Review

MicroRNA as potential Biomarkers in Glioblastoma

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Abstract

Glioblastoma is the most aggressive and lethal tumour of the central nervous system and as such the identification of reliable prognostic and predictive biomarkers for patient survival and tumour recurrence is paramount. MicroRNA detection has rapidly emerged as potential biomarkers, in patients with Glioblastoma. Over the last decade, analysis of miRNA in laboratory based studies have yielded several candidates as potential biomarkers however, the accepted use of these candidates in the clinic is yet to be validated. Here we will examine the use of miRNA signatures to improve glioblastoma stratification into subgroups and summarise recent advances made in miRNA examination as potential biomarkers for glioblastoma progression and recurrence.

Keywords: Micro RNA; biomarker; Glioblastoma

1. Introduction

Gliomas are tumours that arise from glial cells and account for approximately 30% of all CNS and brain tumours and 70-80% of all malignant brain tumours (1, 2). Gliomas include oligodendroglioma, mixed oliogoastrocytoma, ependymoma and astrocytoma; the latter can be further graded into diffuse astrocytoma (WHO Grade I), anaplastic astrocytoma (Grade III) and glioblastoma (Grade IV).

Glioblastoma, also known as glioblastoma multiforme (GBM), is the most common primary tumour of the central nervous system accounting for over 80% of malignant gliomas and is one of the most aggressive (3-5). It is estimated that over 10000 patients will be diagnosed with glioblastoma in the United States annually (6). Maximal safe tumour resection through surgery is beneficial, although microscopic disease is inevitably present, making surgical cure impossible (7). Mortality from glioblastoma is attributed to its invasive nature and destruction of surrounding brain tissue (8). Therefore, a greater understanding of the molecular processes that promote glioblastoma invasion is required to overcome glioblastoma cell infiltration. Surgical resection, followed by concurrent post-operative radiotherapy and temozolomide is now the standard of care for glioblastoma patients (9-11). However, despite this slightly improved survival due to radiotherapy and temozolomide treatment post-surgery, 72% of patients develop tumour recurrence within 17 months post diagnosis (12), and only 9.8% of patients are still alive after 5 years of diagnosis (13).

GBM can be classified as two distinct subtypes: primary and secondary glioblastoma based on clinical presentation and other features. The majority of glioblastomas are primary de novo glioblastomas accounting for more than 90% of total cases (14). Primary glioblastomas are identified without clinical, radiological or histopathological evidence of a precursor lesion (15). In contrast, secondary glioblastomas arise from lower grades of astrocytomas and anaplastic astrocytomas of WHO grade II and III respectively (16). However, identifying

histopathological differences between primary and secondary glioblastomas is extremely difficult (17). Furthermore, classification of WHO grade by microscopic histology is subjective, often lacks reproducibility and cannot confidently predict individual outcomes (18). Therefore, major efforts have been made to identify a molecular classification of glioblastomas and isolate relevant prognostic and predictive biomarkers (16, 19, 20). A Comprehensive study utilizing the Cancer Genome Atlas (TCGA) project examining the molecular characteristics of glioblastomas analysing copy number variations, acquired DNA sequence alterations, gene and miRNA expression and DNA methylation has been reported (4, 21, 22). The extensive TCGA study demonstrated that glioblastomas frequently acquire chromosomes 7 and 19, loss of chromosomes 10 and 13, and contain MDM2 and EGFR amplification (and expression of EGFRvIII (23)), PTEN, NFI and TP53 mutations and CDKN2A/B deletion (21). Genome-wide expression analysis and whole-genome methylation has now further classified glioblastoma into 4 distinct subgroups (classical, mesenchymal, pro-neural and neural), each characterised with distinct genetic mutations and deletions (24, 25). Moreover, the pro-neural subgroup is further divided into 2 groups: neural-CpG island methylator phenotype (G-CIMP) and neural-non-G-CIMP (26, 27).

MicroRNAs (miRNAs) are a group of small (20-25 nucleotides) non-coding RNA fragments that bind to messenger mRNA and stop translation of the corresponding protein leading to mRNA degradation. MiRNAs have been implicated in the initiation and progression of many cancers and therefore hold great potential as diagnostic and therapeutic tools. More than 1500 human miRNAs have been identified and many are now known to be up- or down-regulated in various cancers. Indeed, several miRNA have been implicated in glioblastoma development, progression and as a potential prognostic biomarker (28-30). Several recent reviews have examined the advances made in the role of miRNA in glioblastoma development and progression and hence we will not review this area of research

here (31-34). In particular, the outstanding 2013 review by Hermansen and Kristensen outlined the overall biogenesis, function and discovery of miRNA and clearly described the methodology used to evaluate miRNA expression, incorporated both findings in laboratory and clinical research into their discussions (34). Differing from the review by Hermansen and Kristensen, in this current review, we will focus specifically on research from patient specimens summarising the current literature on the use of miRNA detection in molecular classification of glioblastoma. We will also focus on the validity of using intra-tumoral or circulating miRNA as both prognostic and predictive biomarkers for glioblastoma detection, diagnosis, recurrence and treatment with temozolomide and radiotherapy.

2. Classifying Glioma grade and Glioblastoma sub-groups by differential miRNA expression profiles.

The isolation of specific molecular signatures across glioma grades and sub-groups of glioblastoma may potentially allow for more reliable and consistent diagnosis, staging, prognosis and response to standard and novel therapies. In this section we review the advances made in identifying specific miRNA expression profiles in a glioma setting. A substantial number of studies have performed large-scale miRNA expression analyses reporting both the up-regulation and down-regulation of several miRNAs in patient glioblastoma tumour tissue compared to normal brain tissue (Summarised in Table 1). However, due to inconsistencies in the reported subset of miRNAs that are either up or down-regulated in glioblastoma, a specific glioblastoma miRNA expression signature is not yet well established. Only one miRNA (miR-21) was identified to be up-regulated in glioblastoma versus control brain tissue across all 13 studies listed in Table 1, while another 18 were recognised to be up-regulated in at least 3 of the 13 reports (Table 2). Likewise, 22 miRNA were regularly identified to be down-regulated in glioblastoma compared to normal

or adjacent brain tissue in at least 3 studies, with miR-132 the most consistent (8 out of 13 studies; Table 2). Potential discrepancies between these studies are mainly due to several reasons including: the quality of initial tumour tissue, small samples sizes in some studies, differences in commercial miRNA array profile sets, variation in miRNA extraction techniques, contrasting methods for statistical analysis and the appropriateness of the "control" brain tissue. Nonetheless, many of these initial studies and subsequent investigations have extended the preliminary observations to provide important translational knowledge, identifying critical miRNA signatures distinguishing gliomas of various grades. Meta-analysis performed by other groups examining miRNA expression across several studies may also aid in critically identifying a definitive miRNA gene profile for improved glioma diagnosis and prognosis (35-38). Furthermore, several studies in addition to the high-throughput screens performed above have evaluated individual miRNA as prognostic biomarkers of glioblastoma patient tissue. These include the expression of miR-10b (39) which was shown to be up-regulated and miR-7 (40), miR-34a (41), miR-128 (42), miR-218 (43) and miR-873 (44) which were down-regulated in glioblastoma tissue compared to adjacent or normal brain tissue.

Several studies have examined differences in miRNA expression levels between glioblastoma and anaplastic astrocytoma (AA) tissue samples. Rao and colleagues used a predictive analytical approach of their genome-wide microarray data to distinguish miRNA expression between AA's and glioblastoma (45). Their predictive analysis discovered a 23-miRNA gene signature that could discriminate AA from glioblastoma with 95% accuracy. However, this signature was not impeccable as two glioblastomas out of the 26 examined displayed a miRNA expression profiles more akin to the expression profiles seen in AA's, while 2 of the 13 AA's displayed miRNA signatures more closely resembling the glioblastoma miRNA expression profiles. Similarly, Guan and colleagues identified a 16-

miRNA signature that they believed could be utilized to clearly distinguish AA's from glioblastomas with miR-196a showing the greatest significant difference of approximately 105-fold greater expression in glioblastomas compared to AA's (46). However, once more this signature was not completely robust as two glioblastomas out of the eight examined displayed similar miRNA expression profiles to AA's than to other glioblastomas (46). Further larger-scale studies will be required to validate these current studies. Whether molecular biomarkers such as the miRNA signatures discovered by these reports eventually complement or supersede the currently accepted stratification of grade by tumour histopathological assessment is unclear.

Other researchers have observed differences in a set of miRNA between Grade I, Grade II and Grade III gliomas compared to commercially available or epileptic control brains (47). In addition, they also detected differential expression in several miRNA that distinguish low grade gliomas (Grade I and II) from high grade gliomas (Grade III and IV). Similarly, a report by Zhi et al, found that miR-137 expression was significantly lower in high grade (III and IV) versus low grade (I and II) gliomas (48). Rivera-Diaz and colleagues identified several miRNAs that were associated with grade II, III and IV gliomas (49), whilst another study showed that miR-21 is up-regulated and miR-200a is down-regulated in glioblastomas compared to grade II and III gliomas (50). Finally, a more recent paper identified a 13-miRNA signature distinguishing WHO grade II glioma to high-grade glioma of grade III and IV after large scale assessment of 848 human miRNA (51).

Meanwhile, Lages and co-workers identified 7 miRNA (6 over-expressed and 1 under-expressed) in glioblastoma tumour tissue compared to oligodendrogliomas (52). Interestingly, a total of 26 miRNA was found to be differentially expressed in these glioblastomas and oligodendrogliomas compared to control brain tissue and thus the authors' hypothesis that many miRNA may be regulated by similar pathways in both glioblastoma and

oligodendrogliomas as 19 miRNA (from this set of 26) were found to be similarly expressed in both types of glioma (52). Meanwhile, miR-221 expression has been shown to be significantly higher in gliomas of grade II, III and IV compared to normal brain tissue, with increasing expression correlating to grade (highest levels observed in grade IV glioblastoma tumours) (53).

Several studies have also aimed to differentiate primary and secondary glioblastoma (that are largely indistinguishable histologically) through differential miRNA expression profiles. A study by Rao and colleagues isolated 7 miRNA which were differentially expressed in primary versus secondary glioblastoma samples (45). Likewise, another group identified a set of miRNA with differential expression in diffuse astrocytomas (Grade II) compared to patient matched corresponding recurrent secondary glioblastomas (grade IV) (29). However the diagnostic significance of identifying additional molecular biomarkers including miRNA expression to the existing genetic and epigenetic differences currently identified in primary versus secondary glioblastomas is yet to be determined. Indeed, mutations in IDH1 are observed in greater than 80% of secondary glioblastoma but in less than 5% of primary glioblastomas (14, 54, 55), and thus IDH1 mutations are recognised as a definitive diagnostic genetic marker of secondary glioblastomas with more reliable than clinical and pathological assessment (17).

Finally, separate investigations have determined miRNA profiles that distinguish glioma stem cells from non-stem glioma cells and non-neoplastic stem cells. One study used a combined microarray and deep sequencing approach to identify miRNA gene signature differentiating glioblastoma stem cells from normal neural stem cells (56). Similarly, another study identified differential miRNA profiles in $CD133^+$ versus $CD133^-$ glioblastoma cell lines (57). Another study compared the miRNA profiles of human glioma tissue, embryonic stem cells, neural precursor cells and normal brain tissue (58). In this study, Lavon and

colleagues identified 15 miRNA that showed differential expression in glioma compared to stem cells (58). In summary, large scale assessment and validation of miRNA expression signatures has advanced the understanding of critical molecular mediators and regulatory pathways within all tumours including glioma. Over time it is hoped that these insights will lead to a more accurate and stratified diagnosis of all grades of glioma.

3. Predicting Glioblastoma Patient Outcome by differential miRNA expression profiles.

The identification of miRNA profiles that aid in elucidating glioma grade has been achieved as reviewed here in our previous section. Here we summarise the current literature evaluating miRNA expression signatures or individual miRNA that can predict clinical outcome of glioblastoma patients. Several papers have identified specific miRNA or miRNA profiles that predict survival in glioblastoma. Srinivasan et al, utilized the TCGA database to correlate miRNA expression profiles with survival in 222 glioblastoma patients (59). Their analysis identified a 10-miRNA expression signature that could independently predict survival. The 10 miRNA signature was made up of 7 miRNA that was over-expressed (miR-31, miR-146b, miR-148a, miR-193a, miR-200, miR-221, miR-222) and 3 miRNA that were under-expressed (miR-17-5p, miR-20a, miR-106a) in tumours from glioblastoma patients with shorter median survival. A similar study identified a 5-miRNA gene signature (miR-181d, miR-518b, miR-524-5p, miR-566, miR-1227) that independently predicted survival in both a Chinese cohort of 82 glioblastoma patients and from glioblastoma patients in the TCGA dataset (60). More recently, high-throughput microarray analysis was performed to measure the expression of 1146 miRNA in various grades of glioma (61). In this study, the authors determined that a 5-miRNA signature could distinguish a greater risk of poor outcome for patients with anaplastic gliomas, secondary and pro-neural glioblastomas (61). Finally, another study examined the predictive value of a set of 8 miRNAs which had showed

a differential expression between glioblastoma and control brain tissue (62). They determined that miR-181c and miR-21 expression both individually and in combination was associated with short-term progression of less than 6 months while miR-195 and miR-196b expression correlated with longer overall survival. Disappointingly, these four studies however, did not produce any over-lapping miRNA across the 4 "predictive" signatures and thus isolating a conclusive candidate miRNA expression profile either individually or as a clustered set that predicts patient outcomes for primary glioblastomas still requires further investigation. Furthermore, 2 studies published contradictory findings to those of Lakomy's investigation. Guan and colleagues reported that miR-196a and miR-196b expression were independent and significant predictors of poorer overall survival of glioblastoma patients, which is in direct contrast to Lakomy's study proposing that miR-196b correlated with longer overall survival (46, 62). In addition, Ma et al, showed that the expression levels of miR-196b were inversely correlated with overall survival in GBM patients again in contrast to Lakomy's report (63). Concurring with Lakomy's study however was the correlation of miR-21 expression with poorer survival in astrocytoma patients across all 4 grades (48). Finally, another study showed that high levels of miR-326 and miR130a and low levels of miR-323, miR-329, miR-155 and miR-210 were all significantly associated with better overall survival in Glioblastoma patients (64).

A number of investigations have identified individual miRNA associations with poor survival outcomes in glioblastoma patients. Increased expression of miR-17 (65), miRNA-132 (30), miR-210 (66), miR-224 (67) and miR-155 (68) were significantly associated with low Karnofsky performance score, poor progression-free survival and overall survival rates in high-grade glioma patients. In addition, reduced expression of miR-200b (69), miR-205 (70), miR-326 (71), miR-340 (72) and miR-504 (73) correlated with poor overall survival in high grade glioma patients, whilst reduced miR-375 expression was a predictor of poor overall

survival in glioma patients across all 4 grades (74). Barbano and colleagues showed that over-expression of miR-21, miR-210, miR-22 and miR-155 in glioblastoma all individually associated with a higher risk of death (51). Furthermore, reduced tumour expression of miR-106a and miR-181b in patients with gliomas (across all 4 grades) also correlated to poorer overall survival (48). The study by Men et al (69) demonstrating decreased miR-200b expression correlated with tumour progression and poor prognosis is highly relevant. A genome-wide identification of the miR-200 family revealed a regulatory network that controls cell invasion (75). One of the hallmarks of glioma is their highly invasive and infiltrative nature and Bracken et al (75), showed that the miR-200 family plays a significant role in coordinating actin cytoskeleton dynamics. This is highly pertinent as glioma cells have been shown to possess cell membrane structures known as invadopodia which facilitate the invasion process (76) and an invadopodia related protein known as Tks5 has been demonstrated to be of prognostic importance in glioma patients (77). Thus, one could speculate that miRNA signatures outlining a highly invasive glioma may also show a significant association with miRNA signatures for poorer survival outcome.

Moreover, a clear classification of the various sub-groups of glioblastoma (classical, mesenchymal, pro-neural-G-CIMP, pro-neural non-G-CIMP and neural), may also aid in personalized and improved patient care. It is now becoming evident that each sub-group of glioblastoma has varying, distinguishable genetic and molecular mRNA profiles and may have differing response to therapy and overall survival rates (78). However, some dispute remains regarding the relationship between glioblastoma subclasses and clinical outcome (24, 25, 27, 33). Recently, an important study analysed the TCGA based on miRNA expression profiles and found that these classifications predicted patient outcome far greater than previously reported mRNA expression profiles (79). Their study detected 5 differentially expressed miRNA clusters in 5 distinct glioblastoma sub-classes designated as Oligoneural,

Radial glial, Neural, neuro-mesenchymal and astrocytic glioblastoma based on each type arising from a specific neural precursor cell type. Importantly, they assessed the overall survival rates in each of these sub-groups and found that patients with Oligoneural glioblastomas had significantly better overall survival than patients with radial glial, neural or astrocytic glioblastomas (79). Another group also isolated differences in miRNA profiles between the same 5 glioblastoma subgroups utilising the TCGA database (80). Importantly, they identified a specific miRNA gene signature that correlated with patient clinical outcomes for all 5 glioblastoma sub-groups. Finally, a very recent report used LASSO regression models to identify a 9-miRNA prognostic signature that significantly correlated with survival in all glioblastoma subtypes except the non-G-CIMP pro-neural group (81). Collectively, these reports suggest that stratifying each individual patient based on their differential miRNA expression signatures may result in personalized sub-class specific treatment strategies in the future.

4. Predicting Glioblastoma Patient Response to Therapy by differential miRNA expression profiles.

Despite only offering modest increases in patient survival, the accepted treatment for glioblastoma patients is surgical resection, followed by radiotherapy and adjuvant temozolomide. However, the mechanisms driving glioblastoma resistance to this treatment at a molecular level are not completely understood. Research identifying critical differential miRNA expression profiles between good and poor response may offer greater insight into predicting treatment outcomes and provide increased confidence for therapeutic decision making in the future. Many reports have examined miRNA expression as potential biomarkers in response to the drug treatment of glioblastoma cell lines in laboratory studies. However, in this review we summarize the limited current literature examining miRNA as

possible mediators of resistance to therapy in glioblastoma patients. A recent paper examined the expression levels of miR-125b in 60 glioblastoma patients that were treated with temozolomide and radiotherapy (82). They found that miR-125b expression significantly correlated with survival outcomes for patients treated with temozolomide in combination with radiotherapy. Glioblastoma patients with high miR-125b expression had a median survival of 9 months compared to 18 months for patients low miR-125b expression levels (82). Likewise, another group showed that reduced expression of miR-181b and miR-181c significantly correlated with an improved patient response to temozolomide and radiotherapy (83).

In addition, other studies have examined miRNA profiles in retrospective studies comparing glioblastoma patients treated with or without temozolomide. Zhang and colleagues identified a 5-miRNA gene signature, which they had shown to predict patient survival outcome, to also predict response to temozolomide (60). Patients were grouped based on their expression levels of the 5-miRNA signature (miR-181d, miR-518b, miR-524-5p, miR-566 and miR-1227) into high-risk and low-risk using a risk-score statistical method. Patients that were treated with temozolomide with a low-risk score (n=19) had a significantly better temozolomide response and overall survival versus those that were treated with temozolomide and had high-risks scores (n=21) (60). Similarly, risk-score analysis of a 9-miRNA gene signature established by another group was also associated with temozolomide response in patients with glioblastoma (81).

Conclusive studies verifying these miRNA signatures that facilitate confidence in their predictive biomarker status await further investigation. Importantly, however, these signatures show clear clinical relevance, allowing for the stratification of patients into optimal treatment options that may best improve overall outcomes.

5. Predicting Survival Outcome by differential miRNA expression profiles in patient peripheral blood.

The identification of miRNA gene signatures within patient tumour tissue may potentially assist in predicting progression-free and overall survival of glioblastoma patients and should aid in selecting the most appropriate and effective treatment for each patient. However, identifying miRNA profiles in patient peripheral bodily fluids without the requirement of surgery or biopsy may be more suitable for predicting patient relapse or disease recurrence. Indeed, Baraniskin evaluated differential miRNA expression in the cerebrospinal fluid (CSF) of glioma patients and found that miR-21 and miR-15b were significantly enhanced in CSF samples from patients with gliomas compared to control subjects (84). Similarly, CSF from patients with non-neoplastic neurological conditions (used as controls), glioblastoma and brain tumour metastasis (originating from breast and lung primary tumours) was assessed for the expression of several miRNA known to be strongly associated with glioblastoma development (85). This study found that miR-10b and miR-21 expression were both significantly increased in the CSF of most glioblastoma and metastatic brain tumour patients compared to their levels in the CSF of non-neoplastic patients. Interestingly, the expression of the miRNA 200 family (miR-200a, miR-200b, miR-200c and miR-141) were highly expressed in CSF of most metastatic brain tumour patients but not in primary glioblastoma patients indicating that these miRNA may be a potential biomarker to differentiate between primary brain tumours and tumours that have metastasised to the brain. This is consistent with the studies showing that the miRNA 200 family are linked to tumour invasion and metastasis as we noted earlier in section 3. In addition, Teplyuk and colleagues went on to evaluate whether miRNA levels in patient CSF correlated with remission of disease. Importantly, neither miR-10b nor the miR-200 family of miRNA (and only low levels of miR-21) were detectable in the CSF of patients who were in remission (as defined by no

evidence of tumour by MRI and if CSF cytological analysis was negative) (85). Further work is required however, to determine whether the detection of miRNA in CSF is a better clinical diagnostic tool to establish disease remission (or relapse) compared to the currently used MRI.

Recent studies have demonstrated that glioblastoma exosomes contain similar material to that of their corresponding intracellular tumour mass including miRNA profiles (86, 87) and thus the expression of miRNA in CSF exosomes may make promising biomarkers in glioblastoma patients. Indeed, a large panel of over 50 miRNAs were detected in the CSF exosomes of glioblastoma patients (86). Whether these miRNA can be used as prognostic or predictive biomarkers is yet to be clearly determined. In addition, the use of peripheral blood over CSF for miRNA profiling may be more suitable due to the non-invasive nature, repeatability and ease of blood collection. Studies characterising miRNA expression signatures in serum and peripheral blood from patients with other neurological diseases such as schizophrenia and cerebral ischemic injury have been performed (88, 89), and is therefore also feasible for glioma patients. Here we will review the current knowledge in assessing miRNA expression in glioma patient serum/plasma and discuss the potential of these findings in utilising miRNA expression as prognostic and predictive biomarkers. These studies are summarised in Table 3.

A recent study examined the expression of 752 miRNA by microarray analysis in glioblastoma patient and control sera (90). Following data validation, they identified that 3 miRNA (miR-576-5p, miR-340 and miR-626) were significantly over-expressed and 3 miRNA (miR-320, Let-7g-5p and miR-7) were significantly under-expressed in glioblastoma patient serum compared to normal control serum. Likewise, another group reported a significant differential expression of 2 miRNA (miR-128 and miR-342-3p) from a total of 1158 miRNA tested in glioblastoma patient peripheral blood compared to normal control

blood (91). In addition, Wang and colleagues examined the expression of a small set of miRNA that have been associated with deregulation in glioblastoma tissue and identified 3 miRNA (miR-21, miR-128 and miR-342-3p) that were significantly altered in expression in glioblastoma patient plasma versus health control plasma (92). Interestingly, reduced miR-342-3p expression in glioblastoma patient sera versus control sera was observed in both this study and the study by Roth et al, suggesting that this miRNA may indeed be a suitable biomarker for glioblastoma diagnosis. However, although miR-128 was also reduced in expression in the study by Wang and colleagues, Roth et al, found that miR-128 was conversely up-regulated in glioblastoma patient peripheral blood versus control blood (91, 92). This discrepancy may be attributed somewhat to the use of peripheral blood versus plasma, as it has been suggested that miRNA from blood cells may provide misleading miRNA profiles differing to those seen in patient serum or plasma (92). Nonetheless, these papers offer proof-of-principle support that miRNA expression in patient blood can potentially act as non-invasive biomarkers for disease diagnosis and prognosis.

Two other reports have examined large-scale screening of differential miRNA expression across various grades of glioma (93, 94). An initial screen of 739 miRNA found that 107 miRNA were over-expressed by at least 200-fold in glioma patient serum compared to serum from healthy controls (94). Subsequent qRT-PCR validation in a larger set of serum identified a 9-miRNA signature that were significantly over-expressed in pre-operative serum from patients with Grade II, III and IV glioma (n=90) compared to serum from health controls (n=110). Importantly the expression levels of these 9 miRNA were markedly reduced when comparing patient matched serum pre- and post-surgical resection (n=73), indicating that these miRNA may be suitable serum biomarkers for glioma diagnosis and predicting tumour recurrence. Another study utilized genome-wide solexa sequencing to evaluate the levels of 904 miRNA in the serum of 44 grade III and IV glioma patients and 43

controls (93). A total of 50 miRNA were found to be differentially expressed in patient versus normal control sera. Subsequent validation by qRT-PCR ultimately found the expression levels of 7 miRNA were significantly different in patient versus normal control sera. However, whether this 7-miRNA gene signature in serum also predicted patient outcome across various malignant astrocytomas was not presented in this report (93).

Many other studies have identified individual miRNA as potential diagnostic and prognostic serum or plasma biomarkers for glioma patients. The expression of miR-454-3p in glioma patient plasma was significantly higher than in plasma from normal controls and its expression also correlated with the overall outcomes of glioma patients (95). Importantly, the expression of miR-454-3p was also significantly higher in pre-operative plasma compared to plasma from matched patients 14 days post-surgical resection. Likewise, miR-21 was elevated in glioblastoma patient plasma compared to controls and was enhanced in the pre-operative versus post-operative plasma from matched patients (96). These results suggest that plasma levels of miR-21 and miR-454-3p (and possibly others miRNA) could potentially be used to detect early glioma development or tumour recurrence. Similarly, expression of miR-210 (97), miR-221 and miR-222 (98) were significantly higher in glioma patient serum and plasma compared to normal controls and expression of miR-29 (99) and miR-125b (100) were significantly lower in glioma patient serum compared to normal controls. Finally, others have also reported that miRNA found in circulating exosomes in patient serum may also be utilized as potential diagnostic biomarkers for glioblastoma (101, 102).

6. Conclusions

Currently, gene expression profiles, gene mutational analysis, post-transcriptional epigenetic changes and chromosomal alterations are proposed for clinical practice as biomarkers for patient outcomes and response to treatment. In glioblastoma, these

specifically include isocitrate dehydrogenase 1 and 2 gene mutations and MGMT promoter methylation (103). However, miRNA biomarkers do not currently provide a sufficient degree of prognostic (pre-treatment) or predictive (on treatment) accuracy to guide clinical decision making. Nonetheless, as molecular grading of gliomas is continually improving, traditional histopathological assessment for diagnosis of varying glioma grades may be phased out. The ultimate aim clinically is to characterise and stratify a patient's glioma based on individual genetic profile allowing for a personalized treatment that is based on this classification. The use of reliable miRNA expression signatures should give rise to further confidence for establishing universal molecular signatures for glioma classification and treatment stratification. Indeed the ideal genetic signature that has the greatest diagnostic and prognostic value may involve a combination of current molecular biomarkers and novel miRNA expression profiles in both tumour tissue and more importantly peripheral blood samples. As further advances occur in understanding the role of miRNA expression in glioma development and progression, differential miRNA expression profiles have the capacity to improve glioblastoma sub-type classification, delineate a pattern of disease progression and ultimately improve response to therapy leading to increased patient survival.

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Table 1: Differential miRNA expression in Glioblastoma versus normal brain tissue

No of miRNA tested	Clinical Comparison (Sample size)	No of over-expressed miRNA vs control brain	No of under-expressed miRNA vs control brain	Method Used	(Ref)
245	Glioblastoma (9) vs matched peripheral brain tissue (9)	9	4	Microarray	(104)
180	Glioblastoma (3) vs control brain tissue (8)	5	3	Membrane array hybridisation	(105)
192	Glioblastoma (4) vs control brain tissue (4)	3	13	RT-PCR	(106)
282	Glioblastoma (12) vs control brain tissue (4)	15	11	Membrane array hybridisation and RT-PCR	(52)
245	Glioblastoma (x^a) vs matched peripheral brain tissue (x^a)	8	11	Microarray	(42)
x^b	Glioblastoma (3) vs control brain tissue (3)	5	5	RT-PCR	(107)
875	Glioblastoma (3) vs control brain tissue (3)	33	40	Deep Sequencing	(108)
484	Glioblastoma (6) vs control brain tissue (3)	18	38	Deep Sequencing	(109)
756	Glioblastoma (26)[c] and Anaplastic astrocytomas (13) vs control brain tissue (7)	55	29	Microarray	(45)
754	Glioblastoma (58) vs control brain tissue (10)	108	108	Microarray	(110)
200	Glioblastoma (21), Anaplastic astrocytomas (31), diffuse astrocytoma (26) and pilocytic astrocytoma (6) vs normal adjacent tissue (20)	2	10	RT-PCR	(48)

534	Glioblastoma (156) vs control brain tissue (10)	19	19	Microarray	(64)
534	Glioblastoma (490) vs healthy controls (10)	20[d]	20[d]	Microarray	(111)

[a]*The number of glioblastoma patient samples nor adjacent non-neoplastic tissue was not stated in this report.*
[b]*The number of miRNA assessed was not stated in this report.*
[c]*The 26 patient samples are made up of 13 primary and 13 secondary glioblastoma.*
[d]*miRNA with greatest/lowest contribution to glioblastoma phenotypic state.*

Table 2: Consistent miRNA expression across at least 3 independent studies from Table 1

miRNA Identified	Expression vs control brain	No of studies identified
miR-21	Up-regulated	13 out of 13
miR-10b	Up-regulated	7 out of 13
miR-25	Up-regulated	7 out of 13
MiR-106b	Up-regulated	6 out of 13
miR-155	Up-regulated	6 out of 13
miR-210	Up-regulated	5 out of 13
miR-15b	Up-regulated	4 out of 13
miR-92a	Up-regulated	4 out of 13
miR-92b	Up-regulated	4 out of 13
miR-93	Up-regulated	4 out of 13
miR-9	Up-regulated	3 out of 13
miR-10b*	Up-regulated	3 out of 13
miR-15a	Up-regulated	3 out of 13
miR-16	Up-regulated	3 out of 13
miR-17	Up-regulated	3 out of 13
miR-21*	Up-regulated	3 out of 13
miR-27a	Up-regulated	3 out of 13
miR-130b	Up-regulated	3 out of 13
miR-142-3p	Up-regulated	3 out of 13
miR-132	Down-regulated	8 out of 13
miR-218	Down-regulated	6 out of 13
miR-124	Down-regulated	5 out of 13
miR-128a	Down-regulated	5 out of 13
miR-138	Down-regulated	5 out of 13
miR-7	Down-regulated	4 out of 13
miR-128	Down-regulated	4 out of 13
miR-149	Down-regulated	4 out of 13
miR-124	Down-regulated	3 out of 13
miR-129*	Down-regulated	3 out of 13
miR-129	Down-regulated	3 out of 13
miR-137	Down-regulated	3 out of 13
miR-203	Down-regulated	3 out of 13
miR-323	Down-regulated	3 out of 13
miR-323-3p	Down-regulated	3 out of 13
miR-329	Down-regulated	3 out of 13

miR-330	Down-regulated	3 out of 13
miR-379	Down-regulated	3 out of 13
miR-410	Down-regulated	3 out of 13
miR-432	Down-regulated	3 out of 13
miR-433	Down-regulated	3 out of 13
miR-485p	Down-regulated	3 out of 13

Table 3: Differential miRNA expression in patient versus normal control serum/plasma

Clinical Comparison (Sample size)	Over-expressed miRNA vs control	Under-expressed miRNA vs control	Source	(Ref)
Glioblastoma (20) vs controls (20)	miR-128	miR-342-3p	Peripheral blood	(91)
Glioblastoma (10) vs controls (10)	miR-21	miR-128, miR-342-3p	Plasma	(92)
Glioma (122) vs controls (123)		miR-15b, miR-23a, miR-133a, miR-150*, miR-197, miR-497, miR-548b-5p	Serum	(93)
Glioma (90) vs controls (110)	miR-15b-5p, miR-16-5p, miR-19a-5p, miR-19b-3p, miR-20a-5p, miR-106a-5p, miR-130a-3p, miR-181b-5p, miR-208a-3p		Serum	(94)
Glioma (70) vs controls (70)	miR-454-3p		Plasma	(95)
Glioblastoma (10) vs controls (10)	miR-21		Plasma	(96)
Glioblastoma (42) and AA (46) vs controls (50)	miR-210		Serum	(97)
Glioma (50) vs controls (51)	miR-221, miR-222		Plasma	(98)
Glioma (83) vs controls (69)		miRNA-29	Serum	(99)
Glioma (33)		miRNA-125b	Serum	(100)

vs controls (33)