumor tissue sample obtained from biopsy, real-time quantitative polymerase chain reaction (RT-qPCR) is performed for diagnostic biomarkers such as IDH1/2, CDKN2A, EGFRvIII, PDGFRα, NF1, TP53, and RB1
as well as for the prognosis biomarker MGMT\textsuperscript{10}. Based on these results, three main patient-specific oncogenes are targeted along with Bcl2Like12 (Bcl2L12) oncogene by constructing corresponding siRNAs using the 5'- RACE technique\textsuperscript{11}. Then, nanoparticles are synthesized using (1) a water-soluble, linear cyclodextrin-containing polymer (CDP), (2) an adamantane (AD)-PEG conjugate and (3) targeting ligand of human transferrin receptor (Tfr) and (4) the patient-specific siRNAs\textsuperscript{12}. The nanoparticles are 70 nm in size, biodegradable, and tumor specific via the conjugated Tfr ligand. Conjugated NPs are administered intravenously to the patient according to the clinical trial protocol. Finally, FDG-PET is used to image the patient's tumour as it allows to differentiate between recurrent tumor and scarring tissue\textsuperscript{13}. The volume of the tumor will be the primary assessment of progression-free survival (PFS).

Hypothesized Results

A decrease in tumour volume will be observed along with visible scarring tissue using PET scanning. Thus, this will improve the progression-free survival (PFS) of a patient by a significant amount.

Rationale

The use of RNAi is known to be safe, non-intrusive, and adjustable. The specificity of siRNA allows for treatments to be optimized for the certain individual – removing redundant and ineffective procedures. For instance, the action of temozolomide, main FDA-approved chemotherapeutic drug for GBM, is effective only when the MGMT promoter is methylated and inactive\textsuperscript{14}. This problem will be eliminated with the use of personalized genetic screening to detect which major oncogenes are being highly expressed by the patient's tumour.

In a study, Miller \textit{et al.} utilized RNAi to manipulate chromatin regulators in the context of a tumor environment\textsuperscript{15}. They found that the JMJD6 mRNA and protein is upregulated in gliomas. An inducible shRNA which targeted this mRNA did not remove the tumor wholly but extended the survival of the mouse model. Findings from Davis \textit{et al.} are extremely promising as it was demonstrated that RNAi technology can be delivered intracellularly to specific tissues.

An additional step to make sure that the tumour is not adapting by upregulating other pathways is to enhance apoptosis by knocking down the oncogene Bcl2L12. In fact, in their mice studies, Jensen \textit{et al.} have used siRNAs conjugated to nanogold particles that target Bcl2L12 and have noted impaired tumour growth that likely arise by enhanced apoptosis of tumour cells\textsuperscript{16}.

Although targeting oncogenes is an ideal method, just like drugs, the BBB is the major impediment to the effectiveness of RNAi as single siRNAs are unstable under physiological conditions (Yinjiang citation). To overcome this limitation, nanoparticles have been extensively developed as a delivery tool in vivo. Utilization of biodegradable nanoparticles reduces toxicity and allows treatments such as siRNA to reach the target site with heightened efficiency. (Davis RNAi in humans). They are small enough to breach BBB permeability, without disrupting its integrity, and malleable enough to conjugate various biological agents that can target specific tissues. For example, the transferrin receptor is also known to be overexpressed in GBM\textsuperscript{17}. The receptor’s biochemical mechanism involves endocytosis of the particle containing the Tfr’s ligand. By conjugating the Tfr’s ligand to the nanoparticle, there is increased chance that siRNAs get uptaken in higher quantity by GBM cells over normal cells and that RNA interference acts on tumours cells specifically. Finally, nanoparticles can be injected intravenously such that there is reduced invasiveness of the therapy.

We are combining two innovative techniques, RNAi and nanotechnology, in order to approach the hardbound problems of glioblastoma such as the tumour’s unique molecular profile and the blood-brain-barrier. Both siRNAs and biodegradable nanoparticles are easy and rapid to make, thus the laboratory procedures are economical and can be undertaken during the first months of chemoradiation. Treatment for GBM will be significantly improved with the application of this novel method and also provide health care professionals and patients an additional tool to treat GBM.
References


