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Potential of microRNA based diagnostics and therapeutics in glioma: a patent review

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Abstract

Introduction: Glioma is a group of tumors that are usually derived from the glial cells of the central nervous system and glioblastoma is the deadliest among them. It has a dismal prognosis and no potential cure at this point. Thus, there is an utmost need for novel, more effective therapeutics and early and accurate diagnostics for improved survival of glioma patients. MicroRNAs, having altered expression in glioma and being excellent regulators of gene expression with multi-pathway targeting abilities, offer to be a suitable candidate.

Areas covered: This review summarizes microRNA-based patents that have been granted in the fields of diagnostics and therapeutics of glioma until May 2020. A comprehensive discussion has been attempted, delving into the claims and basis of each patent.

Expert opinion: MicroRNA-based anti-cancer research has been extensively carried out throughout the last decade and the results look promising. These molecules can be efficient biomarkers of glioma and used as therapeutic targets/agents. But, just like any other evolving medical technology, it also faces challenges for moving from the bench to the bedside. However, if correctly addressed, these problems can be overcome, and microRNA-based technologies can advance to be efficient tools for the treatment of glioma.

Keywords: biomarker, cancer, diagnosis, glioblastoma, glioma, MicroRNA, miRNA, patents, prognosis, therapeutics

Article highlights

- miRNAs play a critical role in glioma tumor initiation, progression and therapy response
- Potential of miRNAs as attractive diagnostic/prognostic/predictive biomarkers in glioma.
- miRNA-based therapeutics combat various hallmarks of cancer.
- Expert opinion on future of miRNA research- challenges and possible remedies.

1. Introduction

Gliomas, a group of deadly tumors, constitute about one-third of the total brain tumors and the majority of malignant brain and central nervous system (CNS) tumors [1]. The other forms of primary brain tumors include meningioma, ependymoma, lymphoma, nerve sheath tumors, etc. Gliomas originate from the glial cells of the central nervous system and are histologically more malignant than other brain tumors. They can be broadly classified into two categories: diffuse gliomas and non-diffuse gliomas; with the former being more aggressive and frequent one. Diffuse gliomas extensively infiltrate the dense, interwoven network of neurons and glial cells (the neuropil). Due to its microscopic proliferation and bizarre growth pattern, diffuse gliomas are considered very aggressive and are difficult to remove completely, often leading to tumor recurrence. The 2016 WHO classification of diffuse gliomas has taken into account the various important genetic alterations such as IDH mutation status, 1p/19q co-deletion, H3 K27M mutation, etc. along with the usual histological data; but importantly, grade wise, the diffuse gliomas are still classified as WHO grade II (low grade), grade III (anaplastic) and grade IV (glioblastoma). On the other hand, the non-diffuse gliomas have been classified as WHO grade I, grade II and grade III categories [2]. Among the various forms of gliomas, the most predominant one is glioblastoma (GB). GBs have been proposed to arise from glial cells or their precursors and represent approximately 60-70% of all malignant gliomas. They are further classified into primary and secondary tumors [3]. The recent 2016 WHO classification of CNS tumors have categorized GBs into IDH-wildtype (approximately 90% of cases), IDH-mutant (approximately 10% of cases), and NOS (Not Otherwise Specified), a category of GB tumors where the complete evaluation of IDH status is not possible [2].

GB is the most aggressive form of glioma, and notwithstanding the decades of research and advancement in medical technologies, the disease still has a very poor prognosis (with a median survival of 15 months only) [4]. The majority of affected patients are adult males and suffer from headaches, vomiting, seizure, memory loss, and change in personalities, etc. [5]. Based on the symptoms, patients are diagnosed with the help of tools like computed tomography (CT), magnetic resonance imaging (MRI), etc. to confirm the physical presence of the solid tumor. As a result, the tumor is usually discovered at quite a late stage, and although GB does not usually metastasize to other organs, it spreads locally throughout the CNS, and proliferates microscopically, making complete surgical removal almost impossible. The current mode of

treatment includes surgical removal of tumor, followed by chemo and radiotherapy [6,7]. Aggressive surgery in the brain and therapies often have severe side effects, and invariably lead to the development of chemo/radio-resistance in the patients causing therapy failure, tumor recurrence and eventually death. Discovery of novel diagnostic and prognostic biomarkers as well as advent of focused, personalized gene/molecular therapy is of utmost importance.

MicroRNAs (miRNAs) are short (18-24 nt), non-coding RNAs (ncRNAs) that have emerged as extremely important players of gene regulation, and function primarily by post-transcriptional gene silencing (PTGS). These ncRNAs mainly bind to the 3' UTR of the target mRNAs and lead to the degradation of the transcript or inhibition of translation. The interaction between the seed region of the miRNA and target mRNA does not need to be perfectly complementary, and hence a single miRNA can target multiple genes. On the other hand, a single gene can be targeted by multiple miRNAs [8-10]. This crucial discovery unveiled the significance of miRNAs in tumor progression, as multiple genes are dysregulated in cancer. In glioma too, many miRNAs have been identified to be either oncogenic or tumor suppressive in nature and are associated with the patient survival. Due to its multi-gene targeting abilities, miRNAs control the different properties of cancer cells such as proliferation, migration, invasion, anchorage independence, cell cycle progression, apoptosis etc. [11-15]. miRNAs have also been shown to regulate chemo/radio-resistance and modulate tumor-immune cell interactions [16-18]. Taken altogether, miRNAs undeniably warrant attention as therapeutic agents in glioma. However, despite their biological relevance, miRNA based anti-cancer therapies are yet to be translated into the clinics at a large scale. Naked miRNAs are prone to degradation by serum nucleases, and hence various chemical modifications have been done to engineer mature miRNA mimics or miRNA antagonists to enhance their stability. Various nanocarriers with unique advantages are being developed to carry miRNA-based therapeutics across the blood brain barrier (BBB) in case of brain related disorders or tumors [19].

miRNAs have also caught considerable attention as non-invasive biomarkers of various diseases largely due to their robust stability as compared to large transcripts or proteins and their presence as circulating entities in the body fluids such as serum, saliva and cerebrospinal fluid (CSF). Thus, the levels of specific miRNAs or a set of miRNAs in the patient tissues or body fluids are being tested as cancer biomarkers and hold great promise for detection of early onset/progression

of various cancers [20-24]. A comprehensive view of the steps involved in miRNA based clinical research and its applications in the diagnosis and treatment of glioma patients is depicted in **Figure 1**. Many novel miRNA-based technologies for glioma diagnosis and treatment are being patented across various countries [25].

Considering the promises that miRNA-based technologies hold for glioma treatment and diagnosis, and to predict the future of this technology, it is crucial to have an updated knowledge of the various patents that have been granted based on miRNA and glioma. In this review, we have tried to give a comprehensive view of the patents that have been granted in the field of glioma and miRNA till May 2020 and discuss the future of this technology. For initial patent search, the Google patents database and Espacenet were used, and the results were verified with Patentscope-WIPO/USPTO databases. Only those patents were selected that have direct application of miRNA based technologies in glioma. An overview of the patent search strategy and year wise patents and their applications are depicted in **Figure 2**.

2. MicroRNA Based Patents in Glioma

2.1 MicroRNA Based Diagnostics

The endogenous miRNA signature (both tissue and circulating miRNAs) of a GB patient is significantly altered from that of a healthy individual, and this crucial discovery has laid the foundation of many miRNA-based diagnostic and prognostic tools. We have identified a total of 23 granted patents that involve miRNAs extracted from various sources (tumor tissue/peripheral blood/cerebrospinal fluid/saliva) for detection/grading/predicting prognosis of glioma patients **(Table 1)**.

2.1.1 miRNAs as Diagnostic Biomarkers for Glioma

In 2011, Zou *et al.* patented the application of miR-29a, miR-34c, and miR-219 as detection markers of brain glioma. They identified that these miRNAs are downregulated in glioma tissues as compared to normal tissues [26-28]. In 2012, researchers from Central South University, China patented the use of miR-182 as a glioma molecular marker and devised a kit that measures its level in the peripheral blood of patients. They had found that levels of miR-182 increase with glioma grade and proposed that miR-182 levels in peripheral blood can be used for early diagnosis of glioma [29]. Min *et al.* patented a diagnostic kit for human glioma in 2013, which

measures the levels of miR-183/96/182 in glioma tissues and normal cerebrum. They identified that the above miRNA cluster is upregulated in brain glioma tissues [30]. In the same year, Keller *et al.* obtained an international patent for diagnosing GB based on expression profiles of miRNAs obtained from biological samples of subjects [31]. In 2014, Somasundaram *et al.* an Indian group, obtained a U.S. patent for highlighting set of miRNAs as biomarkers that can differentiate among the various grades of glioma and also pathways of the disease progression. Based on the unique signature of miRNAs, they were able to distinguish normal healthy brain tissue from malignant astrocytoma; anaplastic astrocytoma (AA) from the high-grade GB; primary and secondary GB; and even progressive and de novo pathway. They identified 53 miRNAs that are upregulated (e.g., miR-92b, 25, 195, 193a-3p, 106b etc.) and 24 that are downregulated (e.g., miR-129-3p, 638, 637, 328-3p, 323-3p etc.) in malignant astrocytoma as compared to normal. 56 miRNAs were found to be upregulated (e.g., miR-532-5p, 144, 196, 602, 26a etc.) in GB as compared to AA, while 11 were downregulated (miR-129-5p, 219-2-3p, 638, 125b-1, 625, 128, 637, 933, 659, 338-3p and miRPlus-A1056) in GB as compared to AA. miR-19a and miR-19b were found to be upregulated in secondary GB as compared to primary, while miR-886-3p, miRPlus-A1087, miR-886-5p, miR-222, and miR-221 were downregulated in secondary GB as compared to primary. A total 21 miRNAs were detected to be differentially regulated between the progressive and de novo pathway (miR-886-3p, 886-5p, 221, 222, 146b-5p, 126, 34a, 204, 335, 152, 199a-3p/199b-3p, 193b, 381, 143, 509-3-5p, 339-5p, 193a-3p, 126, 145, 378, miRPlus-A1087) [32]. In 2015, Chunping *et al.* obtained a patent for detecting glioma using miR-29 family as a biomarker. They measured the levels of miR-29a, miR-29b, and miR-29c in the blood of patients. The invention also helps in detecting the grade of glioma [33]. Chiou *et al*. identified miR-142-3p levels to be a decisive factor for a patient to suffer from recurrent GB (rGB) and patented its use thereof. The authors claim that a sudden decrease in miR-142-3p levels in a patient after treatment suggest higher risks of recurrence. They also propose that a therapeutic formulation comprising of chemically modified miR-142-3p can be administered to the tumor site for rGB treatment. In another embodiment, the patent claims to provide a miR-142-3p depleted animal model of GB [34]. Researchers from the City of Hope National Medical Center acquired a U.S. patent by discovering sets of miRNAs that are specific to glioblastoma stem cells (GSCs) and could successfully distinguish them from normal neural stem cells (NSCs) using microarray and deep sequencing technologies [35]. In 2016, a group of

Russian inventors patented a miRNA-based method of differentially diagnosing human gliomas. Total RNA (including miRNA) was isolated from brain tissues of patients, followed by detection of the following miRNAs by qRT-PCR: miR-21, -221, -31, -124, -125b, -16, -451, -191, -181b, -223. Depending on the expression levels of the above miRNAs, a complex criterion (K) was calculated. ($K \geq 1$) indicates the presence of GB; ($-3 \leq K \leq 0$) implies the presence of diffuse astrocytoma, while ($K \leq -4$) is indicative of anaplastic astrocytoma [36]. The pharmaceutical company Advanced Accelerator Applications was granted a patent by the EPO in 2017 for using miR-199b-5p as both diagnostic and therapeutic tools in several cancers. The level of miR-199b-5p and its target genes in a biological sample (preferably tissue) can reveal important information regarding the histopathological stage as well as the presence/absence of metastases in cancers like GB, medulloblastoma, colon carcinoma, lymphoma, and mammary carcinoma [37]. Researchers from Joseph Fourier University were granted a patent by the European Patent Office (EPO) for inventing the use of miRNAs as diagnostic biomarkers of glioma. They scanned the expression levels of 282 miRNAs in healthy brain tissues and glial brain tumors: oligodendroglioma (ODG), and GB. Interestingly, they identified 19 miRNAs whose expression levels were either systematically upregulated or downregulated. The 19 miRNAs were then categorized in two groups. By comparing the expression levels of said miRNAs in patient (suspected) tissue with that of healthy tissue, a reference ODG sample, and GB sample, the inventors were able to diagnose the individual with either one of the above [38].. In 2018, one interesting invention was patented by researchers from the University of California that diagnoses a patient with primary or metastatic brain tumor depending on the levels of a few miRNAs in the cerebrospinal fluid (CSF). They determined the levels of miR-10b, miR-21, and miR-200 in a CSF sample and compared their levels to a reference level. If the levels of said miRNAs are lower than the reference, it indicates the absence of a metastatic or primary brain tumor. If the levels of miR-10b or miR-21 are above the reference, it implies the presence of a metastatic or primary brain tumor. On the other hand, the presence of a miR-200 family member above the reference level indicates the presence of a metastatic brain tumor. They also validated their claim using a computer-based algorithm that compares two different datasets obtained from the miRNA levels [39]. In 2018, a group of Russian inventors patented the use of three miRNAs for the diagnosis and monitoring of cerebral gliomas. Clinical studies were conducted to obtain saliva samples from patients and detecting the levels of miR-21, miR-128 and miR-342 by qRT-

PCR. Patients were diagnosed with cerebral glioma if they had an increased miR-21 and decreased miR-128 and miR-342 levels (compared with a reference value). Depending on the degree of change in gene expression as compared to the reference value, the degree of glioma progression was determined [40]. The Korea Basic Science Institute (KBSI) was granted a patent by the US PTO for inventing a novel method of diagnosing recurring GB with miRNA signature. Their patent claimed that if a GB patient has significantly increased expression of miR-365 and reduced expression of miR-450a as compared to that of a control sample, the patient can be diagnosed with recurring GB. Here, the control sample can be from a patient with newly diagnosed GB, normal GB, primary GB or incipient GB [41]. In 2019, Gang *et al.* patented the use of miR-1246 and/or TERF2IP for the diagnosis of glioma. They also showed it for the first time that miR-1246 is significantly enriched in hypoxic glioma derived exosomes (H-GDEs) as well as in the cerebrospinal fluid of preoperative GB patients [42,43]. In 2019, researchers from Qilu Hospital of Shandong University, China, patented the use of miR-588 as a molecular marker of glioma and also proposed it as a potential therapeutic target. They have shown that the expression of miR-588 gets significantly enhanced by several folds under hypoxic condition in glioma cell lines. Also, ROBO1 (Roundabout Guidance Receptor 1) was established as a direct target of miR-588 [44].

2.1.2 miRNAs as Prognostic Biomarkers for Glioma

A prognostic biomarker is one that provides information regarding the health outcome of a patient. We found only two patents that use miRNAs for the detection of brain cancer and also predicts patients' health outcome.

In 2012, Somasundaram *et al.* obtained an international patent for giving a ten-miRNA signature as prognostic biomarkers of GB. The miRNAs are: miR-20a, miR-106a, miR-17-5p, miR-31, miR-222, miR-148a, miR-221, miR-146b, miR-200b and miR-193a. Apart from predicting the survival of GB patients, the inventors have used the above miRNA signature for distinguishing high risk GB patients from low risk ones [45].

In 2019, researchers from the University of Central Lancashire, UK highlighted a set of miRNAs for the detection of brain cancer. Among them, the most prominent one was miR-34a-5p. The researchers showed that miR-34a-5p levels are higher in the serum of GB patients over the age of

60 as compared to age-matched healthy control and that the high levels of serum miR-34a are correlated with better prognosis of these patients [46].

2.1.3 miRNAs as Predictive Biomarkers for Glioma

Although predictive and prognostic biomarkers are different, yet they are often mistaken for each other. It is important to note that while a prognostic biomarker might hint at the health outcome of a patient irrespective of his/her treatment, a predictive biomarker indicates the likely outcome of a particular therapy the patient is undergoing [47]. We identified two patents that use miRNAs as predictive biomarkers of glioma therapy.

In the first patent, the researchers claim to have formulated a kit for predicting the curative effect of the chemotherapeutic drug temozolomide (TMZ) on GB patients. They discovered an association between miR-181d expression and survival of patients that undergo TMZ treatment. Interestingly, this correlation was not observed in patients that did not undergo TMZ therapy, suggesting that miR-181d can be a promising predictive biomarker. They have also shown that Methyl Guanine Methyl Transferase (MGMT), a crucial protein involved in DNA repair, is a direct target of miR-181d. Since MGMT reverses the effect of the alkylating drug TMZ, miR-181d plays an important role in TMZ responsiveness [48]. In 2019, Zihuang *et al.* obtained a patent for using the serum exosomal miRNAs, miR-208b, miR-873-5p, and miR-6731, as predictive biomarkers of radiotherapy. The inventors highlighted that patients who have better survival following radiotherapy, have higher expression of the above miRNAs in their serum exosomes [49].

Notably, while alterations in the levels of several miRNAs have been reported in GB, only a handful of these have been patented yet as diagnostic biomarkers. This could largely be due to the ignorance/disinterest in patenting by the large part of scientific community that may be further discouraged by the long process of patent granting.

2.2 miRNA Based Therapeutics

Several miRNAs have been shown to function as oncomiRs or tumor suppressive miRNAs in GB. Thus, two types of therapeutic approaches have been attempted: either overexpressing tumor suppressor miRNAs or inhibiting oncomiRs. Many researchers across the globe have proposed the use of miRNAs for GB therapy, either standalone or as an adjuvant to chemo or radiotherapy.

Some of these technologies have been patented by inventors, the majority of which talk about the application of miRNA in GB treatment by targeting cancer cells' uncontrolled proliferation, migration, stemness and chemo/radiosensitivity, **(Table 2)**.

2.2.1 Cellular Proliferation and Migration

Uncontrolled cellular growth and proliferation is probably the most fundamental property of cancer cells. While normal cells tightly regulate the release of growth-promoting signals to maintain cellular homeostasis, cancer cells go into a rapid frenzy of unlimited entry and progression through the cell cycle, resulting in unchecked cellular proliferation [50]. Many researchers have pointed out that several miRNAs are involved in the rapid proliferative ability of glioma. These miRNAs either suppress many important proteins that are involved in maintaining cellular homeostasis and are usually overexpressed, or some miRNAs are downregulated, which restrain unlimited cellular growth and proliferation in a direct/indirect manner [51-54]. Most number of patents that are granted in the field of miRNA therapeutics in glioma involves their role in inhibition of the unlimited cellular proliferation.

Chemotactic migration of cancer cells enables them to invade into neighboring tissues and vasculature, and this builds the cornerstone of a metastatic tumor. However, the report of glial tumors being metastatic is very rare, although the disease spreads locally through the CNS [55]. GB especially are highly infiltrative in nature leading to diffuse invasion into healthy tissue. This is often the major cause for treatment failure and tumor recurrence. Many miRNAs that are dysregulated in glioma were also shown to have a positive or negative effect on cellular migration, however, miRNA patents in glioma targeting migration or invasion is rather rare.

In 2011, Matzuk *et al.* obtained an international patent for using miR-31 as a therapeutic agent for the treatment of GB and some other forms of cancer. They highlighted that miR-31 is a tumor suppressor miRNA, capable of inhibiting rapid proliferation of cancer cells that have mutations in the p53/CDKN2A pathways [56]. miR-21 is arguably one of the most prominent oncomiRs in glioma that is highly overexpressed and is also associated with poor patient survival. In 2012, Gaur *et al.* patented the use of the antagonists of miR-21 for decreasing glial cell proliferation. In the miR-21 targetome, they have focused on Programmed Cell Death 4 (PDCD4) and have shown that, apart from inhibition of endogenous miR-21 levels, activators of PDCD4 can also be used for effective inhibition of glial cell [57]. In 2014, the same group of researchers pointed out

that glial tumor cell proliferation can be decreased by treating them with an effective amount of miR-10a/10b antagonist. Apart from that, activators of Homeobox D10 protein (HoxD10); Zinc finger MYND-type containing 11 (ZMYND11), and RB1 Inducible Coiled-Coil 1 (RB1CC1) can also successfully diminish the tumor cell proliferation [58]. Chunfa *et al.* obtained a patent for inhibiting cellular proliferation of glioma by overexpressing miR-139-5p. They identified that miR-139-5p inhibits cell division cycle 42 (CDC42), and thereby inhibits cellular proliferation [59]. Yanchun *et al.* showed that overexpression of miR-483-5p, miR-219-5p & miR-338-3p can be used for inhibition of glioma cell proliferation [60]. In 2015, Chunping *et al.* showed that overexpressing miR-29a can successfully inhibit proliferation of neuroglioma cells and also induce apoptosis [61]. In 2017, a group of Japanese researchers obtained a patent for identifying miR-340 as a potential therapeutic agent for inhibiting the proliferation of glioma cells. They have proposed a pharmaceutical formulation which contains miR-340 precursor along with a suitable carrier and pharmaceutical excipients and/or additives for overexpressing miR-340 in cancer cells. The patent shows that miR-340 can inhibit glial tumor cells' proliferation, invasion and migration by targeting the tissue plasminogen activator (PLAT). PLAT is reported to be involved in tumorigenesis by performing degradation of extracellular matrix, promoting cellular migration and proliferation [62]. Peruzzi *et al.* patented the use of miR-3189-3p for inhibiting the proliferation of GB cells. They have claimed that a pharmaceutical composition comprising of miR-3189-3p sequence can be administered in suitable doses to a human or animal suffering from GB tumor to effectively reduce the cancer cells' proliferation. This composition can be administered to the subject intravenously, intratumorally or subcutaneously depending on the location of the lesion. Additionally, they have also shown that miR-3189-3p modulates the levels of MYC and the splicing factors p63RoGEF and SF3B2 in GB cells [63]. During the period 2011-2016, Kan *et al.* and Jie *et al.* obtained a series of patents for designing antisense oligonucleotides against thirty one oncomiRs and applying them for therapy against glioma and other tumors that have high expression of said miRNAs **[Table 2]**. These antisense oligos were designed as complementary to the mature miRNA sequences and ranged from 13-22 nt. The bases were either ribonucleotide sequences or deoxyribonucleotides or a chimera of both. This is interesting to note because, although the binding affinity between miRNA and ribonucleotides is higher than that of deoxyribonucleotides, the cost of synthesizing the former is higher than that of the latter. Hence, the synthesis of oligos was cost-effective when chimeras were used. These

oligos had several modifications e.g. a thio/2'methoxy/cholesterol modification. Oligos of varying lengths had varying inhibitory effect on the cancer cells. The authors have claimed that these anti-miRNA oligos have high specificity, low toxicity, decrease the levels of endogenous miRNAs significantly, and when applied in form of a suitable pharmaceutical composition, can inhibit the aggressive proliferation of glial tumor cells [64-94]. Shi *et al.* pointed out that the current methods of inhibition of miRNA levels in cancer cells are expensive and hence a translation to the clinic is difficult. Their group invented a small molecule inhibitor of the highly oncogenic miR-21, characterized by 2,4-diamino-1,3-diazacyclo-5-carbonitrile. It significantly inhibits the endogenous level of miR-21and thereby inhibits the proliferation of glioma cells by arresting the cell cycle at G0/G1 phase and induces apoptosis. Apart from glioma, this inhibitor restricted the growth of breast cancer and gastric cancer cells [95]. Leedman *et al.* from the University of Western Australia obtained a patent for using miR-7 to decrease the expression of Epidermal Growth Factor Receptor (EGFR), a well-known receptor molecule that is overexpressed in various cancers, including glioma, and regulates several important cellular processes such as proliferation, differentiation etc. The inventors claim that overexpressing miR-7 from a precursor molecule can inhibit growth or differentiation of not only brain tumor cells, but also of lung, breast, prostate and colon cancer cells. They have attributed this property to inhibition of EGFR and its downstream signaling pathways by overexpressing miR-7 [96]. Krichevsky *et al.* and Allerson, Charles R. individually showed that inhibiting miR-10a/10b can lead to efficient inhibition of glioma cell proliferation. While the former research group used CRISPR/Cas9 for inhibition of miR-10a/10b, the latter group preferred modified oligonucleotides [97-98].

In 2012, Wang *et al.* obtained a patent for using anti-sense oligonucleotides against miR-210 and thereby inhibiting the invasion of brain glioma cells [99]. In 2019, Rui *et al.* patented the use of miR-588 overexpression in glioma cells to inhibit their migration and invasion. For this purpose, they proposed a pharmaceutical composition consisting of miR-588 mimics, a carrier, excipient and a diluent. They identified Robo1 as a direct target of miR-588 and postulated that upon overexpression of miR-588, Robo1 levels get depleted and in turn, the levels of Matrix Metalloproteinase 2 (MMP2) and MMP9 get decreased. As a result, the invasion and migration of glioma cells get significantly reduced [44]. It is interesting to note that various research groups

have successfully inhibited glioma cells invasion and metastasis by overexpressing miR-29a [61, 100-101].

Thus, a total of 48 miRNA based therapeutic patents targeting glioma cell proliferation/migration utilize miRNA inhibition or replacement therapy. The downregulated miRNAs are overexpressed using either miRNA mimics, or expression vectors (mostly viral vectors); while the upregulated miRNAs are targeted using various mechanisms: e.g. anti-sense oligonucleotides, small molecule inhibitors, CRISPR/Cas9 mediated knockdown etc. In the majority of the cases, the miRNA-based cargo is delivered to the cancer cells using lipid-based transfection agent. Among the tumor suppressor miRNAs, miR-29a, miR-31, miR-3189-3p are notable, while the major oncomiRs that have been targeted in the above patents involve miR-7, miR-21, miR-10a/10b and miR-210.

2.2.2 Stemness and Resistance to Conventional Therapy

Cancer stem cells (CSC) are a subpopulation of cancer cells that have the ability of self-renewal, and differentiation like normal stem cells and can even show tumor-initiating properties when transferred to an animal model [102-104]. Reports have shown that CSCs can endow cancer cells with the property of chemo/radio-resistance. They can also lie dormant and cause a tumor to recur even after apparent successful debulking of tumor mass through surgery. In tumors of glial origin also, presence of stem cells has been detected, and these are known as glial stem cells (GSCs). GSCs are of growing importance in glioma/GB research and interestingly, they have been reported to be regulated by miRNAs as well. Few patents have been granted in glioma therapy where GSCs and miRNAs are directly associated.

Apart from being able to renew itself and differentiate into multiple directions, GSCs have been reported to be highly invasive as well. In 2012, Wang *et al.* patented the use of miR-106a and its inhibitor as a GSC regulating agent. They provided a model for accelerating GSCs' invasion, and came up with an inhibitor to the same, claiming that this can be a novel way of treating glioma. The invasion accelerator model consisted of miR-106a overexpression factor, and the inhibitor model contained an inhibitor to miR-106a. The underlying principle, as outlined by the authors, states that miR-106a inhibits expression of Tissue Inhibitor of Metalloproteinases 2 (TIMP2) in GSCs, which is an inhibitor of MMP2 and MMP9. Therefore, overexpressing miR-106a increases the activity of the MMPs, thereby promoting invasion of GSCs; on the other hand,

inhibition of miR-106a leads to the down regulation of the activity of MMPs, thus leading to inhibition of invasion by GSCs [105]. In 2014, Chiou *et al.* obtained a U.S. patent for applying miR-145 to inhibit cancer stem-like properties of GB stem cells. Initially, they made an interesting observation that the level of miR-145 is inversely correlated with SOX2 and OCT4 in $CD133^+$ GB cells. Delivering a suitable amount of miR-145 overexpressing vector to GB cells using a PU-sbPEI (Polyurethane-short branch polyetherimide) polymer inhibited the stemness and chemo/radio-resistance of GB stem cells [106]. In 2015, Ting *et al.* patented the use of miR-153 for the preparation of a GSC radiosensitizer. The pointed out that miR-153 can sensitize GSCs towards radiation therapy by functioning in the Nrf2/GPx1/ROS signaling pathway [107]. Researchers from Agency for Science, Technology and Research, Singapore observed that miR-138 has increased expression in GSCs isolated from malignant glioma patients as compared to normal neural stem cells (NSCs). They proposed a pharmaceutical composition consisting of oligonucleotide inhibitor of miR-138 for suppressing the proliferation of GSCs or inducing apoptosis. The oligonucleotide is preferably a hairpin that is modified by a 2'-O-methoxyethyl group, a 2'-O-methoxy group, or a phosphorothioate group. They have claimed that the above oligonucleotide-based formulation can be used for treatment of GB and it can prolong the survival of malignant glioma patients [108]. Advanced Accelerator Applications obtained a patent in 2017 for highlighting the use of miR-199b-5p for treatment of tumors having high expression of the prominent stem cell marker CD133, like glioblastoma, medulloblastoma, lymphoma, colon carcinoma and mammary carcinoma. They proposed to deliver an oligonucleotide containing the mature miR-199b-5p sequence in either deoxyribonucleotide or ribonucleotide form with carriers like adenoviral vector, Stable Nucleic Acid Lipid Particles (SNALP) technology to tumor cells. The authors have shown that miR-199b-5p functions as a regulator of the Notch signaling pathway by targeting the transcription factor HES1, and thereby inhibiting the proliferation and anchorage independence of medulloblastoma cells. Overexpression of miR-199b-5p inhibited the expression of several cancer stem cell genes as well as impaired the ability of cancer cells to form xenograft [38]. In 2018, researchers from Dalian University, China patented the use of miR-874 for treatment of GB. They have shown that miR-874 levels are lower in GSCs and GB tissues as compared to NSCs and normal brain tissue, respectively. Delivering a suitable amount of the said miRNA to cells using a suitable pharmaceutical carrier showed that miR-874 inhibits the growth of GB stem cells and causes

apoptosis of GB cells and GB stem cells. The miRNA imparts the above functions by targeting STAT3 (Signal Transducer and Activator of Transcription) [109].

Although stemness plays a crucial role in developing resistance to chemo/radiotherapy, it is not the only governing factor for developing resistance. For example, Kim *et al.* from Seoul National University, R&DB Foundation identified the correlation between a set of miRNAs and radio-resistance in glioma. They discovered that miR-7, miR-26b, miR-203, miR-200c sensitize cancer cells to radiation therapy by delaying the DNA repair mechanism that takes place due to the DNA damage caused by radiation. In their patent published in 2015, they have claimed that delivering a therapeutic amount of the above miRNAs in mature or precursor form to glioma cells render them more susceptible to radiation therapy [110]. Park *et al.* from National Cancer Center, Korea highlighted the role of miR-21 in radiation resistance of glioma and obtained a U.S. patent in 2014 by showing that administering an effective amount of miR-21 inhibitor enhances the sensitivity of cancer cells towards radiation therapy. They showed that miR-21 is upregulated in radiation resistant glioma cells as compared to normal glioma cells. At 8 Gy units of radiation, glioma cells treated with anti-miR-21 showed enhanced cell cycle arrest at G_2/M phase. Also, the survivability of anti-miR-21 treated glioma cells was lower than that of untreated or control treated cells under radiation therapy [111].

2.2.3 Immunotherapy

It is now a well-known fact that tumor cells successfully evade the immune surveillance and can even manipulate the immune system to its own advantages. Modulation of regulatory T cells (Tregs), defective antigen presentation, release of immunosuppressive mediators etc., are some of the mechanisms that tumor cells exploit for evading the mounting immune response against them [112-115]. Apart from these, it is believed that the CNS is an immune privileged region, and absence of an effective lymphatic drainage system makes it even more difficult for the adaptive immune system to function properly. Cancer immunotherapy basically boosts the host's immune system to fight more efficiently against tumor progression. The association of miRNAs and various components of the immune system of a tumor microenvironment is not very well explored. However, in the last decade, researchers across the world have shown that miRNAs might have a potential role in immune responses and immunotherapy against cancer [116-118].

GB and other forms of glioma usually have a very strong immunosuppressed microenvironment. This not only inhibits any immune reaction against the tumor, but also helps tumor invasion and progression. In 2017, Heimberger *et al.* obtained a U.S. patent for showing a novel method of inducing anti-cancer immune response using miRNAs. In their invention, the authors have shown that delivering a pharmaceutically effective amount of miR-124, miR-142 or miR-138 to the immune cells (T-cells, dendritic cells, natural killer (NK) cells) of a subject can enhance the immune response against cancer. The immune cells mentioned above are T-cells, dendritic cells, natural killer (NK) cells. The miRNAs can either be delivered to the immune cells *in vivo* parenterally, or the immune cells can be primed with the miRNAs *ex vivo* and subsequently administered to the patient. The above invention was shown to have quite promising results. For example, in one embodiment of the invention, it was shown that miR-124 reverses the immune suppression and inhibits *in vivo* glioma growth; in another embodiment, it was seen that miR-142-3p inhibits the M2 macrophage. [119]. $CD4^+$ T helper (Th) cells have two subtypes: Th1 and Th2. These two distinct subtypes produce different cytokines and Th2 is usually reported to favor tumor formation, while Th1 helps mount effective immune response against tumor. Researchers from University of Pittsburg, USA obtained a patent in 2013 for identifying Th1 associated miRNAs and applying them for glial tumor immunotherapy. The inventors identified that the miR-17-92 cluster was upregulated in Th1 cells as compared to Th2 cells. They claimed that a heterologous nucleic acid sequence encoding miR-17-92, when administered to glioma-associated antigen specific T-cell isolated from a subject, can elicit effective immune response. The sequence encoding miR-17-92 can be carried by a suitable plasmid DNA or lentiviral vector [120]. Since its discovery in 1995, the Tumor necrosis factor Related Apoptosis Inducing Ligand (TRAIL) had become a very interesting topic in cancer research. Unlike its counterparts, this cytokine from the TNF superfamily has no toxic effect on normal cells and induces apoptosis in cancer cells having the death receptors DR4 and DR5. However, despite all the initial hype, TRAIL therapy did not meet the expectations as approximately 50% of cancer cells were able to bypass TRAIL mediated apoptosis. In view of the above problem, Weiming *et al.* proposed a miRNA-based medicament for lowering the tolerance of glioma to TRAIL. They showed that miR-133a binds to the 3' UTR of DR5 and inhibits its expression. The authors suggested that delivering an anti-sense oligonucleotide of miR-133a to glioma cells would result in increased expression of DR5 and hence effectively reduce the tumor's tolerance to TRAIL therapy [121].

MicroRNA -1246 can induce polarization of M2 macrophages. Gang *et al.* have shown that hypoxic glioma derived exo-miR-1246 inhibition can reduce M2 polarization of glioma tumor associated macrophages [42].

2.2.4 Drug Delivery and Targeted Therapy

An existing challenge in the field of miRNA-based cancer therapy is the efficient delivery of these nucleic acid to the targeted cells and the cells should absorb sufficient amount of the miRNA molecule (or antagonist thereof) for an effective response. The miRNAs or their antisense oligonucleotides face several delivery challenges. In case of an intravascular administration, the abnormal and leaky tumor vasculature can lead to poor bioavailability of the miRNA drug in the actual tumor site. Unmodified and naked miRNAs are prone to degradation by the serum nucleases leading to poor circulation times. Further, naked miRNAs being negatively charged are unable to cross the cellular membrane by themselves. Moreover, if the miRNA can somehow enter the cancer cell, it may get entrapped in the endosome only to be degraded in the lysosome. Among the extravascular barriers, the extracellular matrix (ECM), clearance by the reticuloendothelial (RET) system etc., pose to be the major challenges. [122-123]. Additionally, glioma being a tumor of the brain/CNS, the presence of the blood brain barrier (BBB) poses a significant challenge for efficient delivery.

In order to tackle these problems, researchers have adopted several measures. A number of chemical modifications of the anti-sense oligos have been performed to reduce their chances of nuclease degradation and toxicity and impart increase stability. Locked nucleic acid (LNA) oligonucleotides, 2′-O-methoxyethyl-oligonucleotides (2′-O-MOE), Peptide nucleic acid (PNA) etc. are notable among them. Several lipid or polymer based carriers have also been developed that help carry the negatively charged miRNAs across cell membranes and help escape the endosome [15]. One very crucial point to be noted is that these miRNA molecules, when ectopically overexpressed or knocked down, should not affect the healthy cells. In other terms, targeted delivery of miRNAs to cancer cells is expected to avoid any unintentional side effects due to non-specificity.

Researchers from the Henry Ford Health System, U.S.A obtained a patent for devising a novel cell-derived vesicle-based carrier for delivery of miRNA to GB cells. They identified that miR-146b can target and reduce the expression of EGFR and NF-κB in glioma cells. The authors

proposed to use cell-derived exosomes as a natural carrier of plasmids that contain miR-146b overexpression construct. These plasmids are transfected into producer cells- multipotent mesenchymal stromal cells (MSCs) and exosomes containing the miRNA are harvested and administered to subjects having glioma. Alternatively, MSCs that have produced exosomes containing the said miRNA are selectively harvested and administered to the subject for treatment of glioma [124]. Rosin-Arbesfeld *et al.* obtained a patent for inventing a novel, targeted cancer therapy that can treat cancers which has an abnormally high expression of miR-21. GB and other forms of glioma have a very high level of miR-21 expression. The above invention discloses a novel type of therapy in which the expression of an anti-cancer toxin (e.g. Diphtheria Toxin, fragment A) is put under the transcriptional control of a miR-21 promoter. When the expression of the toxin is operably linked to miR-21 promoter, it is expressed in cancer cells expressing high amounts of miR-21, inhibits *de novo* protein synthesis *in vitro* and also inhibits tumor growth *in vivo.* Overall, the above invention showed an impressive, targeted anti-tumor effect [125]. A unique strategy was adopted by researchers where an anti-tumor agent was targeted to glioma cells having low expression of certain miRNAs. The target sites of the miRNAs were placed in the 3' UTR region of the transgene. Thus, only tissues that had lower expression of the miRNAs had the transgene expressed. Using this strategy, Chunxiao *et al.* and Guocai *et al.* specifically targeted glioma cells having low expression of miR-31, miR-127, miR-143; and miR-124, miR-128, miR-146b, miR-218 with anti-tumor agents [126-127].

3.0 Conclusion

Since its discovery in 1993, and unraveling of roles in development and various diseases, miRNAs have come a long way to be known as regulators of the various hallmarks of cancer. In GB and various forms of glioma, many miRNAs have been reported to have aberrant expression. Researchers across the globe have identified them as either important biomarkers that help in early detection and agents for monitoring course of the disease or as crucial therapeutic targets/agents. From intellectual property rights (IPR) and business point of view, miRNA-based therapeutics and diagnostics look very promising. Various applications of miRNAs as diagnostic, prognostic or predictive biomarkers in glioma have been patented. However, there are more miRNA-based therapeutic patents that reveal novel technologies for controlling the proliferation, migration, therapy resistance and stemness of glioma cells. Patents in miRNA delivery/targeted

therapy and immunotherapy are also notable. Although miRNAs have contributed significantly to brain tumor research and hold great promise, but till now no notable breakthrough has taken place that can cure a highly malignant GB tumor. More miRNA-based multimodal therapies need to be designed and tested for an effective treatment of glioma.

4.0 Expert Opinion

There is now compelling evidence that miRNAs may serve as attractive diagnostic biomarkers or means or targets for cancer therapy. The clinical trials involving specific miRNAs for glioma diagnosis or therapy are now underway. The patent activity in this area has also picked up. Here, we have reviewed various patents pertaining to miRNAs as biomarkers or therapeutic targets in glioma.

The current GB diagnosis is based on neuroimaging techniques or tissue biopsies both of which have certain risks and flaws. The major neuroimaging challenge is its inability to rightly assess treatment response due to the process of pseudoprogression or pseudoresponse, while tissue biopsies are invasive in nature and thus present challenges in monitoring the patient, may lead to brain swelling or affect neurological function, are not reflective of tumor heterogenity of patients and may also be a challenge for tumors present in inaccessible sites. Thus, the need for non-invasive biomarkers becomes important. In this context, miRNAs due to their exceptional stability (well reviewed in) and presence in various bodily fluids hold great promise. There lies a huge interest in identifying diagnostic, prognostic/predictive miRNA biomarkers for GB. This mirrors in the number of publications with “Glioma” and “microRNA” and “diagnosis” keywords totaling to “796”.

Here we have identified a total of 23 patents in glioma patients that involve miRNAs as diagnostic, prognostic or predictive biomarkers. Several miRNAs such as miR-29 family, miR-182 family, miR-21, miR-10b, miR-34c, miR-221/222 etc. have been shown to differentiate the GB patients from control or were shown to correlate with the tumor grade (WHO Grade I-IV). Most of these have also been shown to play a prominent oncogenic (miR-21, miR-10b, miR-221/222) or tumor suppressive role (miR-29a or miR-34c) in GB [15]. Very few patents have claimed miRNA candidates that distinguish recurrent/primary or primary/metastatic glioma or

secondary/primary GB. High levels of miR-19a/b while low levels of miR-886-3p/5p, miR-221/22 etc. were shown to distinguish Primary versus secondary GB. Two patents claim that miR-142 or combination of miR-365 and miR-450 can distinguish Recurrent GB versus Primary GB. Similarly, miR-199b-5p or miR-10b miR-21 and miR-200 (CSF) were shown to distinguish metastatic versus primary brain tumor as part of two separate patents. CSF is considered very useful for the diagnosis of brain disorders/diseases because of its direct contact with the brain. In agreement, miR-10b and miR-21 both shown to be upregulated in GB tissue were also found to be present in higher levels in CSF of GB patients versus control suggesting that their concentration is reflective of the pathological changes in the brain. However, the major drawback with CSF based diagnosis is its invasive nature and potential side effects thus making the screening and follow-up studies difficult. While saliva and urine-based biomarkers are indeed non-invasive in nature, but the problem is the presence of extremely low levels of the analyte miR-21, miR-128, and miR-342 and no clarity regarding their association with brain diseases. miR-21 is the only miRNA that showed its ubiquitious claim as diagnostic biomarker for GB in tumor tissue, CSF as well as saliva in various studies and patents.

The blood/plasma/serum-based biomarkers emerge as the preferred method for GB detection or monitoring largely because of their non-invasive nature facilitating the ease of follow-up and regular screening options. Since tissue miRNAs have been shown to be released by the tumor cells and enter into circulation as free form or mostly as part of exosomes or other vesicular bodies, the extracellular miRNAs in serum too have been shown to correlate with pathological states. Apart from high miR-21 levels, the high miR-182 levels while low levels of miR-29c were shown in both tumor tissue and blood samples of the GB patient. However, the problem with serum miRNA biomarkers such as miR-21, miR-182 or miR-29c is that they are known to be similarly altered in various other cancer types or disease conditions leading to the possible misdiagnosis. Thus, either blood-based biomarkers may be used in conjunction with other diagnostic methods or a spectrum of miRNAs rather than a single miRNA should be used for disease biomarkers to substantially reduce the chances of error. While there are four/five reports on prognostic and predictive miRNA biomarkers for glioma, we could not find any patent claiming a miRNA to be a predisposition biomarker even though the need of a predisposition biomarker for brain glioma cannot be emphasized enough,

An increasing number of reports claim that knocking down or ectopically overexpressing a certain miRNA significantly inhibits tumor progression and even reduce the size and volume of a xenograft to a great extent. A total of 63 patents have been granted for use of miRNAs for glioma treatment. These involved diverse miRNA inhibition strategies such as the use of anti-miR oligonucleotides, LNAs, antagomiRs, small molecule inhibitors of miRNAs or CRISPR/Cas9 strategy to inhibit the levels of specific miRNAs overexpressed in glioma and playing oncogenic roles (such as miR-21, miR31, miR-10a/10b, miR-106a, miR-199b). It was interesting to note that three different groups patented miR-21 inhibition strategy for glioma treatment. While Gaur *et al*., 2012 used anti-miR-21 oligos, Shi *et al*., 2016 used small molecule inhibitor of miR-21 for inhibiting GB cell proliferation and migration. Park *et. al.,* 2014 also patented the use of anti-miR-21 for sensitizing glioma cells for radiation resistance. Interestingly, Rosin-Arbesfeld *et al.,* 2013 found another use of miR-21 where they generated a recombinant construct where an anti-cancer toxin was overexpressed under the control of miR-21 promoter for glioma treatment. Similarly, the inhibition of miR-10a/b has been patented by three different groups for GB treatment [58,97,98]. Thus, miR-21 and miR-10a/b undoubtedly emerge as attractive molecules both for glioma diagnosis or for treatment. Among the patents involving miRNA replacement strategy the major players were miR-7 (two patents), miR-29a (two patents) and miR-3189 (2 patents) among many others for glioma treatment. Many of these were also found to target glioma stem cells and radiation resistance, two major problems responsible for tumor recurrence and poor prognosis. Inhibition of miR-21, miR-10a/b etc. has been shown to be effective inhibitors of tumor growth *in vivo*. The verification in animal models is certainly important as several highly promising cell culture based results, when go through clinical trials, face difficult challenges and are often discontinued.

The successful discovery of a miRNA-based drug and its large-scale production would be rather costly considering the cost in synthesis, chemical modifications, and formulation of a pharmaceutically effective composition. The possible reasons for therapy failure need to be addressed in order to offer a suitable solution. Both miRNA replacement therapy and inhibition therapy have some challenges. In the replacement therapy, the overexpression needs to be carefully monitored, as an excessive dose can lead to toxicity and even organ failure. It is crucial to assess the physiological level of the said miRNA in the patient before ectopically overexpressing it. Non-viral vectors are safer option for delivering precursor miRNAs as they are

less likely to elicit immune response. In the case of miRNA inhibition therapy, off-target effects should be avoided by choosing local delivery methods or designing targeted delivery agents. Rather than using as a standalone technique, miRNA-based therapies would have more chances of success when co-administered with the standard chemo/radiotherapy. This would also help to overcome the therapy resistance that patients develop over time. Several miRNA-based clinical trials for cancer therapy are underway, but to the best of our knowledge, none have been yet granted in the field of glioma treatment.

It is well understood that cancer is a heterogenous disease and every patient having a GB tumor need not have the same miRNA signatures. So, a specific miRNA therapy might not be equally effective against a wide array of GB patients having different ethnicity and genetic makeup. It is extremely important that a miRNA-based medication must be applied to a patient in the form of personalized therapy. But unfortunately, the cost of high-throughput technologies like microarray, deep-sequencing etc., are sky high and very difficult to afford. These techniques should be made more easily available for advancement of miRNA-based diagnosis/therapy. Although sometimes it is reported that GB has a leaky BBB, still the BBB acts as a major obstacle in miRNA delivery and often results in poor bioavailability of drug. However, advanced research in nanotechnology offer carriers that claim to deliver miRNA-based cargo across the BBB. Another crucial issue is that of specificity. MiRNA-based therapies should preferably be targeted in nature to avoid toxicity and side-effects.

Although we observed a decrease in the number of granted miRNA-patents in glioma research of late, in our opinion, it would be wrong to conclude that miRNA-based diagnostics/therapeutics have reached a saturation point. Rather, multimodal therapies based on miRNAs should be pursued to find a breakthrough. However, in small-RNA/RNAi therapeutics new promising candidates are being addressed like- long noncoding RNAs (lncRNAs), circular RNAs (circRNAs) etc. It would not be surprising to find one of these to be leading topics of cancer research in future. However, miRNA-based cancer research still holds great promise. It takes significant amount of time for developing and commercializing any new drug. Given the fact that miRNAs were discovered in the mid 90's, and their association with human disease was first identified in 2002, it would not be wrong to mention that miRNA based diagnostic and therapeutic technologies are still in their infancy. Many pharmaceutical and biotech companies

have recently invested in miRNA-based technologies, and despite the immense potential, it would take some time for miRNA-based theranostics to translate from bench to bedside. We think that the technology will evolve even more, and effective delivery agents will be designed that will be able to deliver miRNA cargo with more specificity and bioavailability. Detection of miRNA biomarkers from body fluids like saliva, blood will become easier and more affordable. Overall, it would not be overestimating to assume that miRNA-based clinical trials might be successful in the coming decade.

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Declaration of interests

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Figure legends:

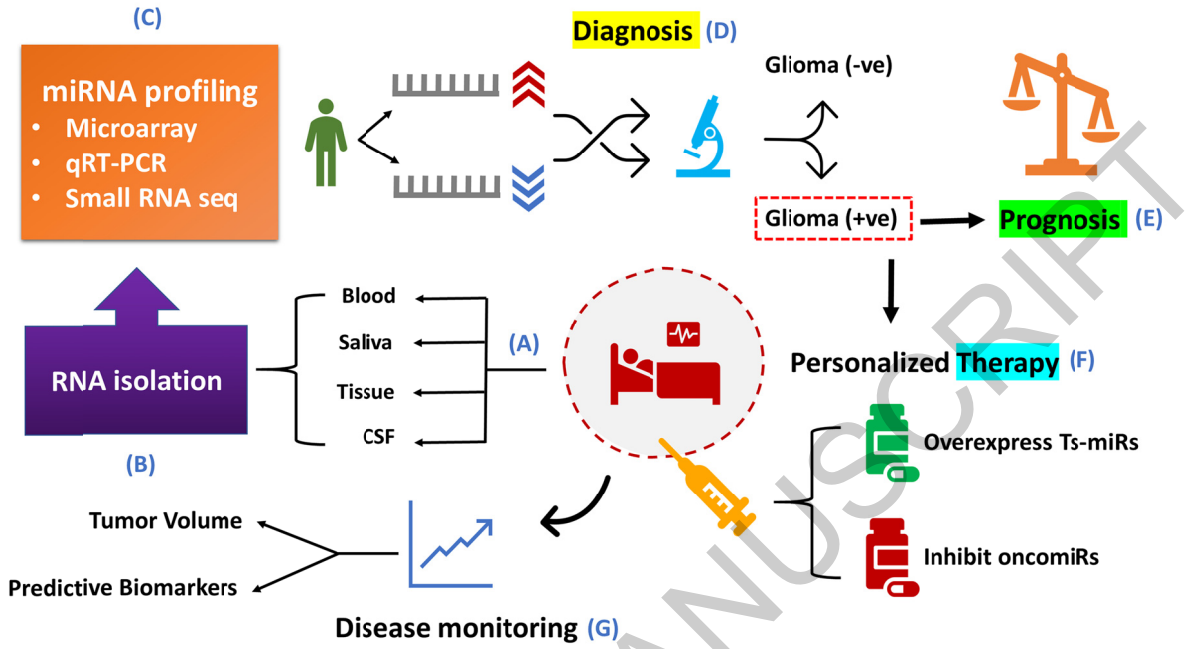

Figure 1: Overview of application of miRNAs in Glioma treatment: Collection of sample from patients (A); Isolation of RNA from patient samples (B); Profiling of miRNA signature of individual patient (C); Diagnosis on the basis of abnormal miRNA expression (D); Prognosis on the basis of miRNA expression (E); Designing and application of personalized medicine- overexpression of tumor suppressor miRNAs, or inhibition of oncomiRs (F); - overexpression of tumor suppressor miRNAs, or inhibition of oncomiRs (F); Monitoring of Disease (G).

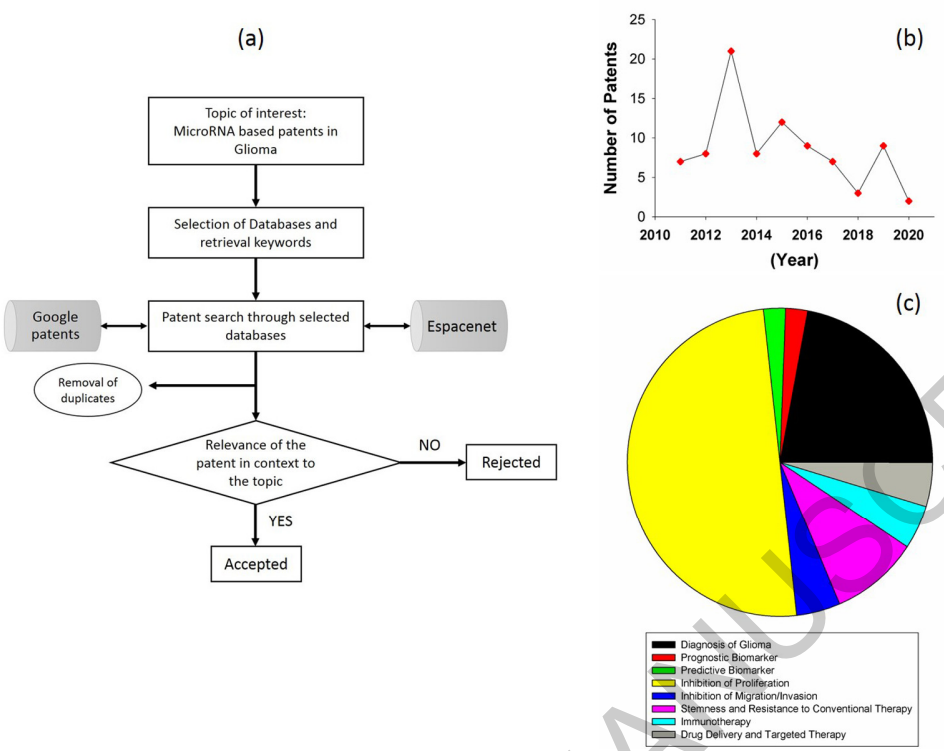

Figure 2: Patent landscape of application of miRNAs in Glioma: Schematic diagram of patent search strategy (a) Year-wise publications of miRNA-based patents in glioma (b); Distribution of miRNA-based patents in various fields of glioma treatment (c).

Table 1: Patents involving miRNAs as diagnostic, prognostic, and predictive biomarkers of glioma

S. No.		Patent No. & Date of Publication	Title of the Patent	Inference	Inventor(s)	Ref. No
1	Diagnosis of Glioma	CN102031308A April 27, 2011	Application of miRNA-29a compound as brain glioma marker	miR-29a downregulated in brain glioma tissues and cell lines	Zou *et al.*	26
2		CN102031309A April 27, 2011	Application of miRNA-34c compound as brain glioma marker	miR-34c downregulated in brain glioma tissues and cell lines	Zou *et al.*	27
3		CN102041316A May 4, 2011	Application of micro ribonucleic acid (miRNA)-219 compound as marker of brain glioma	miR-219 downregulated in brain glioma tissues and cell lines	Zou *et al.*	28
4		CN101792793B May 23, 2012	Application of miR-182 as glioma generating molecular marker and detective reagent kit thereof	Peripheral blood miR-182 for early detection of glioma	Li *et al.*	29
5		CN102424843B March 27, 2013	Application and detection kit of human miR-183/96/182 cluster	miR-183/96/182 levels upregulated in glioma tissues	Min *et al.*	30
6		WO2012089753 A2 October 10, 2013	Complex sets of miRNAs as non-invasive biomarkers for glioblastoma	Diagnosis of GB based on complex sets of miRNAs in patient blood	Keller *et al.*	31
7		US8637241B2 January 28, 2014	MicroRNAs (miRNA) as biomarkers for diagnosing different grades of gliomas and pathways of glioma progression	Grading of glioma based on miRNA signature in tissue	Somasundaram *et al.*	32
8		CN104313171A January 28, 2015	MicroRNA molecular marker for diagnosing glioma and application of microRNA molecular marker	Diagnosis of glioma based on miR-29 family levels in blood	Chunping *et al.*	33
9		US9107934B2 August 18, 2015	MicroRNA as a cancer progression predictor and its use for treating cancer	Diagnosis of recurrent GB based on miR-142-3p levels in patients	Chiou *et al.*	34
10		US9315809B2 April 19, 2016	Differentially expressed microRNA molecules for the treatment and diagnosis of cancer	Identifies miRNAs differentially expressed in GSCs w.r.t. NSCs	Shi *et al.*	35
11		RU2583871C1 May 10, 2016	Method for differential diagnosis of gliomas of human brain	Grading of glioma based on miRNA signature brain tumor tissue	Kolesnikov *et al.*	36
12		EP2411515B1 January 11, 2017	Use of microrna-199b-5p in medical and diagnostic field	Histopathological stage and metastasis marker	Massimo Zollo	38
13		EP2510122B1 Apr 12, 2017	Use of miRNAs as biomarkers for diagnosing gliomas	Diagnosis of GB and ODG based on miRNA signature in tissue samples	Berger *et al.*	37
14		US10100367B2 Oct 16, 2018	Diagnosing and monitoring CNS malignancies using microRNA	Diagnosis of primary or metastatic brain tumor based on levels of miR-21, miR-10b, miR-200 in CSF	Krichevsky *et al.*	39

15		RU2656182C1 May 31, 2018	Method of diagnostics and monitoring of course of cerebral gliomas	Diagnosis and monitoring of cerebral glioma based on levels of miR-21, 128, 342 in saliva	Zaraisky *et al.*	40
16		US10266898B2 Apr 23, 2019	Composition for diagnosing recurring glioblastoma multiforme and method for diagnosing same	Diagnosis of recurring GB based on levels of miR-365, 450a in brain tissue	Kim *et al.*	41
17		CN109762903A May 17, 2019	Application of miR-1246 and/or TERF2IP in diagnosis and treatment of glioma	Detecting, diagnosing, or predicting the progression of glioma based on miR-1246 in CSF	Gang *et al.*	42
18		CN109609653B July 19, 2019	Molecular marker for diagnosing or treating glioma and applications thereof	miR-588, a molecular marker of glioma and is hypoxia inducible	Rui *et al.*	44
19		CN110129445A August 16, 2019	Cerebrospinal fluid exosome miRNA marker related to glioma and application of cerebrospinal fluid exosome miRNA marker	Identification/Diagnosis of glioma based on miR-1246 levels in CSF	Gang *et al.*	43
20	**Prognosis**	WO2012114189 A1 August 30, 2012	Method for predicting survival of glioblastoma patient using a ten-miRNA signature	Ten miRNA signature in blood or tissue as prognostic markers of GB	Somasundaram *et al.*	45
21		EP3019627B1 January 16, 2019	Detection of brain cancer	Serum level of miR-34a-5p as a prognostic biomarker of glioma in patients aged >60	Shaw *et al.*	46
22	**Predictive Marker**	CN102329860B December 11, 2013	Molecular marker associated with temozolomide for treating glioblastoma	miR-181d as a predictive biomarker of TMZ therapy of GB	Tao *et al.*	48
23		CN110616262A December 27, 2019	Application of microRNA in exosome to evaluation of radiation therapy effect of brain glioma	Exosomal miR-208b, miR-873-5p and miR-6731 as predictive biomarkers of glioma radiotherapy	Zihuang *et al.*	49

Table 2: Patents involving miRNA therapeutics in glioma

S. No.		Patent No.& Date of Publication	Title of the Patent	Mode of Therapy	Inventor(s)	Ref. No
1	Inhibition of Cellular Proliferation	WO2011047147A1 April 21, 2011	MicroRNA miRNA-31 as a therapeutic approach for the treatment of cancer	Delivering miR-31 orally, intraperitoneally, or intravenously in a viral vector	Matzuk *et al.*	56
2		CN102140468A August 3, 2011	Human miR-185* antisense nucleic acid and application thereof	Inhibition of miR-185* in cell culture using 2'-methoxy modified antisense oligos	Kan *et al.*	90
3		CN102140463A August 3, 2011	Human miR (microRNA)-1296 antisense nucleic acid and application thereof	Inhibition of miR-1296 in cell culture using thio/2'-methoxy/cholesterol modified antisense oligos	Kan *et al.*	91
4		US8106028B2 January 31, 2012	MicroRNA-21 antagonists and its target PDCD4 for use in the treatment of a glioma	Inhibition of miR-21 in cell lines using commercially available inhibitor or overexpression of PDCD4 & ablation of GB xenograft growth	Gaur *et al.*	57
5		CN102382824A March 21, 2012	Human miR-145 antisense nucleic acid and application thereof	Inhibition of miR-145 in cell culture using thio/2'-methoxy/cholesterol modified antisense oligos	Kan *et al.*	92
6		CN102533755A July 4, 2012	Human miR-328 antisense nucleic acid and application thereof	Inhibition of miR-328 in cell culture using 2'-methoxy modified antisense oligos	Kan *et al.*	93
7		CN102080086B December 26, 2012	Human miR-133a antisense nucleic acid and application thereof	Inhibition of miR-133a in cell culture using 2'-methoxy modified antisense oligos	Kan *et al.*	64
8		CN102080085B January 16, 2013	Human miR-193b antisense nucleotide and application thereof	Inhibition of miR-193b in cell culture using 2'-methoxy modified antisense oligos	Kan *et al.*	65
9		CN102041256B January 16, 2013	Human miR-486-5p antisense nucleic acid and application thereof	Inhibition of miR-486-5p in cell culture using thio- and methoxy-modified antisense nucleic acids	Kan *et al.*	94
10		CN102080083B February 27, 2013	Human miR-149 antisense nucleotide and application thereof	Inhibition of miR-149 in cell culture using thio/2'-methoxy/cholesterol modified antisense oligos	Kan *et al.*	66
11		CN102382825B February 27, 2013	Human miR-1826 antisense nucleic acid and application thereof	Inhibition of miR-1826 in cell culture using thio/2'-methoxy/cholesterol modified antisense oligos	Kan *et al.*	67
12		CN102031256B March 27, 2013	Human miR-485-5p antisense nucleic acid and application thereof	Inhibition of miR-485-5p in cell culture using thio- and	Kan *et al.*	68

				methoxy-modified antisense nucleic acids		
13		CN102140466B March 27, 2013	Human miR-1825 antisense nucleic acid and application thereof	Inhibition of miR-1825 in cell culture using 2'-methoxy modified antisense oligos	Kan *et al.*	69
14		CN102140469B April 10, 2013	Human miR (microRNA)-1233 antisense nucleic acid and application thereof	Inhibition of miR-1233 in cell culture using 2'-methoxy modified antisense nucleic acid	Kan *et al.*	70
15		CN102382823B April 24, 2013	Human miR-515-5p antisense oligodeoxynucleotide and applications thereof	Inhibition of miR-515-5p in cell culture using thio/2'-methoxy/cholesterol modified antisense oligos	Kan *et al.*	71
16		CN102140467B May 15, 2013	Human miR-365 antisense nucleic acid and application thereof	Inhibition of miR-365 in cell culture using 2'-methoxy modified antisense nucleic acid	Kan *et al.*	72
17		CN102080082B May 22, 2013	Human miR-129* antisense nucleic acid and applications thereof	Inhibition of miR-129* in cell culture using thio/2'-methoxy/cholesterol modified antisense oligos	Kan *et al.*	73
18		CN102080084B May 22, 2013	Human miR-125a-5p antisense nucleotide and application thereof	Inhibition of miR-125a-5p in cell culture using thio/2'-methoxy/cholesterol modified antisense nucleic acids	Kan *et al.*	74
19		CN102031255B June 12, 2013	Antisense nucleic acid of human miR-223 and applications of antisense nucleic acid	Inhibition of miR-223 in cell culture using 2'-methoxy substitution, thio modification	Kan *et al.*	75
20		CN102140462B June 12, 2013	Human miR-1260 antisense nucleic acid and application thereof	Inhibition of miR-1260 in cell culture using thio/2'-methoxy/cholesterol modified antisense oligos	Kan *et al.*	76
21		CN102140465B June 12, 2013	Human miR-1249 antisense nucleic acid and application thereof	Inhibition of miR-1249 in cell culture using 2'-methoxy modified antisense oligos	Kan *et al.*	77
22		CN102140470B September 09, 2013	Human miR-1236 antisense ribonucleic acid and application thereof	Inhibition of miR-1236 in cell culture using 2'-methoxy modified antisense nucleic acids	Kan *et al.*	78
23		CN102140464B September 11, 2013	Human miR-1238 antisense nucleic acid and applications thereof	Inhibition of miR-1238 in cell culture using 2'-methoxy modified antisense oligos	Kan *et al.*	79
24		CN102031254B April 2, 2014	Human miR-150 antisense nucleic acid and application thereof	Inhibition of miR-150 in cell culture using 2'-methoxy substitution, thio modification	Kan *et al.*	80

25		CN103898117A July 2, 2014	miRNA simulant inhibiting gene expression and proliferation of human cell division cyclin 42 gene as well as expression vector and application thereof	Overexpression of miR-139-5p in cell culture using commercially available transfection agent	Chunfa *et al.*	59
26		US8778903B2 July 15, 2014	MicroRNA-10 antagonists and MicroRNA-10 targets for use in the treatment of a glioma	Inhibition of miR-10a/b using 2'-O-methyl-modified oligos or activation of target genes; complete ablation of xenograft growth *in vivo*	Gaur *et al.*	58
27		CN104232647A December 24, 2014	miRNA (ribonucleic acid) with neuroglioma inhibition function, vector built by same and application	Overexpression of miR-483-5p, miR-219-5p & miR-338-3p in cell lines using overexpression vector; miR-483-5p significantly inhibited xenograft growth in nude mice	Yanchu n *et al.*	60
28		CN104399088A March 11, 2015	Application of microRNA-29a in preparation of drugs for treating neuroglioma	Overexpression of miR-29a mimics in cell lines	Chunpin g *et al.*	
29		DK2061482T3 April 20, 2015	Procedure for modulating expression of epidermal growth factor receptor (EGFR) concerning miRNA	Overexpression of miR-7 precursor miRNA in cell lines	Leedma n *et al.*	95
30		CN102643807B June 3, 2015	Antisense oligodeoxyncleotide of human miR-484 and application thereof	Inhibition of miR-484 in cell culture using 2'-methoxy modified antisense oligos	Kan *et al.*	81
31		CN102643814B June 17, 2015	Antisense oligonucleotide for human miR-431 and application thereof	Inhibition of miR-431 in cell culture using 2'-methoxy modified antisense oligos	Kan *et al.*	82
32		CN102643813B July 29, 2015	Antisense human miR-504 and its application	Inhibition of miR-504 in cell culture using 2'-methoxy modified antisense oligos	Jie *et al.*	83
33		CN102643811B December 9, 2015	Antisense human miR-1229 and its application	Inhibition of miR-1229 in cell culture using 2'-methoxy modified antisense nucleic acids	Jie *et al.*	84
34		CN102643810B December 16, 2015	Antisense human miR-299-5p and its application	Inhibition of miR-299-5p in cell culture using 2'-methoxy modified antisense nucleic acids	Jie *et al.*	85
35		CN102643808B January 20, 2016	Antisense human miR-1539 and its application	Inhibition of miR-1539 in cell culture using 2'-methoxy modified antisense nucleic acids	Jie *et al.*	86
36		CN102643809B January 20, 2016	Antisense human miR-1274b and its application	Inhibition of miR-1274b in cell culture using 2'-	Jie *et al.*	87

				methoxy modified antisense nucleic acids		
37		CN102643812B February 10, 2016	Antisense human miR-1227 and its application	Inhibition of miR-1227 in cell culture using 2'-methoxy modified antisense nucleic acids	Jie *et al.*	88
38		CN102643806B August 3, 2016	Antisense human miR-1913 and its application	Inhibition of miR-1913 in cell culture using 2'-methoxy modified antisense nucleic acids	Kan *et al.*	89
39		CN103044338B August 3, 2016	Small molecule inhibitors of miR-21 and Application	Inhibition of miR-21 in cell lines using small molecule inhibitors of miR-21	Shi *et al.*	94
40		JP6073775B2 February 1, 2017	microRNA with glioma formation inhibitory action	Overexpression of miR-340 in glioma cells using viral vector; inhibition of tumor formation *in vivo*	Shiro *et al.*	61
41		US10308939B2 June 4, 2019	Use of an miRNA to reduce proliferation of a cancer cell	Overexpression of miR-3189-3p mimic in cell lines using commercially available transfection agent; inhibition of orthotopic GB xenograft	Peruzzi *et al.*	62
42		WO2017123910A1 July 20, 2017	Genome Editing for Treating Glioblastoma	Inhibition of miR-10b/10a using CRISPR/Cas9 in glioblastoma cells; intratumoral injection of lentiviral construct impaired orthotopic tumor growth	Krichevsky *et al.*	96
43		WO2020102142A1 May 22, 2020	MicroRNA compounds and methods for modulating miR-10b activity	Inhibition of miR-10b with modified oligonucleotides having 2'-0-methoxyethyl nucleosides, S-constrained ethyl nucleosides etc., inhibition of tumor growth *in vivo*	Allerson, Charles R.	97
44	Migration/Invasion	CN102475892A May 30, 2012	Application of anti-sense miRNA (Ribonucleic Acid)-210 to preparation of anti-cancer drug	Inhibition of miR-210 using antisense oligonucleotides through miRNA chip technology	Wang *et al.*	98
45		CN104306997A January 28, 2015	Application of microRNA-29a in preparing medicine for inhibiting glioma invasion and metastasis	Overexpression of miR-29a using mimics in cell lines by commercially available transfection agent	Chunping *et al.*	100
46		CN109609653B July 19, 2019	Molecular marker for diagnosing or treating glioma and applications thereof	Overexpression of miR-588 using mimics in cell lines by commercially available transfection agent	Rui *et al.*	99

47		CN110946873A April 3, 2020	Application of microRNA-29a-3p as glioma blood vessel mimesis control target spot	Overexpression of miR-29a-3p in glioma cells & MSCs using commercially available transfection agent; inhibition of *in vivo* tumor growth	Xing *et al.*	101
48	Stemness and Resistance to Conventional Therapy	CN102321585B March 10, 2012	Application of miRNA-106a and miRNA-106a inhibitor in preparing glioblastoma stem cell invasion regulator	Overexpression and inhibition of miR-106a using mimics and inhibitors respectively	Wang *et al.*	105
49		US8846633B2 September 30, 2014	Method for inhibiting cancer stem cell like properties and chemoradioresistant properties of cancer or tumor cells with microRNA145	Overexpression of miR-145 by delivering miR-145 expression vector to cells using PU-PEI delivery agent; inhibition of tumor growth *in vivo*	Chiou *et al.*	106
50		US8883751B2 November 11, 2014	Composition containing microrna-21 inhibitor for enhancing radiation sensitivity	Inhibition of miR-21 by delivering antisense oligonucleotides to cells using transduction	Park *et al.*	111
51		US8980863B2 March 17, 2015	Modulation of radiation response using microRNA	Overexpression of miR-7, 26b, 203, 200c by transfecting cells with precursor miRNAs using commercially available transfection agent	Kim *et al.*	110
52		CN104840974B August 19, 2015	Application of miRNA-153 in preparation of glioma stem cell radiosensitizing agent	Overexpression of miR-153 in cells using lentiviral vector by calcium phosphate-based transfection method; prolonged survival of nude mice	Ting *et al.*	107
53		EP2411515B1 January 11, 2017	Use of microrna-199b-5p in medical and diagnostic field	Overexpression of miR-199b-5p in cells by viral vector or through SNALP technology; inhibition of tumor growth *in vivo*	Massimo Zollo	38
54		EP2654801B1 November 1, 2017	Targeting glioma stem cells by sequence-specific functional inhibition of pro-survival oncomiR-138	Inhibition of miR-138 in GSCs using a hairpin oligonucleotide having a 2'-O-methoxy group, a 2'-O-methoxyethyl group, or a phosphorothioate group; Inhibition of tumor growth *in vivo*	Prabha Sampath	108
55		CN105412140B October 2, 2018	Application of small molecule RNA hsa-miR-874 in the tumor medicament for the treatment of glioblastoma	Overexpression of miR-874 by delivering miRNA expression vector to cells using commercially	Jing *et al.*	109

				available transfection agent		
56	Immunotherapy	US8486911B2 July 16, 2013	Th1-associated microRNAs and their use for tumor immunotherapy	Overexpression of miR-17-92 in glioma associated antigen specific T cells using lentiviral vectors; inhibition of tumor growth in miR-17-92 transgenic mice	Okada *et al.*	120
57		CN103961720B March 2, 2016	Use of microRNA for preparing medicine lowering tolerance of neuroglioma to TRALL	Inhibition of miR-133a using anti-sense oligonucleotides in cells	Weiming *et al.*	121
58		US9675633B2 June 13, 2017	miRNA for treating cancer and for use with adoptive immunotherapies	Overexpression of synthetic/recombinant miR-124, miR-142 or miR-138 in immune cells and administering the immune cells to the patient	Hiemberger *et al.*	119
59		CN109762903A May 17, 2019	Application of miR-1246 and/or TERF2IP in diagnosis and treatment of glioma	Inhibition of miR-1246 in glioma cells by transfecting miR-1246 antagonist	Gang *et al.*	
60	Drug Delivery and Targeted Therapy	CN102099472A June 15, 2011	Nucleic acid molecule and method of targeting gene expression to gliomas	Targeted therapy in glioma cells having low expression of one of miR-31, miR-127 & miR-143 using baculovirus vector *in vitro* or *ex vivo* or *in vivo*	Chunxiao *et al.*	127
61		US8492133B2 July 23, 2013	miR-21 promoter driven targeted cancer therapy	Overexpression of a toxin under transcriptional control of miR-21 promoter inhibiting tumor growth *in vitro* & *in vivo*	Rosin-Arbesfeld *et al.*	126
62		CN103667347A March 26, 2014	Adenovirus capable of expressing anti-cancer gene efficiently, regulated by miRNA and capable of specifically proliferating in glioma cells and application thereof	Targeted therapy in glioma cells having low expression of one of miR-124, miR-128, miR-146b & miR-218; inhibition of tumor growth *in vivo*	Guocai *et al.*	128
63		US9555060B2 January 31, 2017	Methods, systems, and compositions for cell-derived/vesicle-based microRNA delivery	Overexpression of miR-146b using MSCs derived exosomes; significant inhibition of tumor growth in rat 9L gliosarcoma model	Katakowski *et al.*	125