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Current Progress for the Use of miRNAs in Glioblastoma Treatment

Amanda Tivnan · Kerrie Leanne McDonald

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Abstract Glioblastoma (GBM) is a highly aggressive brain cancer with the worst prognosis of any central nervous system disease despite intensive multimodal therapy. Inevitably, glioblastoma is fatal, with recurrence of treatment-resistant tumour growth at distal sites leading to an extremely low median survival rate of 12–15 months from the time of initial diagnosis. With the advent of microarray and gene profiling technology, researchers have investigated trends in genetic alterations and, in this regard, the role of dysregulated microRNAs (highly conserved endogenous small RNA molecules) in glioblastoma has been studied with a view to identifying novel mechanisms of acquired drug resistance and allow for development of microRNA (miRNA)-based therapeutics for GBM patients. Considering the development of miRNA research from initial association to GBM to commercial development of miR-based therapeutics in less than a decade, it is not beyond reasonable doubt to anticipate significant advancements in this field of study, hopefully with the ultimate conclusion of improved patient outcome. This review discusses the recent advancements in miRNA-based therapeutic development for use in glioblastoma treatment and the challenges faced with respect to in vivo and clinical application.

Keywords Glioblastoma · MicroRNA · In vivo · Treatment

Introduction

The term ‘brain tumour’ is a standard idiom for an intracranial solid neoplasm which is located either within the brain itself or within the spinal canal. Although diagnosis of any form of brain cancer is innately serious and life threatening, patient prognosis and overall survival rates differ greatly with respect to the precise classification of the brain tumour type. Glioblastoma (GBM) is a highly aggressive subtype of glioma, designated as a grade IV astrocytoma by the World Health Organization [1, 2]. This form of glioma is extremely invasive with the worst prognosis of any central nervous system disease despite multimodal therapy. Typically, primary care involves mass surgical resection through open craniotomy; however, the infiltrative nature of the glioblastoma cells typically requires post-operative radiotherapy (RT) and concurrent chemotherapy (temozolomide) treatment [3]. Inevitably, glioblastoma is fatal, with recurrence of treatment-resistant tumour growth at distal sites leading to an extremely low median survival rate of 12–15 months from the time of initial diagnosis [3].

Many aspects of glioblastoma contribute to its poor prognosis including the invasive nature of these abnormal cells [4] and the extreme heterogeneity of this cancer both intratumourally and between patients [5]. In addition, treatment progression has been hindered by challenges including drug delivery across the blood–brain barrier (BBB), not to mention the delicate procedures required to remove such tumours completely in the first instance. Despite the extensive clinical differences between individual patients, current treatment regimens are unilaterally applied with the resulting consequence that many patients do not respond to standard therapy and are not reaching median survival times. In this regard, researchers have begun to focus their studies on identifying genetic biomarkers in the hope of developing individual-based treatment options through patient sample gene profiling and deep sequencing for known mutations; for example, our group has previously identified a subset of glioblastoma patients with gene mutations in alternative lengthening of telomeres and isocitrate dehydrogenase 1 which is indicative of improved survival and drug

A. Tivnan (✉) · K. L. McDonald
Cure for Life Neuro-Oncology Group, Lowy Cancer Research Centre, Prince of Wales Clinical School, University of New South Wales, Sydney 2052, Australia
e-mail: a.tivnan@unsw.edu.au

K. L. McDonald
e-mail: k.mcdonald@unsw.edu.au

responsiveness [6] or the clinical associate of miR-124a with reduced glioblastoma survival rates as a result of altered invasive potential [7].

With the advent of microarray and gene profiling technology, new studies have allowed researchers to investigate trends in genetic alterations which may provide result in the development of novel therapeutics for GBM patients [4, 8–15]. Assessment of differential gene expression in glioblastoma patient samples led to the discovery that O6-methylguanione-DNA methyltransferase (MGMT) promoter methylation status is a clinical indicator of a patient's intrinsic resistance to temozolomide treatment [16–18]. Temozolomide is an alkylating chemotherapy which causes cytotoxic DNA damage such as O6-methylguanine [19, 20]. MGMT is a DNA repair enzyme responsible for the removal of an alkyl group from the O^6 position of guanine [21]. MGMT promoter methylation is an epigenetic modification in some glioblastoma resulting in MGMT protein suppression. When expression is silenced, these cancer cells are not capable of repairing the damaging effects induced by alkylating chemotherapy agents thereby rendering them more effective in induction of tumour damage. In phase III clinical trials, MGMT methylation status was shown to be a significant predictive marker of temozolomide (TMZ)/RT treatment response [20, 22]; however, survival rates still remain less than 15 months suggesting that additional genetic biomarkers need to be identified to allow more targeted therapeutic development.

In this regard, over the past decade, the role of dysregulated microRNAs in glioblastoma has been studied with a view to identifying potentially novel mechanisms of acquired drug resistance and allow for development of microRNA (miRNA)-based therapeutics for GBM patients.

MicroRNAs in Glioblastoma

miRNAs are highly conserved endogenous small RNA molecules of approximately 22 nucleotides in length, functioning through complementary messenger RNA (mRNA) base pairing and subsequent gene silencing. Although similar to RNA interference (RNAi), resulting from the use of short interfering RNA (siRNA) or short hairpin RNA (shRNA), complementarity between the target mRNA and the miRNA is not 100 % complete, and therefore, a single miRNA can regulate multiple protein transcripts; unlike an siRNA which is designed to be single-target specific. This mechanism of miRNA function has been referred to as the 'one hit multiple target' mechanism (Fig. 1) [23, 24].

Transport of microRNAs from one cell type to another, to elicit their effect, has recently been associated with bioactive vesicles called exosomes [25]. Studies of these small extracellular vesicles have shown that miRNAs can be functionally transported by such means [26, 27]. Exosomal shuttling of miRNAs has been identified in glioblastoma and malignant glioma [28], thereby identifying a mechanism by which tumour-derived miRNA-loaded exosomes can target specific proteins in surrounding cell types, effecting processes such as angiogenesis and reduced immune response [29]

Although miRNAs were first characterized in the early 1990s by Lee et al. [30], these molecules were not recognized as a distinct class of biological regulators with defined functions until over a decade later. It is now widely accepted that there are over 1,900 miRNAs in humans, regulating over 30 % of genomic RNA [31]. It was not until 2002, however, that miRNAs were first linked to cancer [10] and it was a further 3 years before altered miRNA expression, specifically in glioblastoma, was assessed [32, 33]. Since that date however, the number of peer-reviewed publications has increased exponentially providing insight not only to miRNA expression patterns in differing GBM cell types and patient samples, but also into the function and mechanisms of several key miRNAs and their potential efficacy in GBM treatment (refer to Table 1). Based on the approach of Möller et al. [34], a Medline/PubMed database search on 'microRNA and glioblastoma' was carried out (search date, 29 November 2012). Publication relevance was determined based on title and abstract content, yielding 313 papers, which were of significance to this publication. Of these, 42 were reviews and 136 research-based papers detailed the pro- or anti-apoptotic or proliferative effects of specific miRNAs on glioblastoma, with a subset of 28 papers including in vivo evaluation of individual miRNAs. Of the original 313 papers, 22 papers dealt with the topic of chemoresistance or sensitization caused or induced by miRNAs in GBM and 173 correlated miRNA profiles to patient outcome and/or diagnosis. Notably, of the 313 papers of relevance, 57 % were published since 2011; most likely, as mentioned previously, due to the advancing techniques and availability of microarray technology.

The pattern of over-expression or suppression of specific miRNAs has been of immense interest to researchers working in the glioblastoma field in the hope that reintroduction of a depleted miRNA molecule, or inhibition of a particular miRNA which is over-expressed, may provide a novel pathway through which targeted therapy may be investigated. MiRNA profiles have previously been used to classify tumours based on tissue type and disease state in chronic lymphatic leukaemia, bladder, lung, breast and ovarian cancer [10, 35, 36]. Although still in its infancy with respect to glioblastoma, researchers have highlighted several miRNAs which have been shown to correlate to glioma pathogenesis [20, 37–48] and, as highlighted in Table 1, miRNAs which have been studied in detail have predominantly been shown to be significantly decreased in glioblastoma patient samples or cell lines compared to controls. These miRNAs tend to be encoded in regions of chromosomal loss in glioblastoma patients [49]; for example, miR-34a is encoded at Chr1p36.22, a region with

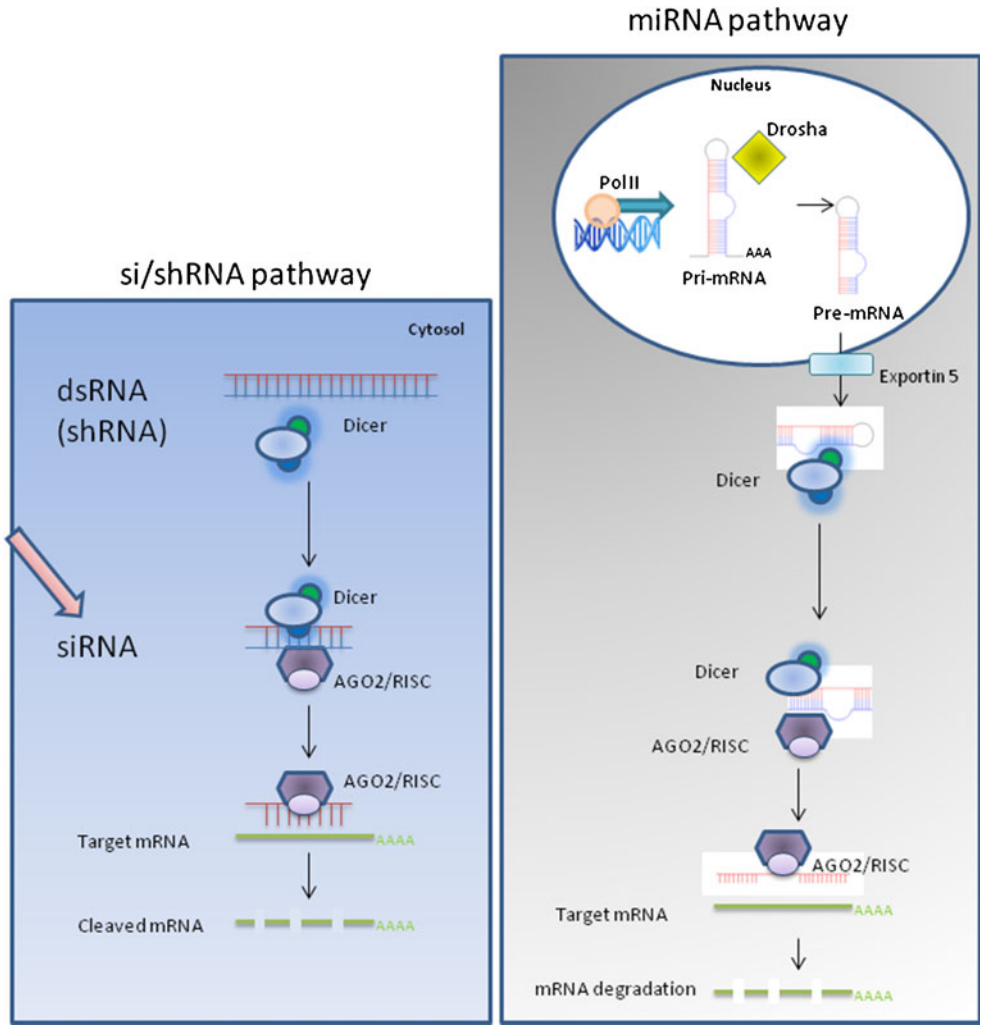

Fig. 1 microRNA and si/shRNA mechanisms of action. RNA interference is a mechanism of gene transcript regulation by transcriptional repression or target transcript degradation. Short interfering RNAs (*siRNAs*), short hairpin RNAs (*shRNAs*) or microRNAs (*miRNAs*) are capable of inducing this mechanism through two similar, but independent, pathways. The microRNA pathway begins with primary microRNA transcripts (*pri-miRNAs*) which are endogenously expressed within the host genome. These pri-miRNAs are transcribed by RNA polymerase II (*Pol II*), processed by Drosha to form precursor miRNAs (*pre-miRNAs*), exported from the nucleus by Exportin 5 and subsequently processed by the Dicer enzyme for loading onto the AGO2–RISC complex. The pre-miRNA which is loaded onto RISC has imperfect sequence complementarity; and therefore, the sense strand is unwound leaving a mature miRNA bound to active RISC. This mature miRNA recognizes target sites typically in the mRNA 3'-UTR, leading to direct translational inhibition or mRNA target degradation in processing (P)-bodies

significant loss in many glioblastoma patient samples [50–53].

Re-expression of these miRNAs in glioblastoma cells has been, in some cases, proven efficacious with respect to reduced proliferation, colony formation, migration and invasion, as is the case for miR-34a [45–48]. Conversely, miR-21 has been extensively studied due to its significant upregulation in glioblastoma and the noted observation that miR-21 inhibition induced apoptosis in glioblastoma cells in vitro [32, 54–56] and in vivo [40, 57, 58]. Although such studies used specific miR-21 inhibitors called anti-miRs, alternative approaches to reduce over-expressed miRNAs include sponge modulators [59]. A sponge modulator acts as a competitive inhibitor of the miRNA binding site. They are transcripts which are expressed from vectors designed with strong promoters and contain multiple binding sites for the microRNA of interest; thereby attracting miRNA binding to the introduced vector as opposed to the native target transcript [60]. This approach has been evaluated by Mei et al. in glioblastoma for the microRNA, miR-146a [61].

Chemoresistance and miRNAs in Glioblastoma

An additional aspect of miRNA study in glioblastoma is the evaluation of miR-based therapeutics as an adjunct therapy in an attempt to improve patient response to current chemotherapeutics. Several groups have evaluated the effect of miRNA

Table 1 Review of experimental miRNA-related papers

miRNA	Expression profile in GBM	Noted effect in vitro	Noted effect in vivo	Reference
524-5p	Decreased	• Expression inhibits cell proliferation, decreases invasion and induces apoptosis	• Subcutaneous tumours reduced after direct injection in lipofectamine	[82]
451	Decreased	• Expression inhibits cell migration but increases proliferation, reduces apoptosis. Expression associated with high glucose levels		[129]
328	Decreased	• Expression suppressed cell proliferation		[130–132]
326	Decreased	• Expression reduces ATP levels through targeting of PKM2. PKM2 siRNA led to reduced colony formation and invasion		[133]
296-3p	Decreased	• Expression leads to reduced proliferation and invasion in GBM cells and increased sensitivity to chemotherapeutics		[134]
181d	Decreased	• Expression induces apoptosis, G1 cell cycle arrest and inhibits proliferation through K-ras and Bcl2 targeting	• Subcutaneous tumours reduced after direct injection in lipofectamine. Once every 3 days for 15 days	[92]
146-5p	Decreased	• Expression reduced invasion and migration but proliferation effect required several days before a significant effect was noted		[135]
146a	Decreased	• Expression enhanced neuronal differentiation, inhibited proliferation, increased cell cycle exit and reduced sphere formation	• Pre-treatment with miR-146a prior to orthotopic implantation of GBM cells lead to increased survival and reduced tumour burden	[61]
145	Decreased	• Treatment of GBM cells with PU-PEI-miR-145 reduced proliferation, invasion and soft agar colony formation. Enhanced sensitivity to radiation and TMZ/cisplatin treatment • Expression of miR-145 as an adjunct therapy to AAV mediated suicide gene therapy (Ad5CMVSVtk retrovirus)	• Pre-treatment of cells or intracranial injection of miRNA reduced tumour growth in orthotopic tumour model. Enhanced sensitivity to radiation and TMZ/cisplatin treatment • In vivo work was done via intracranial administration of 'drug' (Hsvtk + miR-145) showing significant reduction in GBM tumour growth	[63, 96, 136, 137]
128	Decreased	• Expression reduced proliferation in GBM cell lines, with a decrease in cells in S phase but no change in apoptotic G0 phase	• Subcutaneous tumour burden reduced in miR-128 U87 cells • Pre-treatment of cells with miR-128 in subcutaneous model and also a H-RasV12 induced transgenic model of glioma. miR-128 was significantly decreased in these tumours • When H-RasV12 is intracranially injected, miR-128 is co-expressed. Reintroduction of miR-128 significantly increased survival	[138]
125b	Decreased	• Expression resulted in reduced proliferation, sphere formation through direct E2F2 targeting		[139, 140]
124 and 137	Decreased	• Expression of miR-124 and miR-137 enhances neuronal-like differentiation of adult mNSCs and inhibition of proliferation in GBM cell lines		[141]
124/124a	Decreased	• Expression of miR-124 led to reduced sphere formation and inhibition of invasion through SNAI2 reduction • Ectopic expression of miR-124a led to reduced invasion and retrospective association with reduced survival rates	• Subcutaneous injection of pre-treated cells showed that miR-124 lessened tumour burden	[7, 93]

Table 1 (continued)

miRNA	Expression profile in GBM	Noted effect in vitro	Noted effect in vivo	Reference
101	Decreased	• Expression of miR-101 target (EZH2) induced glioma proliferation, migration/invasion and angiogenesis • Inhibition by a peptide inhibitor (DZNep), comparable to miR-101 activity reduced GBM growth	• IV injection of DZNep reduced subcutaneous GBM tumour growth	[90, 91]
34a	Decreased	• Expression of miR-34a inhibited cell proliferation, colony formation, induced cell cycle arrest and inhibited transwell invasion of glioblastoma cells	• Pre-treatment of GBM cells with miR-34a lead to reduced orthotopic brain tumour burden	[50–53]
23b	Decreased in migratory GBM	• Expression of miR-23b inhibited migration in 4 GBM cell lines tested, whereas anti-miR23b enhanced migration		[142]
18	Decreased	• Forced miR-18 re-expression reduced CTGF expression, mediated through Smad3		[143]
7	Decreased	• Expression reduced migration and invasion in GBM cells	• Reintroduction inhibited subcutaneous tumour formation and reduced lung metastasis	[88, 144]
26	Increased	• MiR-26a directly targets the 3'UTR of PTEN, and also EZH2 and SMAD1. Inhibition of miR-26 leads to partial inhibition of GBM proliferation and growth but not as much as PTEN deletion	• Reintroduction leads to partial reduction in tumour latency	[86]
21	Increased	• MiR-21 inhibition induced apoptosis, and the presence of taxol enhanced this effect further, depressing viability and increasing caspase-3 expression. Also miR21 inhibition +/− taxol resulted in reduced invasive potential	• Pre-treatment and intracranial injection lead to reduced tumour burden in glioma cells where miR-21 was inhibited. Inhibition of miR-21 allowed re-expression of Pdcd4, and reduction in tumour growth in subcutaneous model of GBM	[32, 40, 54, 56–58]
21 30b/c	Increased	• Expression of miR-21 and 30b/c induced resistance to TRAIL and anti-miR-21 or anti-miR-30b/c induced sensitivity to TRAIL. MiR21 acts directly on TAp63 and miR-30b/c on caspase 3		[55]
10b	Increased	• Inhibition reduced cell growth, led to cell cycle arrest and apoptosis through Bim, TFAP2c, p16 and p21 targeting • As an alternative to miR-10b inhibition, a 'decoy vector' which had 13 repeats of the miR-10b binding site was generated. Inhibition of miR-10b decreased invasiveness of the U87-2MI cells, decreased angiogenicity and enhanced apoptosis	• Subcutaneous tumour burden reduced after 3 intratumoural injections (1 per day for 3 days) and reduced proliferation (Ki-67) and increased TUNEL positive cells • Pretreatment with miR-10b decoy versus control vector reduced GBM cell invasion, growth and vascularisation	[84, 87]

modulation in enhancing glioblastoma chemosensitivity to temozolomide [62–64], taxol [56, 65] and cisplatin [66], reducing the intrinsic resistance of glioblastoma cells to current therapeutics. Although this approach would benefit patients from the point of initial diagnosis, acquired resistance remains a major obstacle to improvements in current glioblastoma treatment. As mentioned previously, distal tumours often occur in these patients after primary TMZ/RT treatment which are refractory to additional chemotherapy; and although several hypothesis have been proposed as to why such a resistance occurs after initial treatment [67], there are currently no biomarkers or molecular indicators to suggest genetic predisposition to acquired resistance in glioblastoma. Initial studies have assessed the differential expression of miRNAs in acquired resistance in vitro providing potential avenues of miR-based targeting to improve patient treatment response [68, 69].

Multidrug resistance proteins (MRPs) are ATP-binding cassette transporters (ABC transporters) which have previously been identified to play a significant role in drug resistance in several forms of cancer [67, 70, 71], and in 1994, Abe et al. identified a potential role for MRP transporter proteins in drug-resistant glioma cells [72], specifically over-expression of MRP1, 3, 4, 5 (ABCC1, ABCC3, ABCC4 and ABCC5, respectively) and ABCG2 [71, 73]. Although small molecule inhibition of the MRP1 transporter has been evaluated with respect to other forms of cancer such as neuroblastoma [74], and although high grade glioblastoma patients exhibit vascular permeability [75], effective delivery across the BBB in glioblastoma has yet to be evaluated. Although there is no absolute cut-off with respect to molecular size of compound transport across the BBB [76, 77], passive transport is facilitated in molecules under 500D, with low hydrogen bonding capability and lipophilicity, criteria which many current chemotherapies fail to achieve [78]. As a means of evaluating alternative anti-cancer molecules, with potential of crossing the BBB, miRNAs which have altered expression in glioblastoma have been identified and studied. With respect to ABC transporters, miRNA-328 targeting of ABCG2 [79] or miR-9 effect on ABCC3 [80] was assessed in an attempt to improve glioblastoma cell exposure to chemotherapy treatment. Notably, however, such studies were carried out in vitro.

In Vivo Research of miRNAs in GBM

In reviewing several papers detailing the effects of miRNAs in glioblastoma, it was obvious that many of these studies are focused on in vitro work, and the in vivo applications of many of these regulatory molecules in glioblastoma progression have not yet been evaluated. Of significant importance is the fact that those publications which did undertake in vivo research focused on evaluation of subcutaneous tumour formation subsequent to glioblastoma cell inoculation [40, 58, 81–95], orthotopic intracranial injection of glioblastoma cells which were pre-treated with a particular miRNA [52, 53, 57, 61, 63, 96] or direct intracranial injection of a chosen miRNA to an orthotopic model of glioblastoma [57, 63]. A single study evaluated the effect of systemic delivery of a miRNA target protein (EZH2) inhibitor (DZNep), as a comparative molecule to the miRNA (miR-101) itself, with respect to orthotopic glioblastoma tumour formation [91]. This approach, however, eliminated the multi-gene target effect which miR-101 may possess in glioblastoma.

Major limitations in using microRNAs for in vivo therapeutics are their rapid degradation in serum conditions, their lack of reliable delivery to the intracellular space [97] and, when combined with the intrinsic challenges of delivery across the BBB, many researchers have focused on direct, rather than systemic, administration of miR-based therapeutics [57, 63]. Notably, the angiogenic and migratory potential of glioblastoma cannot be reflected in subcutaneous tumour models due to the absence of the brain microenvironment which is known to play a significant role in glioblastoma pathogenesis and drug resistance [98–105]. Therefore, translational research requires the use of orthotopic models of glioblastoma in combination with systemic delivery of a potential therapeutic. In a clinical setting, systemic intravenous delivery of potential therapeutics to glioblastoma patients is more favourable with respect to willing involvement in clinical trials due, in part, to the minimal disruption experienced by the patient as the drug of interest may be administered in conjunction with currently established chemotherapy treatment regimens.

When questioning the potential which miR-based therapeutics holds for chronic diseases such as cancer, attention must be drawn to the recent initiation of a phase IIa clinical trial using an anti-miR-122-based drug, miravirsen (SPC3649), in patients with hepatitis C [106] by Regulus Therapeutics™; also additional research into miR-21 for hepatocellular carcinoma, kidney fibrosis, miR-33 for atherosclerosis and miR-10b for glioblastoma research progression is the primary focus within this company's therapeutic pipeline [107]. Similarly, Mirna Therapeutics™ focuses its research on miRNAs which affect solid and blood-borne tumours, such as hepatocellular carcinoma, non-small cell lung cancer, colorectal cancer and pancreatic carcinoma, with MRX34 (miR-34a mimic) due to progress to clinical development in 2013 [108]. Additionally, Miragen Therapeutics™ has several miRNAs which are nearing the end of preclinical assessment in a variety of chronic and cancer-related conditions. Although such commercialized research shows translational promise for miR-based therapeutics, a major challenge for such therapeutics however, as with many drugs, will be stability, and in the case of miR-10b for glioblastoma, successful delivery across the BBB.

Potentials for miRNA Delivery Across BBB

Several studies have been undertaken to assess the delivery of exogenously administered microRNAs in various forms of cancer. In most cases, miRNAs have been encapsulated in lipid- or nanoparticle-based molecules. The system of choice for miRNA, or anti-miR, delivery plays a critical role in the delivery of an effective molecule capable of modulating gene expression. Studies involving siRNA molecules have found that delivery systems possessing an external cationic charge can lead to stimulation of the host immune system [109]. Additional studies have shown that certain delivery systems such as polypropylenimne dendrimer encapsulation may lead to induction of particular 'gene signatures' not normally present within the target cell, thereby leading to an increased potential of off-target effects of the siRNA or miRNA being delivered [110–112]. The choice of an efficient delivery system is also dependent upon controlled release of the miRNA/siRNA molecule into the intracellular cytosol without degradation in endosomes or lysosomes [113]. Similar to previously described studies using miRNAs, or anti-miRs, for brain tumour research, CNS delivery of siRNA has been through localised intratumoural or intrathecal injection [114] due to challenges in BBB penetration and biodistribution after systemic administration. Notably, the first and only clinical trial for RNAi-based treatment of inoperable brain tumours required invasive surgery and direct injection of the siRNA to the brain [115], although several studies have been evaluated in vitro as reviewed by Guo et al. [116].

More recently, scientific studies have been undertaken to evaluate the potential use of nanoparticles for a variety of applications including cancer imaging [117, 118] and molecule delivery [119]. Several studies have used nanoparticles which have been modified to enable encapsulation of miRNAs for tumour-specific delivery and cancer cell death in numerous cancers including neuroblastoma [66, 120–122], lymphoma [123] and head and neck squamous cell carcinoma [124]. Such encapsulation of miRNAs, or indeed anti-miRs, within biodegradable nanoparticles may be applied to numerous miRNAs of interest in glioblastoma research. The chemistry of nanoparticle composition plays an important role with respect to cargo encapsulation, transport across the BBB and successful endocytosis, content release and degradation within the tumour cell. As discussed previously, exosomes are naturally occurring RNA nano-vesicles which may be harnessed for miRNA transport across the BBB. Alvarez-Erviti et al. delivered Cy5-labelled siRNA molecules contained in immunological inert exosomes to brain regions in an in vivo model of Alzheimer's disease after systemic administration [125]. Loading of exosomes may potentially be exploited for efficient systemic delivery of miRNAs to an orthotopic model of glioblastoma. Hwang do et al. carried out a study using cysteine residue-modified polyethyleneimine (SSPEI) to transport miR-124 to the brain in vivo. This study was focused on the delivery of a functional miRNA molecule across the BBB after systemic administration via tail vein injection. Notably, this study utilized an isomer of sorbitol (Mannitol) which temporarily shrinks the endothelial cells of the BBB, stretching the tight junction between them [126], facilitating temporary 'opening' of the BBB. This study proved that SSPEI-miR124 was successfully transported across the BBB to the brain parenchyma in vivo [97]. Alternative methodologies including siRNA targeting of the tight junction protein, claudin-5, have been used to deliver small peptide-based molecules into the brain [127]. To date, however, nanoparticle delivery of miRNA molecules in glioblastoma has only been assessed in vitro [56].

The aggressive progression and dismal prognosis associated with glioblastoma diagnosis have led clinicians and researchers alike to explore new avenues of potential treatment for these patients. MicroRNAs are involved in crucial biological processes, not only in healthy cells but also in cancer cell biology, including differentiation, proliferation and apoptosis. As detailed in Table 1, exogenous altered expression of several miRNAs in glioblastoma leads to significant effects on glioblastoma cell morphology, invasiveness and tumourigenicity. The findings of such studies yield great promise to the hypothesis that miRNAs may be used to target specific traits of glioblastoma cell biology, for example migration and invasion potential, angiogenic capabilities, heterogeneity and resistance to current treatment regimes [128] (refer to Table 1). The advent of nanoparticle-mediated delivery and recent progression in RNAi- molecule delivery across the BBB to both CNS disease and cancer models proves encouraging to the progression of miR-based therapeutic efficacy in this debilitating disease. Considering the development of miRNA research from initial association to glioblastoma [32, 33] to commercial development of miR-based therapeutics [106–108] in less than a decade, it is not beyond reasonable doubt to anticipate significant advancements in this field of study, hopefully with the ultimate conclusion of improved patient outcome.

References

1. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P (2007) The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 114(2):97–109. doi:10.1007/s00401-007-0243-4
2. Fuller GN (2008) The WHO classification of tumours of the central nervous system, 4th edition. Arch Pathol Lab Med 132(6):906. doi:10.1043/1543-2165
3. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO

(2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352(10):987–996. doi:10.1056/NEJMoa043330
4. Monticone M, Daga A, Candiani S, Romeo F, Mirisola V, Viaggi S, Melloni I, Pedemonte S, Zona G, Giaretti W, Pfeffer U, Castagnola P (2012) Identification of a novel set of genes reflecting different in vivo invasive patterns of human GBM cells. BMC cancer 12:358. doi:10.1186/1471-2407-12-358
5. Okajima K, Ohta Y (2012) Diagnostic imaging of high-grade astrocytoma: heterogeneity of clinical manifestation, image characteristics, and histopathological findings. Brain and Nerve = Shinkei kenkyu no shinpo 64(10):1151–1157
6. McDonald KL, McDonnell J, Muntoni A, Henson JD, Hegi ME, von Deimling A, Wheeler HR, Cook RJ, Biggs MT, Little NS, Robinson BG, Reddel RR, Royds JA (2010) Presence of alternative lengthening of telomeres mechanism in patients with glioblastoma identifies a less aggressive tumor type with longer survival. J Neuropathol Exp Neurol 69(7):729–736. doi:10.1097/NEN.0b013e3181e576cf
7. Fowler A, Thomson D, Giles K, Maleki S, Mreich E, Wheeler H, Leedman P, Biggs M, Cook R, Little N, Robinson B, McDonald K (2011) miR-124a is frequently down-regulated in glioblastoma and is involved in migration and invasion. Eur J Cancer 47(6):953–963. doi:10.1016/j.ejca.2010.11.026
8. Gautam P, Nair SC, Gupta MK, Sharma R, Polisetty RV, Uppin MS, Sundaram C, Puligopu AK, Ankathi P, Purohit AK, Chandak GR, Harsha HC, Sirdeshmukh R (2012) Proteins with altered levels in plasma from glioblastoma patients as revealed by iTRAQ-based quantitative proteomic analysis. PLoS One 7(9):e46153. doi:10.1371/journal.pone.0046153
9. Persano L, Rampazzo E, Basso G, Viola G (2013) Glioblastoma cancer stem cells: role of the microenvironment and therapeutic targeting. Biochem Pharmacol 85(5):612–622. doi:10.1016/j.bcp.2012.10.001
10. Calin GA, Croce CM (2006) MicroRNA signatures in human cancers. Nat Rev Cancer 6(11):857–866. doi:10.1038/nrc1997
11. Delfino KR, Serao NV, Southey BR, Rodriguez-Zas SL (2011) Therapy-, gender- and race-specific microRNA markers, target genes and networks related to glioblastoma recurrence and survival. Cancer Genomics Proteomics 8(4):173–183
12. Huse JT, Holland E, DeAngelis LM (2013) Glioblastoma: molecular analysis and clinical implications. Ann Rev Med 64:59–70. doi:10.1146/annurev-med-100711-143028
13. Liu G, Yuan X, Zeng Z, Tunici P, Ng H, Abdulkadir IR, Lu L, Irvin D, Black KL, Yu JS (2006) Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. Mol Cancer 5:67. doi:10.1186/1476-4598-5-67
14. Srinivasan S, Patric IR, Somasundaram K (2011) A ten-microRNA expression signature predicts survival in glioblastoma. PLoS One 6(3):e17438. doi:10.1371/journal.pone.0017438
15. Visnyei K, Onodera H, Damoiseaux R, Saigusa K, Petrosyan S, De Vries D, Ferrari D, Saxe J, Panosyan EH, Masterman-Smith M, Mottahedeh J, Bradley KA, Huang J, Sabatti C, Nakano I, Kornblum HI (2011) A molecular screening approach to identify and characterize inhibitors of glioblastoma stem cells. Mol Cancer Ther 10(10):1818–1828. doi:10.1158/1535-7163.MCT-11-0268
16. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, Bromberg JE, Hau P, Mirimanoff RO, Cairncross JG, Janzer RC, Stupp R (2005) MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 352(10):997–1003. doi:10.1056/NEJMoa043331
17. Hegi ME, Liu L, Herman JG, Stupp R, Wick W, Weller M, Mehta MP, Gilbert MR (2008) Correlation of O6-methylguanine methyltransferase (MGMT) promoter methylation with clinical outcomes in glioblastoma and clinical strategies to modulate MGMT activity. J Clin Oncol Off J Am Soc Clin Oncol 26(25):4189–4199. doi:10.1200/JCO.2007.11.5964
18. Parkinson JF, Wheeler HR, Clarkson A, McKenzie CA, Biggs MT, Little NS, Cook RJ, Messina M, Robinson BG, McDonald KL (2008) Variation of O(6)-methylguanine-DNA methyltransferase (MGMT) promoter methylation in serial samples in glioblastoma. J Neurooncol 87(1):71–78. doi:10.1007/s11060-007-9486-0
19. Yoshimoto K, Mizoguchi M, Hata N, Murata H, Hatae R, Amano T, Nakamizo A, Sasaki T (2012) Complex DNA repair pathways as possible therapeutic targets to overcome temozolomide resistance in glioblastoma. Frontiers in oncology 2:186. doi:10.3389/fonc.2012.00186
20. Lakomy R, Sana J, Hankeova S, Fadrus P, Kren L, Lzicarova E, Svoboda M, Dolezelova H, Smrcka M, Vyzula R, Michalek J, Hajduch M, Slaby O (2011) MiR-195, miR-196b, miR-181c, miR-21 expression levels and O-6-methylguanine-DNA methyltransferase methylation status are associated with clinical outcome in glioblastoma patients. Cancer Sci 102(12):2186–2190. doi:10.1111/j.1349-7006.2011.02092.x
21. Pegg AE (1984) Methylation of the O6 position of guanine in DNA is the most likely initiating event in carcinogenesis by methylating agents. Cancer investigation 2(3):223–231
22. Wick W, Hartmann C, Engel C, Stoffels M, Felsberg J, Stockhammer F, Sabel MC, Koeppen S, Ketter R, Meyermann R, Rapp M, Meisner C, Kortmann RD, Pietsch T, Wiestler OD, Ernemann U, Bamberg M, Reifenberger G, von Deimling A, Weller M (2009) NOA-04 randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with procarbazine, lomustine, and vincristine or temozolomide. J Clin Oncol Off J Am Soc Clin Oncol 27(35):5874–5880. doi:10.1200/JCO.2009.23.6497
23. Wurdinger T, Tannous BA (2009) Glioma angiogenesis: towards novel RNA therapeutics. Cell Adhes Migr 3(2):230–235
24. de Fougerolles A, Vornlocher HP, Maraganore J, Lieberman J (2007) Interfering with disease: a progress report on siRNA-based therapeutics. Nat Rev Drug Disc 6(6):443–453. doi:10.1038/nrd2310
25. Thery C (2011) Exosomes: secreted vesicles and intercellular communications. F1000 biology reports 3:15. doi:10.3410/B3-15
26. Hu G, Drescher KM, Chen XM (2012) Exosomal miRNAs: biological properties and therapeutic potential. Front Genet 3:56. doi:10.3389/fgene.2012.00056
27. Montecalvo A, Larregina AT, Shufesky WJ, Stolz DB, Sullivan ML, Karlsson JM, Baty CJ, Gibson GA, Erdos G, Wang Z, Milosevic J, Tkacheva OA, Divito SJ, Jordan R, Lyons-Weiler J, Watkins SC, Morelli AE (2012) Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes. Blood 119(3):756–766. doi:10.1182/blood-2011-02-338004
28. Skog J, Wurdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, Curry WT Jr, Carter BS, Krichevsky AM, Breakefield XO (2008) Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat Cell Biol 10(12):1470–1476. doi:10.1038/ncb1800
29. Katakowski M, Buller B, Wang X, Rogers T, Chopp M (2010) Functional microRNA is transferred between glioma cells. Cancer Res 70(21):8259–8263. doi:10.1158/0008-5472.CAN-10-0604
30. Lee RC, Feinbaum RL, Ambros V (1993) The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75(5):843–854
31. Kozomara A, Griffiths-Jones S (2011) miRBase: integrating microRNA annotation and deep-sequencing data. Nucleic acids research 39 (Database issue):D152-157. doi:10.1093/nar/gkq1027
32. Chan JA, Krichevsky AM, Kosik KS (2005) MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res 65(14):6029–6033. doi:10.1158/0008-5472.CAN-05-0137

33. Ciafre SA, Galardi S, Mangiola A, Ferracin M, Liu CG, Sabatino G, Negrini M, Maira G, Croce CM, Farace MG (2005) Extensive modulation of a set of microRNAs in primary glioblastoma. Biochem Biophys Res Commun 334(4):1351–1358. doi:10.1016/j.bbrc.2005.07.030
34. Moller HG, Rasmussen AP, Andersen HH, Johnsen KB, Henriksen M, Duroux M (2013) A systematic review of microRNA in glioblastoma multiforme: micro-modulators in the mesenchymal mode of migration and invasion. Mol Neurobiol 47(1):131–144. doi:10.1007/s12035-012-8349-7
35. Esquela-Kerscher A, Slack FJ (2006) Oncomirs—microRNAs with a role in cancer. Nat Rev Cancer 6(4):259–269. doi:10.1038/nrc1840
36. Ryan BM, Robles AI, Harris CC (2010) Genetic variation in microRNA networks: the implications for cancer research. Nat Rev Cancer 10(6):389–402. doi:10.1038/nrc2867
37. Wang Q, Li P, Li A, Jiang W, Wang H, Wang J, Xie K (2012) Plasma specific miRNAs as predictive biomarkers for diagnosis and prognosis of glioma. Journal of experimental & clinical cancer research : CR 31:97. doi:10.1186/1756-9966-31-97
38. Lages E, Guttin A, El Atifi M, Ramus C, Ipas H, Dupre I, Rolland D, Salon C, Godfraind C, deFraipont F, Dhobb M, Pelletier L, Wion D, Gay E, Berger F, Issartel JP (2011) MicroRNA and target protein patterns reveal physiopathological features of glioma subtypes. PLoS One 6(5):e20600. doi:10.1371/journal.pone.0020600
39. Novakova J, Slaby O, Vyzula R, Michalek J (2009) MicroRNA involvement in glioblastoma pathogenesis. Biochem Biophys Res Commun 386(1):1–5. doi:10.1016/j.bbrc.2009.06.034
40. Gabriely G, Wurdinger T, Kesari S, Esau CC, Burchard J, Linsley PS, Krichevsky AM (2008) MicroRNA 21 promotes glioma invasion by targeting matrix metalloproteinase regulators. Mol Cell Biol 28(17):5369–5380. doi:10.1128/MCB.00479-08
41. Ilhan-Mutlu A, Wagner L, Wohrer A, Jungwirth S, Marosi C, Fischer P, Preusser M (2012) Blood alterations preceding clinical manifestation of glioblastoma. Cancer Investig 30(9):625–629. doi:10.3109/07357907.2012.725443
42. Ilhan-Mutlu A, Wagner L, Wohrer A, Furtner J, Widhalm G, Marosi C, Preusser M (2012) Plasma MicroRNA-21 concentration may be a useful biomarker in glioblastoma patients. Cancer Investig 30(8):615–621. doi:10.3109/07357907.2012.708071
43. Ma R, Yan W, Zhang G, Lv H, Liu Z, Fang F, Zhang W, Zhang J, Tao T, You Y, Jiang T, Kang X (2012) Upregulation of miR-196b confers a poor prognosis in glioblastoma patients via inducing a proliferative phenotype. PLoS One 7(6):e38096. doi:10.1371/journal.pone.0038096
44. Zhang W, Zhang J, Hoadley K, Kushwaha D, Ramakrishnan V, Li S, Kang C, You Y, Jiang C, Song SW, Jiang T, Chen CC (2012) miR-181d: a predictive glioblastoma biomarker that downregulates MGMT expression. Neuro-Oncology 14(6):712–719. doi:10.1093/neuonc/nos089
45. Niyazi M, Zehentmayr F, Niemoller OM, Eigenbrod S, Kretzschmar H, Schulze-Osthoff K, Tonn JC, Atkinson M, Mortl S, Belka C (2011) MiRNA expression patterns predict survival in glioblastoma. Radiat Oncol 6:153. doi:10.1186/1748-717X-6-153
46. Roth P, Wischhusen J, Happold C, Chandran PA, Hofer S, Eisele G, Weller M, Keller A (2011) A specific miRNA signature in the peripheral blood of glioblastoma patients. J Neurochem 118(3):449–457. doi:10.1111/j.1471-4159.2011.07307.x
47. Wuchty S, Arjona D, Li A, Kotliarov Y, Walling J, Ahn S, Zhang A, Maric D, Anolik R, Zenklusen JC, Fine HA (2011) Prediction of associations between microRNAs and gene expression in glioma biology. PLoS One 6(2):e14681. doi:10.1371/journal.pone.0014681
48. Guan Y, Mizoguchi M, Yoshimoto K, Hata N, Shono T, Suzuki SO, Araki Y, Kuga D, Nakamizo A, Amano T, Ma X, Hayashi K, Sasaki T (2010) MiRNA-196 is upregulated in glioblastoma but not in anaplastic astrocytoma and has prognostic significance. Clin Cancer Res Official J Am Assoc Cancer Res 16(16):4289–4297. doi:10.1158/1078-0432.CCR-10-0207
49. Wooten EC, Fults D, Duggirala R, Williams K, Kyritsis AP, Bondy ML, Levin VA, O'Connell P (1999) A study of loss of heterozygosity at 70 loci in anaplastic astrocytoma and glioblastoma multiforme with implications for tumor evolution. Neuro-Oncology 1(3):169–176
50. Guessous F, Zhang Y, Kofman A, Catania A, Li Y, Schiff D, Purow B, Abounader R (2010) microRNA-34a is tumor suppressive in brain tumors and glioma stem cells. Cell Cycle 9(6):1031–1036
51. Li WB, Ma MW, Dong LJ, Wang F, Chen LX, Li XR (2011) MicroRNA-34a targets notch1 and inhibits cell proliferation in glioblastoma multiforme. Cancer Biol Ther 12(6):477–483. doi:10.4161/cbt.12.6.16300
52. Li Y, Guessous F, Zhang Y, Dipierro C, Kefas B, Johnson E, Marcinkiewicz L, Jiang J, Yang Y, Schmittgen TD, Lopes B, Schiff D, Purow B, Abounader R (2009) MicroRNA-34a inhibits glioblastoma growth by targeting multiple oncogenes. Cancer Res 69(19):7569–7576. doi:10.1158/0008-5472.CAN-09-0529
53. Silber J, Jacobsen A, Ozawa T, Harinath G, Pedraza A, Sander C, Holland EC, Huse JT (2012) miR-34a repression in proneural malignant gliomas upregulates expression of its target PDGFRA and promotes tumorigenesis. PLoS One 7(3):e33844. doi:10.1371
54. Papagiannakopoulos T, Shapiro A, Kosik KS (2008) MicroRNA-21 targets a network of key tumor-suppressive pathways in glioblastoma cells. Cancer Res 68(19):8164–8172. doi:10.1158/0008-5472.CAN-08-1305
55. Quintavalle C, Donnarumma E, Iaboni M, Roscigno G, Garofalo M, Romano G, Fiore D, De Marinis P, Croce CM, Condorelli G (2012) Effect of miR-21 and miR-30b/c on TRAIL-induced apoptosis in glioma cells. Oncogene. doi:10.1038/onc.2012.410
56. Ren Y, Zhou X, Mei M, Yuan XB, Han L, Wang GX, Jia ZF, Xu P, Pu PY, Kang CS (2010) MicroRNA-21 inhibitor sensitizes human glioblastoma cells U251 (PTEN-mutant) and LN229 (PTEN-wild type) to taxol. BMC cancer 10:27. doi:10.1186/1471-2407-10-27
57. Corsten MF, Miranda R, Kasmieh R, Krichevsky AM, Weissleder R, Shah K (2007) MicroRNA-21 knockdown disrupts glioma growth in vivo and displays synergistic cytotoxicity with neural precursor cell delivered S-TRAIL in human gliomas. Cancer Res 67(19):8994–9000. doi:10.1158/0008-5472.CAN-07-1045
58. Gaur AB, Holbeck SL, Colburn NH, Israel MA (2011) Downregulation of Pdcd4 by mir-21 facilitates glioblastoma proliferation in vivo. Neuro-Oncology 13(6):580–590. doi:10.1093/neuonc/nor033
59. Ebert MS, Neilson JR, Sharp PA (2007) MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. Nature methods 4(9):721–726. doi:10.1038/nmeth1079
60. Sumazin P, Yang X, Chiu HS, Chung WJ, Iyer A, Llobet-Navas D, Rajbhandari P, Bansal M, Guarnieri P, Silva J, Califano A (2011) An extensive microRNA-mediated network of RNA-RNA interactions regulates established oncogenic pathways in glioblastoma. Cell 147(2):370–381. doi:10.1016/j.cell.2011.09.041
61. Mei J, Bachoo R, Zhang CL (2011) MicroRNA-146a inhibits glioma development by targeting Notch1. Mol Cell Biol 31(17):3584–3592. doi:10.1128/MCB.05821-11
62. Wong ST, Zhang XQ, Zhuang JT, Chan HL, Li CH, Leung GK (2012) MicroRNA-21 inhibition enhances in vitro chemosensitivity of temozolomide-resistant glioblastoma cells. Anticancer Res 32(7):2835–2841
63. Yang YP, Chien Y, Chiou GY, Cherng JY, Wang ML, Lo WL, Chang YL, Huang PI, Chen YW, Shih YH, Chen MT, Chiou SH (2012) Inhibition of cancer stem cell-like properties and reduced chemoradioresistance of glioblastoma using microRNA145 with

cationic polyurethane-short branch PEI. Biomaterials 33(5):1462–1476. doi:10.1016/j.biomaterials.2011.10.071
64. Asuthkar S, Velpula KK, Chetty C, Gorantla B, Rao JS (2012) Epigenetic regulation of miRNA-211 by MMP-9 governs glioma cell apoptosis, chemosensitivity and radiosensitivity. Oncotarget 3(11):1439–1454
65. Meng W, Jiang L, Lu L, Hu H, Yu H, Ding D, Xiao K, Zheng W, Guo H, Ma W (2012) Anti-miR-155 oligonucleotide enhances chemosensitivity of U251 cell to taxol by inducing apoptosis. Cell Biol Int 36(7):653–659. doi:10.1042/CBI20100918
66. Ryan J, Tivnan A, Fay J, Bryan K, Meehan M, Creevey L, Lynch J, Bray IM, O'Meara A, Davidoff AM, Stallings RL (2012) MicroRNA-204 increases sensitivity of neuroblastoma cells to cisplatin and is associated with a favourable clinical outcome. Br J Cancer 107(6):967–976. doi:10.1038/bjc.2012.356
67. Gottesman MM (2002) Mechanisms of cancer drug resistance. Annu Rev Med 53:615–627. doi:10.1146/annurev.med.53.082901.103929
68. Sasaki A, Udaka Y, Tsunoda Y, Yamamoto G, Tsuji M, Oyamada H, Oguchi K, Mizutani T (2012) Analysis of p53 and miRNA expression after irradiation of glioblastoma cell lines. Anticancer Res 32(11):4709–4713
69. Ujifuku K, Mitsutake N, Takakura S, Matsuse M, Saenko V, Suzuki K, Hayashi K, Matsuo T, Kamada K, Nagata I, Yamashita S (2010) miR-195, miR-455-3p and miR-10a(*) are implicated in acquired temozolomide resistance in glioblastoma multiforme cells. Cancer Lett 296(2):241–248. doi:10.1016/j.canlet.2010.04.013
70. Takeuchi K, Shibata M, Kashiyama E, Umehara K (2012) Expression levels of multidrug resistance-associated protein 4 (MRP4) in human leukemia and lymphoma cell lines, and the inhibitory effects of the MRP-specific inhibitor MK-571 on methotrexate distribution in rats. Exp Ther Med 4(3):524–532. doi:10.3892/etm.2012.627
71. Quezada C, Peignan L, Segura R, Riquelme F, Melo R, Rojas ZD, Ayach F, San Martin R, Carcamo JG (2011) Study of resistance to chemotherapy mediated by ABC transporters in biopsies of glioblastoma multiforme. Revista medica de Chile 139(4):415–424. doi:S0034-98872011000400001
72. Abe T, Hasegawa S, Taniguchi K, Yokomizo A, Kuwano T, Ono M, Mori T, Hori S, Kohno K, Kuwano M (1994) Possible involvement of multidrug-resistance-associated protein (MRP) gene expression in spontaneous drug resistance to vincristine, etoposide and adriamycin in human glioma cells. Int J Cancer J Int du cancer 58(6):860–864
73. Decleves X, Fajac A, Lehmann-Che J, Tardy M, Mercier C, Hurbain I, Laplanche JL, Bernaudin JF, Scherrmann JM (2002) Molecular and functional MDR1-Pgp and MRPs expression in human glioblastoma multiforme cell lines. Int J Cancer J Int du cancer 98(2):173–180
74. Burkhart CA, Watt F, Murray J, Pajic M, Prokvolit A, Xue C, Flemming C, Smith J, Purmal A, Isachenko N, Komarov PG, Gurova KV, Sartorelli AC, Marshall GM, Norris MD, Gudkov AV, Haber M (2009) Small-molecule multidrug resistance-associated protein 1 inhibitor reversan increases the therapeutic index of chemotherapy in mouse models of neuroblastoma. Cancer Res 69(16):6573–6580. doi:10.1158/0008-5472.CAN-09-1075
75. Yuan F, Salehi HA, Boucher Y, Vasthare US, Tuma RF, Jain RK (1994) Vascular permeability and microcirculation of gliomas and mammary carcinomas transplanted in rat and mouse cranial windows. Cancer Res 54(17):4564–4568
76. Nau R, Sorgel F, Eiffert H (2010) Penetration of drugs through the blood-cerebrospinal fluid/blood–brain barrier for treatment of central nervous system infections. Clin Microbiol Rev 23(4):858–883. doi:10.1128/CMR.00007-10
77. Yoon CH, Kim SJ, Shin BS, Lee KC, Yoo SD (2006) Rapid screening of blood–brain barrier penetration of drugs using the immobilized artificial membrane phosphatidylcholine column chromatography. J Biomol Screen 11(1):13–20. doi:10.1177/1087057105281656
78. Gabathuler R (2010) Approaches to transport therapeutic drugs across the blood–brain barrier to treat brain diseases. Neurobiol Dis 37(1):48–57. doi:10.1016/j.nbd.2009.07.028
79. Li WQ, Li YM, Tao BB, Lu YC, Hu GH, Liu HM, He J, Xu Y, Yu HY (2010) Downregulation of ABCG2 expression in glioblastoma cancer stem cells with miRNA-328 may decrease their chemoresistance. Med Sci Monit Int Med J Exp Clin Res 16(10):HY27–30
80. Jeon HM, Sohn YW, Oh SY, Kim SH, Beck S, Kim S, Kim H (2011) ID4 imparts chemoresistance and cancer stemness to glioma cells by derepressing miR-9*-mediated suppression of SOX2. Cancer Res 71(9):3410–3421. doi:10.1158/0008-5472.CAN-10-3340
81. Almog N, Ma L, Schwager C, Brinkmann BG, Beheshti A, Vajkoczy P, Folkman J, Hlatky L, Abdollahi A (2012) Consensus micro RNAs governing the switch of dormant tumors to the fast-growing angiogenic phenotype. PLoS One 7(8):e44001. doi:10.1371/journal.pone.0044001
82. Chen L, Zhang W, Yan W, Han L, Zhang K, Shi Z, Zhang J, Wang Y, Li Y, Yu S, Pu P, Jiang C, Jiang T, Kang C (2012) The putative tumor suppressor miR-524-5p directly targets Jagged-1 and Hes-1 in glioma. Carcinogenesis 33(11):2276–2282. doi:10.1093/carcin/bgs261
83. Choudhury Y, Tay FC, Lam DH, Sandanaraj E, Tang C, Ang BT, Wang S (2012) Attenuated adenosine-to-inosine editing of microRNA-376a* promotes invasiveness of glioblastoma cells. J Clin Investig 122(11):4059–4076. doi:10.1172/JCI62925
84. Gabriely G, Yi M, Narayan RS, Niers JM, Wurdinger T, Imitola J, Ligon KL, Kesari S, Esau C, Stephens RM, Tannous BA, Krichevsky AM (2011) Human glioma growth is controlled by microRNA-10b. Cancer Res 71(10):3563–3572. doi:10.1158/0008-5472.CAN-10-3568
85. Godlewski J, Nowicki MO, Bronisz A, Williams S, Otsuki A, Nuovo G, Raychaudhury A, Newton HB, Chiocca EA, Lawler S (2008) Targeting of the Bmi-1 oncogene/stem cell renewal factor by microRNA-128 inhibits glioma proliferation and self-renewal. Cancer Res 68(22):9125–9130. doi:10.1158/0008-5472.CAN-08-2629
86. Huse JT, Brennan C, Hambardzumyan D, Wee B, Pena J, Rouhanifard SH, Sohn-Lee C, le Sage C, Agami R, Tuschl T, Holland EC (2009) The PTEN-regulating microRNA miR-26a is amplified in high-grade glioma and facilitates gliomagenesis in vivo. Genes Dev 23(11):1327–1337. doi:10.1101/gad.1777409
87. Lin J, Teo S, Lam DH, Jeyaseelan K, Wang S (2012) MicroRNA-10b pleiotropically regulates invasion, angiogenicity and apoptosis of tumor cells resembling mesenchymal subtype of glioblastoma multiforme. Cell death & disease 3:e398. doi:10.1038/cddis.2012.134
88. Lu ZJ, Liu SY, Yao YQ, Zhou YJ, Zhang S, Dai L, Tian HW, Zhou Y, Deng HX, Yang JL, Luo F (2011) The effect of miR-7 on behavior and global protein expression in glioma cell lines. Electrophoresis 32(24):3612–3620. doi:10.1002/elps.201100230
89. Papagiannakopoulos T, Friedmann-Morvinski D, Neveu P, Dugas JC, Gill RM, Huillard E, Liu C, Zong H, Rowitch DH, Barres BA, Verma IM, Kosik KS (2012) Pro-neural miR-128 is a glioma tumor suppressor that targets mitogenic kinases. Oncogene 31(15):1884–1895. doi:10.1038/onc.2011.380
90. Smits M, Mir SE, Nilsson RJ, van der Stoop PM, Niers JM, Marquez VE, Cloos J, Breakefield XO, Krichevsky AM, Noske DP, Tannous BA, Wurdinger T (2011) Down-regulation of miR-101 in endothelial cells promotes blood vessel formation through reduced repression of EZH2. PLoS One 6(1):e16282. doi:10.1371/journal.pone.0016282

91. Smits M, Nilsson J, Mir SE, van der Stoop PM, Hulleman E, Niers JM, de Witt Hamer PC, Marquez VE, Cloos J, Krichevsky AM, Noske DP, Tannous BA, Wurdinger T (2010) miR-101 is down-regulated in glioblastoma resulting in EZH2-induced proliferation, migration, and angiogenesis. Oncotarget 1(8):710–720
92. Wang XF, Shi ZM, Wang XR, Cao L, Wang YY, Zhang JX, Yin Y, Luo H, Kang CS, Liu N, Jiang T, You YP (2012) MiR-181d acts as a tumor suppressor in glioma by targeting K-ras and Bcl-2. J Cancer Res Clin Oncol 138(4):573–584. doi:10.1007/s00432-011-1114-x
93. Xia H, Cheung WK, Ng SS, Jiang X, Jiang S, Sze J, Leung GK, Lu G, Chan DT, Bian XW, Kung HF, Poon WS, Lin MC (2012) Loss of brain-enriched miR-124 microRNA enhances stem-like traits and invasiveness of glioma cells. J Biol Chem 287(13):9962–9971. doi:10.1074/jbc.M111.332627
94. Zhang C, Kang C, You Y, Pu P, Yang W, Zhao P, Wang G, Zhang A, Jia Z, Han L, Jiang H (2009) Co-suppression of miR-221/222 cluster suppresses human glioma cell growth by targeting p27kip1 in vitro and in vivo. Int J Oncol 34(6):1653–1660
95. Zhang CZ, Zhang JX, Zhang AL, Shi ZD, Han L, Jia ZF, Yang WD, Wang GX, Jiang T, You YP, Pu PY, Cheng JQ, Kang CS (2010) MiR-221 and miR-222 target PUMA to induce cell survival in glioblastoma. Molecular cancer 9:229. doi:10.1186/1476-4598-9-229
96. Lee SJ, Kim SJ, Seo HH, Shin SP, Kim D, Park CS, Kim KT, Kim YH, Jeong JS, Kim IH (2012) Over-expression of miR-145 enhances the effectiveness of HSVtk gene therapy for malignant glioma. Cancer Lett 320(1):72–80. doi:10.1016/j.canlet.2012.01.029
97. Hwang do W, Son S, Jang J, Youn H, Lee S, Lee D, Lee YS, Jeong JM, Kim WJ, Lee DS (2011) A brain-targeted rabies virus glycoprotein-disulfide linked PEI nanocarrier for delivery of neurogenic microRNA. Biomaterials 32(21):4968–4975. doi:10.1016/j.biomaterials.2011.03.047
98. Guo J, Niu R, Huang W, Zhou M, Shi J, Zhang L, Liao H (2012) Growth factors from tumor microenvironment possibly promote the proliferation of glioblastoma-derived stem-like cells in vitro. Pathology oncology research : POR 18(4):1047–1057. doi:10.1007/s12253-012-9543-7
99. Candolfi M, King GD, Yagiz K, Curtin JF, Mineharu Y, Muhammad AK, Foulad D, Kroeger KM, Barnett N, Josien R, Lowenstein PR, Castro MG (2012) Plasmacytoid dendritic cells in the tumor microenvironment: immune targets for glioma therapeutics. Neoplasia 14(8):757–770
100. Hardee ME, Marciscano AE, Medina-Ramirez CM, Zagzag D, Narayana A, Lonning SM, Barcellos-Hoff MH (2012) Resistance of glioblastoma-initiating cells to radiation mediated by the tumor microenvironment can be abolished by inhibiting transforming growth factor-beta. Cancer Res 72(16):4119–4129. doi:10.1158/0008-5472.CAN-12-0546
101. Jamal M, Rath BH, Tsang PS, Camphausen K, Tofilon PJ (2012) The brain microenvironment preferentially enhances the radioresistance of CD133(+) glioblastoma stem-like cells. Neoplasia 14(2):150–158
102. Jackson C, Ruzevick J, Phallen J, Belcaid Z, Lim M (2011) Challenges in immunotherapy presented by the glioblastoma multiforme microenvironment. Clin Dev Immunol. doi:10.1155/2011/732413
103. Heddleston JM, Hitomi M, Venere M, Flavahan WA, Yang K, Kim Y, Minhas S, Rich JN, Hjelmeland AB (2011) Glioma stem cell maintenance: the role of the microenvironment. Curr Pharm Des 17(23):2386–2401
104. Charles N, Holland EC (2010) The perivascular niche microenvironment in brain tumor progression. Cell Cycle 9(15):3012–3021. doi:10.4161/cc.9.15.12710
105. Hoelzinger DB, Demuth T, Berens ME (2007) Autocrine factors that sustain glioma invasion and paracrine biology in the brain microenvironment. J Natl Cancer Institute 99(21):1583–1593. doi:10.1093/jnci/djm187
106. Inc RT (2012) Regulus is focused on the discovery and development of miRNA therapeutics. http://www.regulusrx.com/therapeutic-areas/
107. Therapeutics M (2013) Pipeline of miRNA therapeutics. http://www.miragentherapeutics.com/7/Pipeline/
108. Therapeutics M (2012) Pipeline of miRNA therpaueitcs lead candidates. http://www.mirnatherapeutics.com.
109. Judge AD, Sood V, Shaw JR, Fang D, McClintock K, MacLachlan I (2005) Sequence-dependent stimulation of the mammalian innate immune response by synthetic siRNA. Nat Biotechnol 23(4):457–462. doi:10.1038/nbt1081
110. Hollins AJ, Omidi Y, Benter IF, Akhtar S (2007) Toxicogenomics of drug delivery systems: exploiting delivery system-induced changes in target gene expression to enhance siRNA activity. Journal of drug targeting 15(1):83–88. doi:10.1080/10611860601151860
111. Akhtar S, Benter I (2007) Toxicogenomics of non-viral drug delivery systems for RNAi: potential impact on siRNA-mediated gene silencing activity and specificity. Advanced drug delivery reviews 59(2–3):164–182. doi:10.1016/j.addr.2007.03.010
112. Omidi Y, Hollins AJ, Benboubetra M, Drayton R, Benter IF, Akhtar S (2003) Toxicogenomics of non-viral vectors for gene therapy: a microarray study of lipofectin- and oligofectamine-induced gene expression changes in human epithelial cells. Journal of drug targeting 11(6):311–323. doi:10.1080/10611860310001636908
113. Omidi Y, Hollins AJ, Drayton RM, Akhtar S (2005) Polypropylenimine dendrimer-induced gene expression changes: the effect of complexation with DNA, dendrimer generation and cell type. Journal of drug targeting 13(7):431–443. doi:10.1080/10611860500418881
114. Akhtar S, Benter IF (2007) Nonviral delivery of synthetic siRNAs in vivo. J Clin Invest 117(12):3623–3632. doi:10.1172/JCI33494
115. Zukiel R, Nowak S, Wyszko E, Rolle K, Gawronska I, Barciszewska MZ, Barciszewski J (2006) Suppression of human brain tumor with interference RNA specific for tenascin-C. Cancer biology & therapy 5(8):1002–1007
116. Guo D, Wang B, Han F, Lei T (2010) RNA interference therapy for glioblastoma. Expert Opin Biol Ther 10(6):927–936. doi:10.1517/14712598.2010.481667
117. Kirui DK, Khalidov I, Wang Y, Batt CA (2012) Targeted near-IR hybrid magnetic nanoparticles for in vivo cancer therapy and imaging. Nanomedicine: nanotechnology, biology, and medicine. doi:10.1016/j.nano.2012.11.009
118. Ryvolova M, Chomoucka J, Drbohlavova J, Kopel P, Babula P, Hynek D, Adam V, Eckschlager T, Hubalek J, Stiborova M, Kaiser J, Kizek R (2012) Modern micro and nanoparticle-based imaging techniques. Sensors (Basel) 12(11):14792–14820. doi:10.3390/s121114792
119. Nduom EK, Bouras A, Kaluzova M, Hadjipanayis CG (2012) Nanotechnology applications for glioblastoma. Neurosurgery clinics of North America 23(3):439–449. doi:10.1016/j.nec.2012.04.006
120. Tivnan A, Orr WS, Gubala V, Nooney R, Williams DE, McDonagh C, Prenter S, Harvey H, Domingo-Fernandez R, Bray IM, Piskareva O, Ng CY, Lode HN, Davidoff AM, Stallings RL (2012) Inhibition of neuroblastoma tumor growth by targeted delivery of microRNA-34a using anti-disialoganglioside GD2 coated nanoparticles. PLoS One 7(5):e38129. doi:10.1371/journal.pone.0038129
121. Bray I, Tivnan A, Bryan K, Foley NH, Watters KM, Tracey L, Davidoff AM, Stallings RL (2011) MicroRNA-542-5p as a novel tumor suppressor in neuroblastoma. Cancer Lett 303(1):56–64. doi:10.1016/j.canlet.2011.01.016
122. Foley NH, Bray IM, Tivnan A, Bryan K, Murphy DM, Buckley PG, Ryan J, O'Meara A, O'Sullivan M, Stallings RL (2010) MicroRNA-184 inhibits neuroblastoma cell survival through targeting the serine/threonine kinase AKT2. Molecular cancer 9:83. doi:10.1186/1476-4598-9-83

123. Babar IA, Cheng CJ, Booth CJ, Liang X, Weidhaas JB, Saltzman WM, Slack FJ (2012) Nanoparticle-based therapy in an in vivo microRNA-155 (miR-155)-dependent mouse model of lymphoma. Proc Natl Acad Sci U S A 109(26):E1695–1704. doi:10.1073/pnas.1201516109
124. Piao L, Zhang M, Datta J, Xie X, Su T, Li H, Teknos TN, Pan Q (2012) Lipid-based nanoparticle delivery of Pre-miR-107 inhibits the tumorigenicity of head and neck squamous cell carcinoma. Molecular therapy: the journal of the American Society of Gene Therapy 20(6):1261–1269. doi:10.1038/mt.2012.67
125. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ (2011) Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol 29(4):341–345. doi:10.1038/nbt.1807
126. Ikeda M, Bhattacharjee AK, Kondoh T, Nagashima T, Tamaki N (2002) Synergistic effect of cold mannitol and Na(+)/Ca(2+) exchange blocker on blood–brain barrier opening. Biochem Biophys Res Commun 291(3):669–674. doi:10.1006/bbrc.2002.6495
127. Campbell M, Kiang AS, Kenna PF, Kerskens C, Blau C, O'Dwyer L, Tivnan A, Kelly JA, Brankin B, Farrar GJ, Humphries P (2008) RNAi-mediated reversible opening of the blood–brain barrier. The journal of gene medicine 10(8):930–947. doi:10.1002/jgm.1211
128. Singh SK, Vartanian A, Burrell K, Zadeh G (2012) A microRNA link to glioblastoma heterogeneity. Cancers 4:846–872
129. Godlewski J, Bronisz A, Nowicki MO, Chiocca EA, Lawler S (2010) microRNA-451: A conditional switch controlling glioma cell proliferation and migration. Cell Cycle 9(14):2742–2748
130. Hegi ME, Rajakannu P, Weller M (2012) Epidermal growth factor receptor: a re-emerging target in glioblastoma. Curr Opin Neurol 25(6):774–779. doi:10.1097/WCO.0b013e328359b0bc
131. Kang MK, Kang SK (2007) Tumorigenesis of chemotherapeutic drug-resistant cancer stem-like cells in brain glioma. Stem cells and development 16(5):837–847. doi:10.1089/scd.2007.0006
132. Wu Z, Sun L, Wang H, Yao J, Jiang C, Xu W, Yang Z (2012) MiR-328 expression is decreased in high-grade gliomas and is associated with worse survival in primary glioblastoma. PLoS One 7(10):e47270. doi:10.1371/journal.pone.0047270
133. Kefas B, Comeau L, Erdle N, Montgomery E, Amos S, Purow B (2010) Pyruvate kinase M2 is a target of the tumor-suppressive microRNA-326 and regulates the survival of glioma cells. Neuro-Oncology 12(11):1102–1112. doi:10.1093/neuonc/noq080
134. Bai Y, Liao H, Liu T, Zeng X, Xiao F, Luo L, Guo H, Guo L (2013) MiR-296-3p regulates cell growth and multi-drug resistance of human glioblastoma by targeting ether-a-go-go (EAG1). Eur J Cancer 49(3):710–724. doi:10.1016/j.ejca.2012.08.020
135. Katakowski M, Zheng X, Jiang F, Rogers T, Szalad A, Chopp M (2010) MiR-146b-5p suppresses EGFR expression and reduces in vitro migration and invasion of glioma. Cancer investigation 28(10):1024–1030. doi:10.3109/07357907.2010.512596
136. Lee HK, Bier A, Cazacu S, Finniss S, Xiang C, Twito H, Poisson LM, Mikkelsen T, Slavin S, Jacoby E, Yalon M, Toren A, Rempel SA, Brodie C (2013) MicroRNA-145 is downregulated in glial tumors and regulates glioma cell migration by targeting connective tissue growth factor. PLoS One 8(2):e54652. doi:10.1371/journal.pone.0054652
137. Speranza MC, Frattini V, Pisati F, Kapetis D, Porrati P, Eoli M, Pellegatta S, Finocchiaro G (2012) NEDD9, a novel target of miR-145, increases the invasiveness of glioblastoma. Oncotarget 3(7):723–734
138. Zhang Y, Chao T, Li R, Liu W, Chen Y, Yan X, Gong Y, Yin B, Qiang B, Zhao J, Yuan J, Peng X (2009) MicroRNA-128 inhibits glioma cells proliferation by targeting transcription factor E2F3a. J Mol Med (Berl) 87(1):43–51. doi:10.1007/s00109-008-0403-6
139. Wan Y, Fei XF, Wang ZM, Jiang DY, Chen HC, Yang J, Shi L, Huang Q (2012) Expression of miR-125b in the new, highly invasive glioma stem cell and progenitor cell line SU3. Chinese journal of cancer 31(4):207–214. doi:10.5732/cjc.011.10336
140. Wu N, Xiao L, Zhao X, Zhao J, Wang J, Wang F, Cao S, Lin X (2012) miR-125b regulates the proliferation of glioblastoma stem cells by targeting E2F2. FEBS Lett 586(21):3831–3839. doi:10.1016/j.febslet.2012.08.023
141. Silber J, Lim DA, Petritsch C, Persson AI, Maunakea AK, Yu M, Vandenberg SR, Ginzinger DG, James CD, Costello JF, Bergers G, Weiss WA, Alvarez-Buylla A, Hodgson JG (2008) miR-124 and miR-137 inhibit proliferation of glioblastoma multiforme cells and induce differentiation of brain tumor stem cells. BMC medicine 6:14. doi:10.1186/1741-7015-6-14
142. Loftus JC, Ross JT, Paquette KM, Paulino VM, Nasser S, Yang Z, Kloss J, Kim S, Berens ME, Tran NL (2012) miRNA expression profiling in migrating glioblastoma cells: regulation of cell migration and invasion by miR-23b via targeting of Pyk2. PLoS One 7(6):e39818. doi:10.1371
143. Fox JL, Dews M, Minn AJ, Thomas-Tikhonenko A (2013) Targeting of TGFbeta signature and its essential component CTGF by miR-18 correlates with improved survival in glioblastoma. RNA 19(2):177–190. doi:10.1261/rna.036467.112
144. Wu DG, Wang YY, Fan LG, Luo H, Han B, Sun LH, Wang XF, Zhang JX, Cao L, Wang XR, You YP, Liu N (2011) MicroRNA-7 regulates glioblastoma cell invasion via targeting focal adhesion kinase expression. Chinese medical journal 124(17):2616–2621