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Original Investigation

Systematic Evaluation of Promising Clinical Trials-Gene Silencing for the Treatment of Glioblastoma

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ABSTRACT

AIM: To systematically investigate the role of artificial small interfering RNA (siRNA) molecules in glioblastoma treatment and to give a detailed overview of the literature concerning studies performed in this field worldwide in the last 31 years.

MATERIAL and METHODS: Articles about clinical trials conducted between December 1, 1949 and November 8, 2017, were identified from the Cochrane Collaboration, the Cochrane Library, Ovid MEDLINE, ProQuest, the National Library of Medicine, and PubMed electronic databases, using the terms "post transcriptional gene silencing," "small interfering RNA," "siRNA," and "glioblastoma," either individually or combined ("OR" and "AND"), without language and country restrictions. Articles that met the examination criteria were included in the study. After descriptive statistical evaluation, the results were reported in frequency (%).

RESULTS: After scanning 2.752 articles, five articles were found that met the research criteria. Examination of full texts of the five identified articles provided no sufficient evidence for research conducted with regard to the use of gene silencing via siRNAs in glioblastoma treatment.

CONCLUSION: To be able to evaluate the clinical use of siRNAs, there is an urgent need for in vivo studies and for trials with randomized, controlled, and clinical designs that provide long-term functional outcomes.

KEYWORDS: Brain tumor, Glioblastoma, Posttranscriptional gene silencing, siRNA vector

INTRODUCTION

Gliomas are common primary malignant brain tumors of adults and, despite current treatment modalities, they have a poor prognosis. Glioblastomas (GBs) account for 55.4% of the gliomas, which constitute 24.7% of all brain tumors (26). Males are more often affected by this type of tumor than females (38).

The median age of diagnosis has been reported as 64 years and the incidence may increase with age (27). Other research has indicated that GBs develops through various genetic pathways and may be seen at an average age of 45 to 62 years (25). Factors associated with the risk of development of GB are reported as previous therapeutic radiation (14), reduced allergic sensitivity (35), immunological factors and immunological genes (36), and some single nucleotide polymorphisms (42) detected by extensive genomic cohort studies as well.

GBs are most frequently observed in supratentorial regions and are rarely seen in the cerebellum and spinal cord (6). Compared with supratentorial tumors, cerebellar tumors tend to be smaller and more frequent at early ages (2). Although

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leptomeningeal spread of GBs may occur from time to time, hematogenous and lymphatic spreads are reported to be very rare (2).

Median survival of GB has been reported to be three months in patients who received no treatment (23). Based on randomized phase III trials conducted by the European Organization for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada (NCIC), radiotherapy (RT) with concurrent temozolomide (TMZ), followed by six cycles of adjuvant TMZ is now the standard therapy for newly-diagnosed GB (31). After treatment, including maximal surgical resection within safe limits (60 Gy at 2 Gy/fraction), followed by RT with concomitant TMZ (75 mg/m^2/day, every day, synchronized with RT), an alkylating agent, followed by adjuvant TMZ (150 mg/m^2/day for 5 days, every 28-day cycle), the median survival is reported to be 15–20 months, with 1-, 2- and 3-year overall survival (OS) rates of 40%, 15%, and 7%–8%, respectively (9,37).

Variables such as patient age, preoperative performance status, tumor location, preoperative imaging features, and resection size affect prognosis (17,18,24), and tumor progression remains a substantial problem. Cytotoxic agents fail to halt progression, due to poor drug penetrability and molecular complexity of the disease (41). Recently, bevacizumab, an anti-angiogenic monoclonal antibody developed against vascular endothelial growth factor, has been used in GB treatment. Despite increased positive response rates, this biological agent was not able to give a survival advantage (13,32).

The most important factor affecting GB prognosis is the degree of surgical resection. Resection of the maximal tumor mass, within the safe limits, increases the RT and TMZ efficacy to be applied afterwards. Thus, it prolongs the progression period of the tumor. Despite the technological advances in surgical microscopes, current technological advances that allow safe surgical interventions to facilitate intraoperative tumor resection (such as the use of specific fluorescent stains for neoplastic cells during the operation), and the use of neuronavigational devices, surgical resection of the tumor provides a limited contribution to patient survival.

Despite the multimodal treatment of GB, overall survival has not been improved, and mean survival rates have remained very low. Therefore, scientists have turned to regenerative and reparative medicine, and the application of molecular research has gained popularity. In particular, research on post-transcriptional gene silencing has gained momentum.

Genetic manipulation in model systems has led to many inventions. However, cancer studies have been adversely affected, since no response has been found about the fundamental problems underlying the molecular pathways in mammals (29). Ribonucleic acid (RNA) interference, known as the small interfering RNA (siRNA) gene silencing technique, offers a different approach to the treatment of diseases by silencing genes after transcription, to reveal their functions and to stop unwanted genetic activity at the target (34). With increasing efficacy and reliability of siRNA gene silencing, which is used for the investigation of the functions of molecules involved in cancer, studies about the applicability of the method have intensified (1,16,21,28,30). siRNA silencing of the SATB1 gene, which effects cancer metastasis, halted tumor growth and reversed the process (11,15). Also, silencing of the STAT3 gene with RNA interference (RNAi) prevented tumor growth in an experimental study conducted in mice with hepatocellular carcinoma (12).

For the siRNA gene silencing technique to be effective, it is important to adjust the amount of mRNA that is destroyed and the duration of the gene silencing. siRNA expression vectors have been developed to control these two important limitations. These vectors usually express siRNAs that will cleave the target mRNA by using human H1 or mouse U6 promoters, which are RNA polymerase III promoters (8,47). These vectors, which have been used in therapy, are composed of bacterial plasmids and lentiviral vectors (1). They act as mini-chromosomes and replicate in the host organism independently of the host cellular replication.

The aim of this study was to systematically review the use of artificial siRNA molecules in GB treatment.

■ MATERIAL and METHODS

Literature Search Strategy

The logic of this study was to explain the subject through information from previously published research. Reports of clinical trials conducted between December 1, 1949 and November 8, 2017, were identified in the Cochrane Collaboration, the Cochrane Library, Ovid MEDLINE, ProQuest, the National Library of Medicine, and the PubMed electronic databases, using the keywords “post transcriptional gene silencing,” “small interfering RNA,” “siRNA,” and “glioblastoma,” either individually or in combinations. The included studies were based on clinical trials conducted for post-transcriptional gene silencing using siRNAs as a vector in GB treatment. Non-double blind, non-randomized, and non-clinical studies were excluded from this study. Comments, letters, editorials, protocols, guides, meta-analyses, and compilations were also excluded. Unpublished studies found in the informal electronic databases were excluded in the evaluation (Table I).

Of all the studies, those with high-evidential value were selected. The study carried out by Lijmer et al.(20) was used to determine the level of evidence required for the studies. To classify the level of scientific evidence, we used the classification system of scientific evidence developed by the Scottish Intercollegiate Guidelines Network.

Accordingly, the evidence levels of randomized clinical trials or multiple clinical trials with significant treatment effects were as follows:

A) Randomized clinical trials with low or moderate treatment effects

B) Non-prospective, non-controlled, non-randomized cohort studies

C) Unimportant, non-randomized cohort studies or case-control studies

Table I: The Frequency of the Studies by Years

Keyword(s)	Amount of total manuscript	Date range	Only clinical trials	Date range
Post Transcriptional gene silencing	64,323	2017 Nov 3 - 1949 Dec	82	2017 Aug 31 - 1995 Feb
small interfering RNA	66,259	2017 Nov 7 – 1953	121	2017 Apr 13 - 2002 Oct 10
Post Transcriptional gene silencing + small interfering RNA	21,774	2017 Oct 26 - 1973 Dec	29	2017 Jan 5 - 2007 Feb
Post Transcriptional gene silencing+ siRNA	25,153	2017 Oct 26 - 1973 Dec	31	2017 Jan 5 - 2007 Feb
siRNA	86,788	2017 Nov 7 – 1953	169	2017 Apr 13 - 2002 Oct 10
Glioblastoma	32,087	2017 Nov 8 - 1929 Sep	1,461	2017 Oct – 1965
Glioblastoma + Post Transcriptional gene silencing	667	2017 Nov 8 - 1993 Dec 1	3	2014 Oct - 2008 Jan
Glioblastoma + small interfering RNA	904	2017 Oct 2 - 2004 Feb 6	2	2014 Oct - 2014 Apr
Glioblastoma + siRNA	1,181	2017 Nov 6 - 2003 Oct 10	3	2015 Jun - 2014 Apr

D) Case series or compiled case series of patients without control groups

E) Data or predictions obtained from analysis based on assumptions collected for other reasons

G) Common approaches to logical predictions

H) Frequently applied daily practices before accepting evidence-based protocols

We excluded studies with a level of evidence of "F" and which were performed on animals, and mechanical models, since we were interested only in clinical trials. While creating a flow chart revealing the number and reasons of excluded and included studies at each stage of the selection process, the standards for reporting the results of a systematic review, the transparency in the presentation of the results, and determining the common issues amongst the reviews were provided by "the Transparent Reporting of the Systematic Review (PRISMA)" (10,19,20,38,39,46). In this way, all the articles were examined during the design phase and the form of reporting was determined.

All bibliographies thought to have been missed during the database search were reviewed again. The reference lists were also re-evaluated in terms of the availability of appropriate articles. Frequently cited articles were identified in Web of Science and Scopus databases. The references and citations of all articles were examined to avoid possible repetitions (Figure 1) (10,44,46).

Accumulation and Evaluation of Data

The authors independently selected the included studies. The risk of selection bias, which could be caused by potentially masking, was also investigated. All studies were examined by five authors (NK, TC, DYS, ST, and IY) to ensure accuracy. In the event of disagreement between at least three authors, consensus was reached on the issue by re-consulting all authors in the presence of senior authors (HO and OA).

Table II: Distribution of Studies by Years After Full Text Review

First Author	Date
Blázquez et al. (3)	2008
Wyszko et al. (42)	2008
Fan et al. (7)	2014
Wang et al. (40)	2014
Ma et al. (22)	2015

Statistical Analyses

The data obtained were listed in Microsoft Excel (version 2013) and descriptive statistical evaluations were then carried out. The results are reported in frequency (%).

■ RESULTS

Five articles containing the key words were included in this systematic review. Three, two, and three clinical trials were encountered, respectively, when the keywords were entered as "glioblastoma and/or post transcriptional gene silencing" (3,7,43), "glioblastoma and/or small interfering RNA" (41,43), or "glioblastoma and/or siRNA" (7,22,40). After a full text review, the distribution of studies by years was presented (Table II). Our literature search found only five studies involving all of the search criteria.

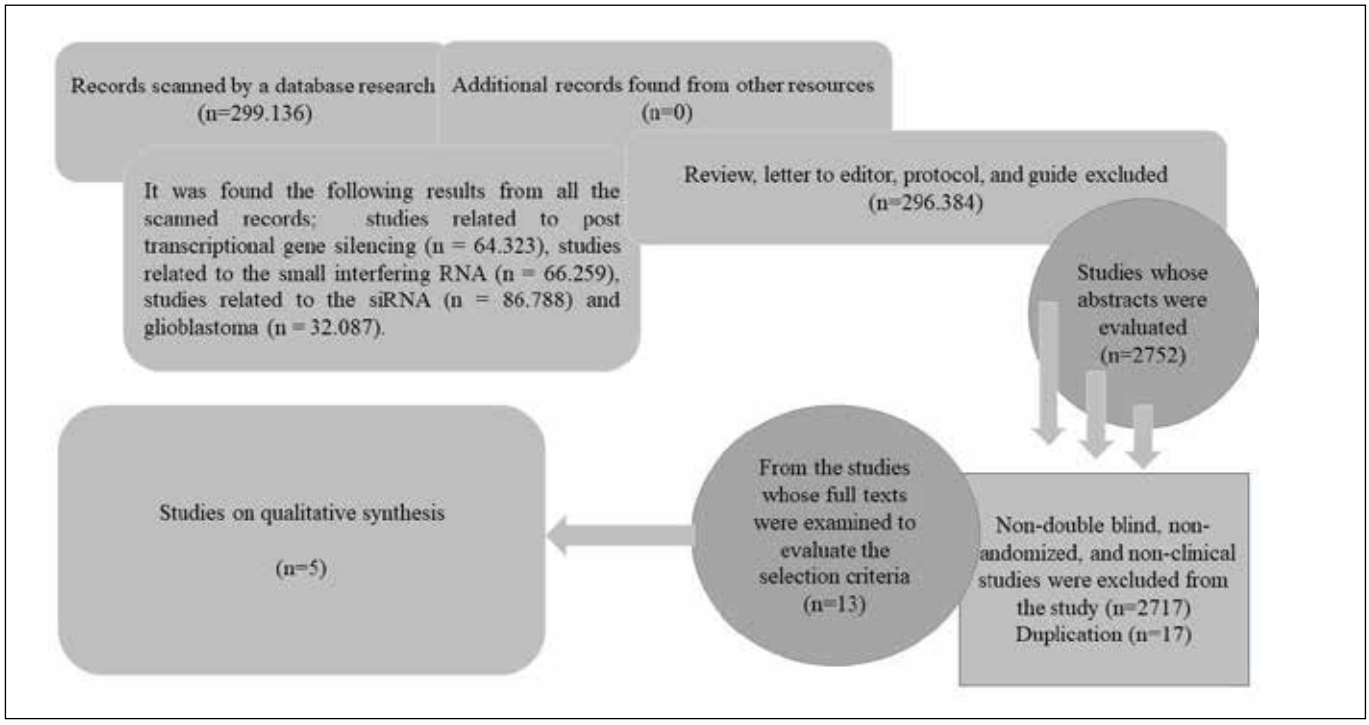

Figure 1: Flow chart of literature identification.

■ DISCUSSION

Progression of diagnosed GB is an ongoing problem. Treatment of tumor progression with cytotoxic agents continues to fail due to poor drug penetrability and the molecular complexity of the disease (13,17,18,24,32). Despite the increased response rates of GB to biological agents, including anti-angiogenic monoclonal antibodies and tumor-vasculature-targeting agents such as bevacizumab, these biological agents do not provide a survival advantage. In addition to all currently available conservative treatment modalities, the degree of surgical resection provides a limited contribution to the survival of the patients (4,45). The failure to improve overall survival with multimodal treatment methods and the low average survival rate remain major problems in the treatment of malignant GB. These problems encourage scientists to explore different treatment methods.

In the search of new treatment methods for GB, research into the efficacy of using artificial RNA molecules has gained in popularity. Boado (5) reported that human epidermal growth factors (EGFR) played an important oncogenic role in solid cancers, including primary brain and metastatic cancers. He also indicated that trans-vascular non-viral gene therapy in combination with EGFR-RNAi might be presented as a new treatment for silencing of oncogenic genes in solid cancers. He reported that EGFR decreased tumor expression and increased life expectancy by 88% in mice with advanced intracranial brain cancer. In addition, he pointed out that the healing efficacy of the new period cancer drugs might be increased, and the treatment of brain tumors might be accelerated, thanks to the technologies where the RNAi methodology is used (5).

Fan et al. (7) examined the role of Cullin1 (Cul1) in the pathogenesis of human glioma and investigated the role of Cul1 in the growth, migration and invasion of glioma cells. In this study, in which they attempted to knock down Cul1 expression in human GB cells with specific siRNAs, they demonstrated that Cul1 was significantly increased in tissues from both the benign tumor and malignant tumor in comparison with Cul1 levels in tumor-adjacent normal brain. However, they found no correlation between Cul1 expression and clinicopathologic parameters. They also underlined that the knockdown of Cul1 by RNAi significantly inhibited cell proliferation, and that matrix metalloproteinases-2 and -9 downregulated gene expressions and caused cell cycle arrest. In the light of these findings, they determined that Cul1 expression was significantly increased in human glioma, and that some conclusions about proliferation, migration, and invasion in glioma cells might be obtained (7).

Sandmair et al. (33) reported that a herpes simplex virus thymidine kinase (HSV tk) gene therapy combined with ganciclovir (GCV) medication might be a new method for the treatment of malignant GB. They used retrovirus-packaging cells (PA317/tk) and adenoviruses (Adv/tk) for gene therapy for malignant glioma. Retrovirus-packaging cells were used for eight tumors in seven patients, while adenoviruses were used for seven tumors in seven patients. As a control group (n=7), seven tumors in seven patients were transduced with the lacZ marker gene 4–5 days before tumor resection. To evaluate the efficacy of the gene therapy, clinical findings, laboratory parameters and radiological tests such as magnetic resonance imaging were used to assess patients' survival. Four patients with adenovirus injections experienced significant

increases in anti-adenovirus antibody and two of them had a short-term fever reaction. In addition, an increase in epileptic seizures in two patients was reported, but they did not detect any other adverse effect of gene therapy. They noted that all treated gliomas of patients in the retrovirus-treated group had positive developments at the 3-month time point, when their radiological imaging was examined. However, three of the seven patients treated with Adv/tk remained stable, and these results were statistically significant. The average survival time was prolonged for retrovirus-treated, adenovirus-treated, and control groups. They found that HSV tk gene therapy was safe and well tolerated, and they inferred that this research would shed some light on future studies, especially on adenovirus vectors (33).

Wyszko et al. (43) conducted a study on 46 patients with GB, which is the most common form of malignant glioma, characterized by genetic imbalance, intra-tumoral histopathological variability, and unpredictable clinical behavior. In this study, they applied double-stranded RNA (dsRNA) (ATN-RNA), which was completed with tenascin-C mRNA, to the tissues resected from the patients. This treatment slowed the growth of the tumor and relieved the symptoms of recurrence, due to the inhibition of tenascin-C synthesis. More importantly, there was a significant increase in overall survival without a decrease in the quality of life of the patients. They indicated that such novel RNAi-based methodologies might have great therapeutic potential in the treatment of GB (43). They also reported that this was the first protocol study of application RNAi in human disease treatment.

A prospective and clinical study performed by Hegi et al. (13) evaluated the methylation status of the O-6-methylguanine-DNA methyltransferase (MGMT) promoter for outcome in GB patients treated with the alkylating agent TMZ. The methylation status of MGMT was evaluated in the tumor biopsies in 38 patients and they determined the epigenetic silencing of MGMT using methylation-specific polymerase chain reaction. They found that the survival time at 18 months was 62% for patients testing positive for a methylated MGMT promoter and suggested that long-term survival could be achieved by silencing the MGMT gene by promoter methylation (13). In conclusion, after evaluating the data obtained from our literature review, only five studies containing all our search criteria were found (3,7,22,40,43).

Clinical guidelines for diagnosis and treatment have become a part of medical practice in many parts of the world recently. These guidelines provide guidance to clinicians at the highest level of healthcare delivery. It is inevitable for clinicians to require some current information about diagnosis, treatment, or prognosis of almost every patient in their daily practice. Since the time period to reach this data is often limited, it is important that evidence-based practices can be achieved through effective, readily-available, and current sources. In carrying out this research, we aimed to create an infrastructure for future therapeutic guidelines by combining clinical trials where GB was treated using the siRNA vector. However, in the articles we evaluated, we did not find common data on how to treat GB by silencing a damaged gene in the post-transcriptional stage with the siRNA vector. This can be considered as a weakness of this study. Increasing the number of studies to be examined could be achieved by extending our search criteria. However, this situation may give rise to further confusion among the results and it may prevent making binding inferences. Therefore, we believe that it is appropriate to present the data we have obtained from the study as designed.

CONCLUSION

To reduce treatment complications in GB and to increase the success rate of treatment, the number of pharmacogenomic therapy studies targeting the pathways of tumor progression should be increased. There is an urgent need for further studies to clinically reveal the applicability of these therapies. For the future treatment of GB, alone or combined with surgical treatment, it will be important to discover the means by which the direct and/or locally effective delivery of oligonucleotides to the damaged site can be performed. After these discoveries, treatment options which are target-oriented and have less systemic side effects will be possible in tumor therapy.

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