Chapter

MicroRNA Biomarkers in Primary Brain Malignancies

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Abstract

Despite the concerted efforts within the management of brain malignancies over the past few decades, primary brain cancers remain an obscure challenge with unfavourable outcomes for the patients. Glioblastomas (GBM) and medulloblastomas afford the most prevalent brain tumours and account for markedly high mortality rates within affected patients. The unmet clinical requirements for an early diagnostic biomarker and effective treatment have shed light onto microRNAs (miRNAs). These are small, endogenous noncoding RNAs involved in a wide spectrum of biological processes, such as post-translational modification, tumorigenesis, angiogenesis, invasiveness, and apoptosis. Increased expression of miR-21 has been shown to have devastating effects upon patients with brain tumours, and it could be used as a diagnostic biomarker and an early relapse indicator. miRNAs such as miR-128a, miR-34a, miR-7 and miR-1253 have demonstrated tumour suppressive properties and could serve as putative therapeutic agents. MiRNA signatures, such as miR-21 and miR-10b could be incorporated as potential prognostic indicators for advanced and metastatic brain malignancies, whereas miR-221/222 cluster has a therapeutic potential to sensitise cancerous cells towards radiotherapy. Herein, we summarised current knowledge on how miRNAs with significant role in glioblastomas and medulloblastomas specifically can be effectively used as promising brain cancer diagnostics, prognostics, and therapeutics.

Keywords: microRNA, signalling pathways, biomarker, diagnosis, prognosis, therapy, glioblastoma (GBM), medulloblastoma

1. Introduction

1.1 Research background

Within the current field of primary brain cancer research, a complimentary class of potential biomarkers, known as microRNAs (miRNAs), are becoming increasingly favoured upon their pleiotropic advantages; from adopting potential diagnostic, prognostic, and therapeutic properties. In the contemporary study of the adult brain tumour, glioblastoma (GBM), several miRNAs are seen to act as potential biomarkers of the debilitating cancer. Through the revelation of current studies, miR-21,

miR10b, miR-221, and miR-222 are seen to have promising usages of becoming novel diagnostic and prognostic markers. Besides the use of individual miRNAs to act as biomarkers, the cluster, miR-221/222, has also been seen to additionally possess therapeutic potentials within the treatment of high-grade gliomas. Similarly, miRNAs can also serve as biomarkers within medulloblastomas, a paediatric brain cancer. The miRNA, miR-10b, is seen to own diagnostic and therapeutic potentials within a subgroup of medulloblastoma, known as Sonic Hedgehog (SHH). Additionally, miR-466-3p can act both therapeutically and diagnostically, dependent on the regulation pattern of the miRNA. The review will expand upon the potential usages of the miRNAs mentioned above but will also introduce additional potential miRNAs to highlight the beneficial impacts this class of noncoding RNAs can play within primary brain malignancies.

2. Primary brain malignancies

Classed as a heterogenous set of tumours, primary brain cancers are termed as abnormal cellular growths within the cavity of the brain [1]. Although the malignancy is classified as rare, primary brain cancers owe to significantly high mortality and poor survival rates, with only 40% of patients surviving over a year [2]. The incidence rates for brain cancers in the UK alone has increased by 39% since the 1990s [3]. In fact, by 2035, it is estimated that incidence rates will increase by a further 5% and 8% for males and females in the UK from 2014 [3]. Similarly, individuals affected with brain cancers are more likely to suffer from prolonged life-changing cognitive, physical and psychological impairments unlike other types of cancers, with one study observing approximately 90% of patients with brain metastases displaying substantial cognitive deficits prior to treatment [4, 5]. Thus, there is a demand for novel interventions to deliver successful treatment and improve prognosis outcomes for patients.

Brain malignancies in adults currently stand as the eighth most common cancer [4]. In fact, the tenth leading source of deaths for men and women is from brain and central nervous system (CNS) cancers [6]. Serving as a highly heterogeneous tumour, GBM is a rare aggressive adult primary brain cancer [7]. Native to gliomas, the malignancy subtype collectively constitutes 81% of malignant intracranial tumours in adults [4]. Despite significant efforts, the five-year survival rates for GBM patients remain low standing at only 6.8%, with an 8-month median survival period on average [4]. Often a full brain tumour resection is not achievable due to the anatomical structure of the cranial and brainstem nerves surrounding the brain [8]. Even with surgical interventions to increase prognosis outcomes, the majority of GBM tumours remain obstinate to chemotherapeutics; a primary cause for the reduced efficacy outcomes for GBM patients [9].

Among children to adolescent years, paediatric brain cancers stand as the leading source of cancer-related deaths [4]. Categorised as solid tumours, risk factors to the young are thought to be influenced from environmental and genetic factors; where a family history and a maternal age over 40 during birth, as well as high radiation exposure commonly seen in leukaemia patients, all pose high risks to the child [10]. Medulloblastoma, a high-grade tumour, accounts for approximately 10% of all paediatric brain malignancies [11]. Associated with significantly high morbidity rates, the malignant tumour originates within the posterior fossa of the brain [11]. Common treatment plans for medulloblastoma patients include chemotherapy and/or radiotherapy. Although, a study observed the nutritional effects young children gained from chemotherapy, where the nutritional status of patients began to fall; inclining

them to a mean weight loss of 8.2% during the second course of chemotherapy treatment from diagnosis [12]. Additionally, surgical interventions are also primarily used to exile tumours; however, it has been reported that up to 40% of patients suffer from neurological losses from surgery [13]. Even with successful treatment outcomes, both children and adults can endure long-term debilitating and neurological effects. Paediatrics can suffer from learning and growth difficulties, while adults tend to be stranded with cognitive and neurological impairments [13]. Since current findings highlight the significant association between morbidity and current treatments for medulloblastoma patients, there is a need for novel interventions which allow for long-term effective treatment plans for patients.

In the management of brain malignancies, the location of primary brain tumours has shown to pose mainstream challenges to current therapeutic interventions, restricting efficacy outcomes in patients. Majority of current drug and chemotherapeutic drug delivery strategies have been shown to have difficulty in the passive movement across the blood brain barrier (BBB), due to intracranial endothelial cells forming tight junctions which limits the passive diffusion to only small sized gas and lipophilic molecules [14]. In turn, this poses constraints for chemotherapy treatments and the readily delivery of therapeutic drugs to brain tumours, thus restricting treatment efficacy. In order to overcome the microvasculature structure, current studies have begun to observe the successful usages of nanoparticles, intra-arterial and intranasal methods of treatment deliveries through the BBB [15]. However, further research is required to optimise the scope of new techniques for drug deliveries to patients to improve current survival rates post treatment.

3. MicroRNA biogenesis

MiRNA biogenesis begins with post-or co- transcription of transcripts of RNA polymerase II/III [16]. At present, the majority of miRNA identified are intragenic and commonly processed from noncoding regions of protein coding genes, known as introns [17]. The remaining miRNAs are referred to as intergenic; where both, transcription occurs independently from a host gene and regulation occurs from their own independent promoters [17]. From the study of miRNAs, the pleiotropic nature of these noncoding RNAs, which have significant utilities within disease states and drug resistance, elucidates the potential of these molecules to serve as important biomarkers within the diagnosis and treatment of a wide range of diseases [18].

The classification of miRNA biogenesis is separated into two distinct pathways: non-canonical and canonical. The primary route for miRNA processing occurs in the canonical biogenesis pathway. The recognition of multiple motifs and N6-methyladenylated GGAC occurs primarily from the DiGeorge Syndrome Critical Region 8 (DGCR8) [19]. The protein alongside Drosha, a ribonuclease III enzyme, forms a microprocessor complex, allowing the processing of transcribed primary miRNA (pri-miRNA) into precursor miRNA (pre-miRNA) [20]. The resulting catalytic subunit of the microprocessor complex, Drosha, can cleave pri-miRNA from its hairpin assembly, forming 2-nucleotide 3′ overhang on the pre-miRNA [21]. The overhang of pre-miRNA exports from the nucleus into the cytoplasm with the assistance of exportin 5 (XPO5)/RanGTP complex [22]. The pre-miRNA becomes processed by Dicer, a RNase III endonuclease, where a mature miRNA duplex is biosynthesised through the elimination of the terminal loop [23]. Depending on whether the guide strand arises from the 3′ or 5′ end which is reliant on the either use of 3p or 5p strand, the Argonaute protein (AGO 1–4)

facilitates the loading of both strands onto the protein [24]. This results in the formation of an additional complex known as the miRNA-induced silencing complex (RISC), where miRNAs have the ability to bind to 3′ untranslated regions (UTR) and thus, become regulators at a post-transcriptional level [17].

Conversely, the non-canonical pathway utilises predominant proteins from the canonical biogenesis pathway, such as Dicer, AGO2, Drosha and exportin 5 [16]. However, the pathway can be further sub-categorised into Drosha/DGCR8-independent and Dicer-independent routes [25]. Among the Drosha/DGCR8 independent pathway, pre-miRNAs such as mirtrons feature substrates of the RNase III endonuclease, Dicer to complete cytoplasmic maturation [26]. Unlike the canonical pathway, Drosha cleavage is not utilised and instead the nascent RNAs leave the nucleus to the cytoplasm via exportin 1 [16]. Similarly, the Dicer-independent pathway processes miRNA from transcripts of endogenous short hairpin RNA (shRNA) via the ribonuclease III enzyme, Drosha [27]. Within the cytoplasm, AGO2 allows maturation of the pre-miRNA, since they cannot resemble the sufficient length of Dicer substrates [27]. The maturation process ends when the 3p strand is sliced via AGO2 and the 5p strand becomes trimmed via 3′ to 5′ trimming [28].

Following the production of minimal miRNA-induced silencing complex (miRISC) in the cytoplasm, miRNA response elements (MREs) allow miRISC to maintain target specificity [29]. The 3′ untranslated region is typically where a regulatory potential is provoked, though MREs are situated throughout a mRNA molecule [30]. The degree in which a given target can be controlled is crucially dependent on miRNA and MRE affinity for one another, subcellular location and number of miRNA and MRE present in the cell [30]. However, the vast number of miRNA:MRE interactions within animal cells are not entirely correlative to each other. Since, MREs commonly have at least one central mismatch on the guide miRNA, the function of AGO2 endonuclease is inhibited [16].

4. MicroRNAs in brain malignancies

MiRNAs are a novel class of endogenous noncoding molecules of RNA composed of a nucleotide length of 18–22 [7]. These single stranded molecules facilitate the expression of target genes at a post transcriptional level through complementary base pairing with specific regions in target mRNAs [9]. Initially discovered in the nematode *Caenorhabditis elegans*, miRNAs are becoming recognised, with an upward interest in miRNA novel therapies in cancers, upon their diagnostic and therapeutic potentials. The expression of various functioning genes can become downregulated by a single miRNA. Hence, the development of novel methods to potentially identify and alter miRNA pathways can lead to a new discovery in cancer treatment, since cancers contain numerous gene aberrations [31]. In contrast to conventional drug therapies, miRNAs can pass more easily through the BBB into the intracranial space, particularly in disease states [32]. Thus, miRNAs can become optimally used as potential biomarkers, as well as in novel drug delivery mechanisms into intracranial tumours. **Figure 1** below outlines the scope of this chapter demonstrating the possible uses of miRNAs in combating brain malignancies.

With poor long-term survival outcomes for primary brain cancers, the standard treatments of surgery and radiotherapy remain unsatisfactory. However, miRNA studies provide potential hopes from numerous novel approaches in the treatment of primary brain malignancies. Firstly, current miRNA studies highlighted the potential ability of a miRNA to class tumours, with a study by Lu et al., finding expression

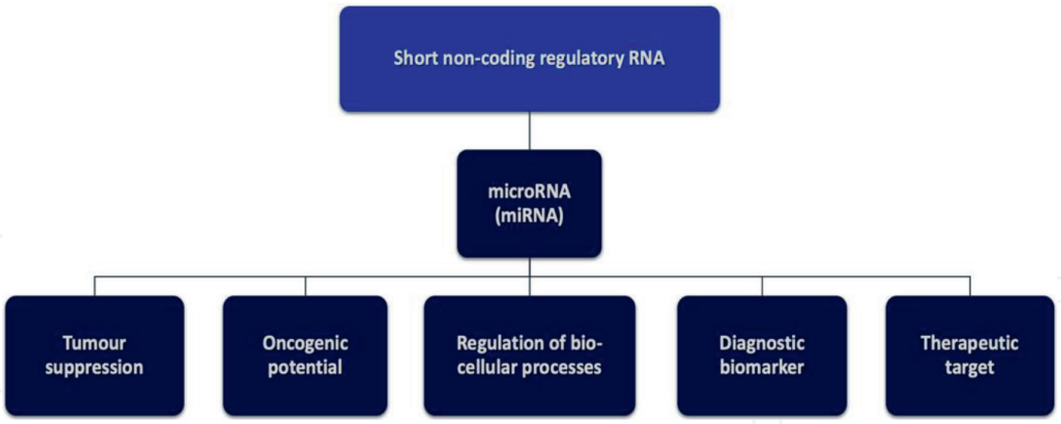

Figure 1.
Scope of the possible implications of miRNAs in combating brain malignancies. These small, non-coding RNAs have shown the ability to act as oncomiRs or tumour suppressors. Thus, their elevated or decreased levels could be possibly used as diagnostic, prognostic, and therapeutic markers in the management of CNS tumours.

profiles of miRNA to be effective in classifying poorly differentiated cancers [33]. The miRNA profiles exhibited greater levels of knowledge regarding tumour states and lineage progression of tumours [33]. Thus, from the successful usages of miRNAs in the differentiation of cancers, these pleiotropic molecules can potentially become used within the development of future novel therapeutics in cancer.

Additionally, miRNAs are increasingly becoming known to exhibit tumour suppression properties within brain cancers, a potential approach for treatment. A recent study by Xue et al. found that the tumour development from medulloblastoma was downregulated by two exosomal miRNAs; miR-101-3p and miR-423-5p. These tumour suppressors targeted the *FOXP4* gene and the histone methyltransferase, *EZH2* [34]. Furthermore, a contemporary study revealed the therapeutic potential usages of the tumour suppressor miR-138, for treating primary glioblastoma, by directly decreasing the regulation of CD44 to suppress proliferation [35]. Moreover, a study carried out by Costa et al. observed the potential advantages of anti-miR-21 oligonucleotide within a glioblastoma mouse model. The miRNA was found to decrease cellular proliferation and tumour growth, while being able to also increase apoptosis within the model [36].

Therein, miRNAs hold therapeutic capabilities to differentiate tumour grades, excel the current standards of knowledge regarding these cancers, as well as acting as tumour suppressors in the treatment of primary brain tumours. Such novelties should be explored in the treatment of such debilitating tumours, to provide personalised therapies for many primary brain malignancies. Although additional research is required to demonstrate miRNA therapeutic potentials in clinical context, the discovery of miRNAs to possess multiple advantageous properties promise a forward approach towards the treatment and outcomes for primary brain cancers.

5. MiRNAs and glioblastomas

5.1 miRNAs as biomarkers within glioblastomas

The unmet clinical requirements for an early diagnostic tool and effective treatment for glioblastoma via the existing routine strategies have initiated the need of

novel approaches for the early and correct diagnosis of GBM, followed by an adequate prognostic plan and a possible treatment strategy. The extensive role of miRNAs in the regulation of GBM tumorigenesis has made these small, non-coding RNAs an attractive source of information for researchers. MiRNAs found in GBM are involved in a wide spectrum of biological processes ranging from neurone differentiation and maturation, post-translational modification of genetic information, tumorigenesis, angiogenesis, invasiveness, resistance to treatment, apoptosis, and immune system modulation. In a systematic review Møller and colleagues demonstrated that more than 300 miRNAs are deregulated in GBM, with miR-253 being overexpressed and miR-95 under-expressed [37]. Faulty events during the biogenesis of miRNAs can lead to their deregulation in many cancers. Such events include amplifications, deletions, epigenetic modifications, translocations, and silencing of miRNAs [38].

5.2 Overexpressed miRNAs in GBM and their possible implications as biomarkers

Some miRNAs can act as tumour suppressors and others as oncogenes (oncomiRs). Detection of miRNA signatures in primary brain tumours has revealed unique avenues for assessing the diagnosis, prognosis, and monitoring of patients [39]. Some of the well-studied examples of overexpressed miRNAs in high grade glioblastomas with diagnostic and prognostic properties include miRNA-21, miRNA-10b, miR-221, and miR-222. The molecular mechanisms via which these miRNAs act are under extensive evaluation with some of the affected genes and pathways shown in **Figure 2**.

miR-21 is aberrantly expressed in many types of cancer, such as colorectal, lung, pancreas, leukaemia and GBM. Located within the vacuole membrane protein 1 (VMP1) locus on chromosome 17, the mature miR-21 is transcribed in a complex

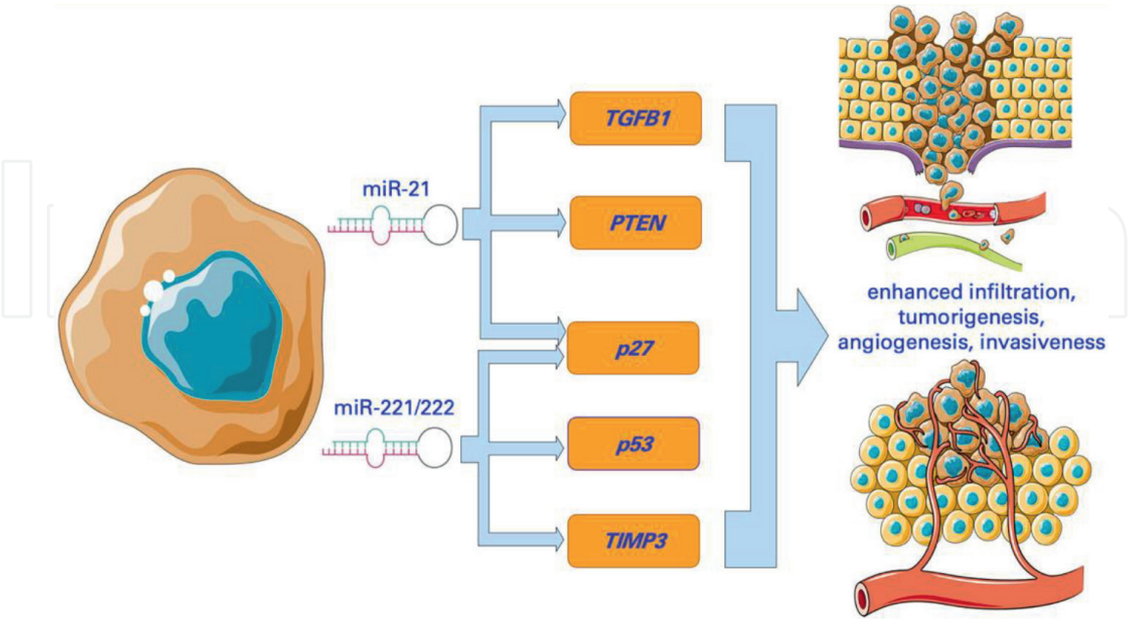

Figure 2.
Molecular pathways affected by miR-21 and miR-221/222. Overexpression of oncomiR-21 leads to enhanced cell survival, and thus proliferation, due to the inhibiting ability of miR-21 towards pro-apoptotic genes such as PTEN. miR-221/222 directly regulate glioma cells invasion via the tissue inhibitor of metalloproteinase 3 (TIMP3). miR-221/222 lower the gene expression and diminish the protein levels of p27 and 57, thus promoting S-phase progression and cell proliferation.

manner from two pri-miR-21 of an approximate size 3.5 kb and 4.3 kb [40]. miR-21 plays a pivotal role in the tumorigenesis of GBM and is the only miRNA, which to date has clearly defined diagnostic and prognostic properties. The levels of miR-21 drastically decrease post tumour resection, allowing the biomolecule to be used as monitoring agent for patients and detect early relapse [41]. High levels of miR-21 in blood samples predict a poor prognosis for GBM patients, due to the pro-tumorigenic properties of miR-21, acting as a down-regulator for insulin-like growth factor-binding protein 3 (IGFBP3) and other caspases, thus inhibiting programmed cell death [42]. Multiple studies have demonstrated the up-regulated profile of miR-21 in several types of cancers, indicating its potential role as an oncomiR [43, 44]. Extracellular miR-21 has demonstrated its relevance as a diagnostic biomarker of GBM. A meta-analysis study has revealed that miR-21 can predict GBM with high accuracy and specificity [45]. The unique properties of miR-21 in gliomas differentiated between other brain tumours and have been validated by Ivo D'Urso and colleagues who showed that miR-21, alongside miR-16 possessed a 90% sensitivity and 100% specificity in doing so [46]. The detection of miR-21 in cerebrospinal fluid (CSF) and its diagnostic relevance has also been evaluated via its direct action upon the TGF-β/Smad3 signalling pathway. The researchers found out that the inhibitor for GF-β type I receptor kinase, named galunisertib, decreased the expression of miR-21 and thus supressed its oncogenic properties [47]. However, screening CSF samples is not an easy task and might lead to complications, if not performed precisely. Elevated levels of miR-21 were also reported following examination of the plasma samples collected from GBM patients in which the results showed increased expression of miR-21 [48]. Mao and colleagues also studied the expression of miR-21 in serum samples from control and GBM patients and demonstrated that miR-21 was significantly upregulated in all GBM samples [49]. The overexpression of miR-21 in glioblastoma tumours has shown to lead to enhanced cell survival, and thus proliferation, due to the inhibiting ability of miR-21 towards pro-apoptotic genes such as *PTEN*, consequently leading to avoiding apoptosis [44, 50].

Another miRNA with a high oncogenic potential and universal to GMB is miR-10b. miR-10b is virtually undetectable in normal brain tissue; however, in low- and high-grade brain tumours from different subtypes this miRNA becomes abundantly expressed [51]. Located within the *HOXD* genomic locus, miR-10b is involved in various cancerogenic pathways including proliferation, invasion, and metastasis of malignant glioblastomas. Although miR-10b has been found to be deregulated in different types of tumours such as ovarian and gastric tumours, alongside glioblastomas, the regulation of the miRNA appears to be cell- and context-specific [52]. The detection of high levels miR-10b in the serum of GBM patients who have undergone a treatment therapy with bevacizumab has indicated the potential of miR-10b as a prognostic marker for monitoring therapy. The researchers identified that miR-10b, alongside miR-21, were highly expressed in GBM patients' post-treatment in comparison to pre-treatment levels of both miRNAs [53]. A negative correlation between the highly expressed miR-10b and miR-21 and the size of glioblastomas was also evaluated throughout this research. The same correlation was not observed in patients who have undergone a temozolomide therapy. Thus, miR-10b, in a combination with miR-21, might be incorporated in monitoring patients treated with bevacizumab. While miR-10b is specifically detectable in the CSF of patients with advanced and metastatic brain tumours, miR-21 is expressed in various cancer types and normal brain tissue and lacks exclusive specificity for GBM [54]. Low or absent levels of miR-10b and miR-21 were found in the CSF of GBM patients in remission, with an increase of both miRNAs during relapse rates and progression of the tumour with an accuracy 91–99% [51]. Thus,

the utilisation of miR-10b alongside miR-21 could be a possible monitoring and prognostic signature biomarker of advanced and metastatic GBM.

The overexpressed miR-221/222 cluster is associated with the degree of glioblastoma infiltration and poorer overall survival [55]. The collectively encoded miR-221 and miR-222 in a gene cluster located on chromosome X (Xp11.3) are highly conserved in vertebrates with an identical seed region separated by 727 bases [56]. The role of the miRNA cluster as prognostic marker in glioblastomas has been demonstrated by Zhang and colleagues, who identified significantly high plasma levels ($p = 0.0001$) of miR-221/222 in glioma patients which positively correlated with poorer survival rates within 95% of the studied cohort [57]. Some of the molecular mechanisms via which the miR-221/222 family acts during glioblastoma carcinogenesis are by promoting the S-phase of the cell cycle, inhibiting apoptosis, or regulating the invasiveness of the cancer. The up-regulation of miR-221/222 is closely related with the cell cycle check points *p27* and *p57*. Both miRNAs bind to their 3' UTR regions ensuring lower gene expression and diminish the protein levels of p27 and 57. This in turn promotes S-phase progression and cell proliferation [58]. miR-221/222 were shown to directly regulate glioma cells invasion via the tissue inhibitor of metalloproteinase 3 (TIMP3). The researchers demonstrated that TIMP3 is a direct target for miR-221/222 and knockdown of miR-221/222 in xenograft mouse models restored the normal levels of TIMP3 and reduced tumour growth [55]. Researchers also demonstrated that the cluster could potentially serve as a therapeutic agent by increasing the radiosensitivity within glioblastoma cells via *PTEN* independent activation of the Akt pathway [59]. Tokudome and colleagues have identified low levels of *PTEN* after radiotherapy, suggesting the miR-221/222 cluster could act as an inhibitor for glioblastoma cells post radiation and thus supress tumour growth [60]. The significance of the prognostic and potential therapeutic implications of miR-221/222 in high-graded gliomas is supported by their interactions with the tumorigenic genes *TIMP3*, *p27*, *p53* and *PTEN*. The plasma levels of miR-222 and miR-21 were shown to be reduced after total tumour resection within GBM patients. Thus, a signature of miR-222, miR-21 and miR-124-3p could find an implication in monitoring patients with post tumoral resection and possibly identify early relapse [61].

5.3 Under-expressed miRNAs in GBM and their possible implications as biomarkers

MiRNAs with potential diagnostic properties include miR-128, miR-34-3p, and miR-7 [45]. These miRNAs are downregulated in glioblastoma patients and act as tumour suppressive agents that could be used for diagnostic, prognostic and therapeutic purposes. The molecular mechanisms via which these miRNAs regulate some of the affected genes and pathways shown in **Figure 3**.

The brain-enriched miR-128 is a type of an intronic miRNA encoded by two different genes, miR-128-1 and miR-128-2, located on chromosomes 2q21.3 and 3p23.p, respectively [62]. The normal expression miR-128 has been linked to normal brain development [63]. However, miRNA assays, quantitative Real-Time Polymerase Chain Reaction (RT-qPCR) and Western blot analyses indicated that miR-128 was under-expressed in aggressive solid brain tumours, including glioblastomas and medulloblastomas, when compared to normal adjacent brain tissue [64]. miR-128 exerts its role in glioblastoma tumorigenesis via different pathways, including inhibition of proliferation, influencing apoptosis and drug resistance, regulating epithelial to mesenchymal transition and inhibiting tumour cell invasion and motility. Previous

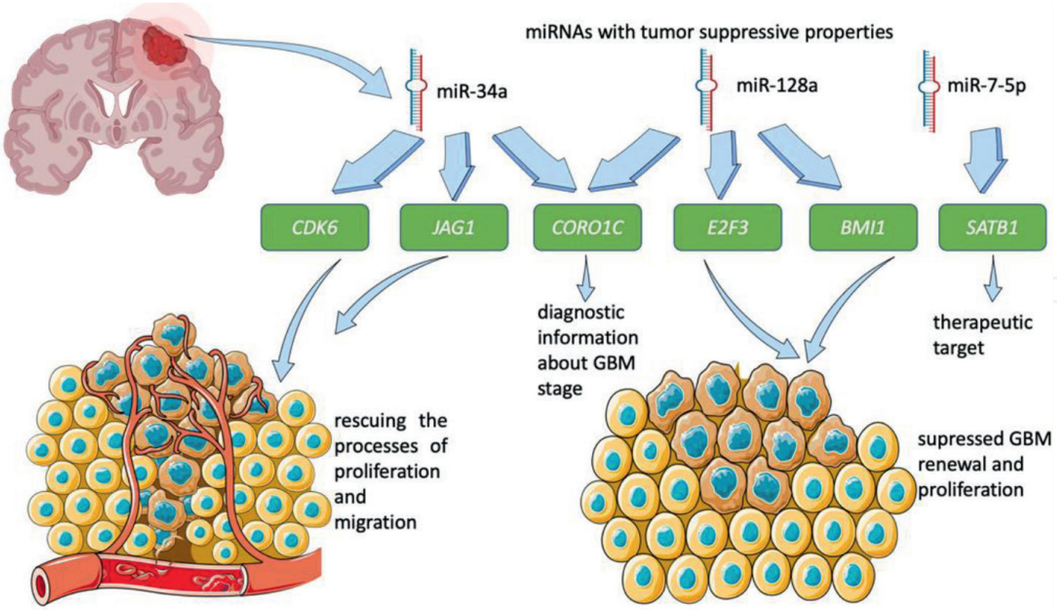

Figure 3.
Molecular pathways affected by miR-128a and miR-34a. miR-128a is associated with the polycomb complex protein BMI1 and E2F transcription factor 3, E2F3. miR-128a can inhibit proliferation of glioblastoma cells by directly targeting E2F3a. miR-128a could supress GBM renewal and proliferation by directly targeting BMI1. The relevance of CORO1C in glioblastoma has demonstrated a link to the grade of the malignancy. The consequences of the downregulation of miR-34a in GBM has been associated with the suppression of several oncogenes, including CDK6, Notch1 and Notch2. The induced overexpression of miR-34a possess the potential to supress the functions of distorted genes, such as JAG1 and CDK6, rescuing the processes of proliferation, cell cycle progression, survival, and migration.

research has confirmed the high analytical specificity and sensitivity of both, miR-21 and miR-128, in their use as diagnostic markers for GBM. Roth and colleagues demonstrated the significant downregulation of miR-128 in the peripheral blood samples of 20 GBM patients with an accuracy of 81%, and sensitivity and specificity of 79% and 81%, respectively [65]. However, the number of studied patients was only 20, indicating the need of validating these results in a larger cohort of GBM patients. The forced expression of miR-128a demonstrated inhibition of GBM development via the promotion of apoptosis in the U-87 MG GBM cell line [66]. However, the molecular mechanism via which miR-128a acts in glioblastoma multiform tumours is not yet fully understood. Multiple studies have suggested that the expression of miR-128a is differentiated in various types of cancer and might exert distinctive roles in cancer development. For instance, miR-128a has been found to be overexpressed in acute lymphoblastic leukaemia [65, 67]. miR-128a was mainly found to be associated with the polycomb complex protein BMI1 and E2F transcription factor 3, *E2F3*. Previous experiments have demonstrated that miR-128a can inhibit proliferation of glioblastoma cells by directly targeting *E2F3a* which could subsequently lead to rescuing the supressed proliferation mechanism found in GBM [68]. The link between BMI1, a stem cell renewal factor and miR-128a has also been documented by Godlewski and colleagues, who demonstrated that miR-128a could supress GBM renewal and proliferation by directly targeting BMI1 [69]. The relevance of coronin-1 C, (*CORO1C*), in glioblastoma has demonstrated a link to the grade of the malignancy [70]. Cell cycle progression, cell transduction and apoptosis are possible pathways via which the gene might exert its tumorigenic potential. The expression of the gene has been examined in different types of brain malignancies. Hence, CORO1C might provide

useful diagnostic information about GBM stage and could possibly be blocked when miR-128a is overexpressed, and thus modify its oncogenic activity.

The tumour suppressor miR-34a has emerged as a possible therapeutic agent for GBM patients. Located on the second exome of chromosome 1p36, miR-34a is encoded by its own, two transcripts, which are highly conserved in humans [71]. This miRNA is thought to act as a tumour suppressor via the p53 pathway as its gene promoters contain *p53* binding sites. Researchers identified significantly lower levels of miR-34a in patients with mutated *p53* status in comparison to wild-type *p53* GBM samples [72]. Gao and his colleagues also identified that the levels of miR-34a in high grade gliomas were significantly lower when compared to normal adjacent brain tissue. This finding allowed for the consideration of miR-34a as a possible diagnostic and predictive biomarker in GBM. The under expression of miR-34a has been shown to correlate with poorer prognosis in GBM patients. The consequences of the down-regulation of miR-34a in GBM has been associated with the suppression of several oncogenes, including *CDK6, Notch1* and *Notch2* [71]. Researchers have suggested that *Notch1* and *Notch2* are frequently overexpressed in glioblastomas and medulloblastomas [73]. As a key player in cell-to-cell communication, normal neuronal development and differentiation, and *de novo* blood vessel formation, the dysregulated Notch signalling pathway is a very important tumorigenic factor in GBM. For the Notch signalling pathway to transduce signals between cells, a family of Jagged protein receptors embedded in the membranes of adjacent cells are required. One of these protein receptors is Jagged1, transcribed from the *JAG1* gene. The oncogene cyclin dependant kinase 6, *CDK6*, a serine/threonine protein kinase that regulates transition through the cell cycle has been shown to be overexpressed in brain tumours. The expression of *CDK6* at later tumour stages was found to be increased in 12 out of 14 glioblastoma tumour samples [74]. Increased levels of miR-34a *in vitro* could induce apoptosis and inhibit proliferation in GBM cell lines [75]. Thus, the induced over-expression of miR-34a possess the potential of a therapeutic miRNA that could be used to supress the functions of distorted genes, such as *JAG1* and *CDK6*, rescuing the processes of proliferation, cell cycle progression, survival, and migration. The lack of research investigating these genes indicates a gap in this field of cancer research, with the potential for discovering new molecular mechanism via GBM tumorigenesis affecting possible targeted therapeutics.

Another miRNA that has been found to be downregulated in glioblastoma tissues is miR-7. In humans, an identical mature sequence of this miRNA can be encoded by, miR-7a-1, miR-7a-2, and miR-7b, located on different chromosomes [76]. miR-7 is highly expressed in normal brain tissue and plays an important role in many physiological and pathological processes within the brain. miR-7-5p was found to inhibit cell migration and invasion in glioblastomas by targeting the special AT rich sequence binding protein (SATB1) [77]. Yin and colleagues demonstrated that miR-7-5p has a suppressive effect upon SATB1 within the U87 and U373 glioblastoma cell lines expressed in inhibited migration and invasion of the cells. An immunohistochemical analysis of a microarray with 122 glioma samples has indicated that high-grade gliomas were associated with significant expression of phosphorylated SATB1, which in turn also correlated with poorer overall survival rates indicated by Kaplan-Maier analysis [78]. The suppressive effect of miR-7-5p upon SATB1 provides a potential avenue for treatment. Delivery of miR-7-5p in DNA-cationic liposome complexes to glioblastoma cells demonstrated significant growth and metastasis inhibition *in vivo* [79]. The direct inhibiting action of this tumour suppressive miRNA upon EGFR antagonises downstream effectors such as *ERK*, *Akt* and *Stat3* which subsequently

leads to enhanced apoptosis and ceased inhibition, proliferation, and migration within glioblastoma cells. Kefas and colleagues demonstrated low levels of miR-7 in glioblastoma tissues and further evaluated its inhibiting action towards the EGF receptor subsequently leading to impaired viability and metastatic properties of GBM cells [80]. miR-7-5p has the potential to be incorporated into new, targeted therapies within patients expressing wild type EGFR glioblastoma molecular profiles.

Despite the efforts made to combat the deadly glioblastoma multiform tumours, this type of brain cancer is still associated with poor prognosis and low overall survival rates. MiRNAs have demonstrated promising outcomes in their use as prognostic, diagnostic biomarkers and treatment targets. However, their clinical adaptation is still far from accepted, due to uncertainties in the experimental findings caused by the limited number of patient samples used in research [81].

6. MiRNAs in medulloblastomas

6.1 MicroRNAs as potential biomarkers in paediatric medulloblastomas

Similar to several types of paediatric brain cancers, miRNAs are involved in the regulation of different cellular and physiological processes in medulloblastoma including CNS development related processes. Over 60% of the reported miRNAs are detected in the adults' brain and their expression changes as the brain goes through maturation and develops from embryonic to adult stages [82]. They are involved in the regulation of the post translational process that controls the neural development and morphology. Thus, they play a pivotal role in cellular events related to promoting or supressing tumour growth and proliferation either as oncogenes or tumour suppressors [82].

For instance, miR-124 is reported to be one of the most expressed miRNAs in the mature CNS. It also plays a crucial role in the neural differentiation and maturation [83].

MiR-124 was also reported to have an important role in normal prefrontal cortex (PFC) and brain functioning as it regulates the Dopamine D2 receptor (Drd2) pathway which is responsible for dopamine regulation and secretion. Studies have reported a relation between decreased mi-124 expression and brain disorders including Alzheimer's disease and frontotemporal dementia (FTD) [83, 84]. In mice exposed to chronic ultra-mild stress, an overall of 80% decrease -compared to non-stress exposed mice- in mir-124 expression was observed and depression-like behaviours were exhibited [84–86].

Despite the unclear mechanism of action of miR-124 in normal brain and based on the previously reported findings and studies, miR-124 can afford to be a potential diagnostic biomarker and therapeutic target for CNS disorders and brain cancer [87].

6.2 MiRNAs in different medulloblastoma subgroups

As the most common severe paediatric brain malignancy, medulloblastoma has four molecular subtypes: Wingless (WNT), Sonic Hedgehog (SHH), Group 3 (Gr3) and Group 4 (Gr4). Based on the aforementioned discussion, miRNAs play a crucial role in the neuron development and maturation. Hence, they have a share in promoting or supressing tumour growth by their aberrant expression. However, an entire clear and detailed role description of miRNAs in tumours remains to date unclear [82].

6.2.1 Wingless (WNT) subtype

Several miRNAs were reported to be downregulated in WNT subtype including miR-383, miR-206, miR-183, miR-128a/b, miR-449, and miR-133b. Tumour formation initiated by miRNA downregulation indicated that they act as tumour suppressors [82, 88]. Thus, miRNAs with tumour suppressive effect could afford potential diagnostic biomarkers and promising therapeutic targets by upregulating their expression and restoring their tumour-suppressing function. For instance, miR-148a expression was reported to reduce Neuropilin (NRP1) expression that is involved in several pathways promoting tumour growth and metastasis. Considering its suppressive effect on tumour promoting pathways and factors including NRP1, miR-148a is considered one of the main reasons behind the lower metastatic incidence and good survival rates of the WNT subtype patients. The downregulation of the NRP1 by the miR-148a suggested a good diagnostic biomarker and a highly promising therapeutic agent for this medulloblastoma subtype [88–90].

6.2.2 Sonic hedgehog (SHH) subtype

The SHH subtype has a moderate prognosis that depends on the molecular mutation and the metastatic status. Alteration in the SHH signalling pathway results in tumour formation, development, and proliferation [82]. Among these mutations, protein patched homologue (PTCH) inactivating and smoothened homologue (SMO) activating mutations are the two most common mutations. Patients diagnosed with SHH medulloblastoma and have additionally a *TP53* gene mutation have the worse outcome. Around 80% of SHH cases combined have mutations in the downstream SMO pathway, resulting in tumours that are resistant to SMO inhibitors. Also, the Nrp2 receptor and its ligand Vegfa are up regulated in SHH's cancer stem cells (CSCs) promoting their self-renewal ability and viability [10]. Among the validated inhibiting molecules of Nrp2 and Vegfa molecules is the miR-446-3p. Stated that, an upregulated expression of miR-466-3p could potentially be considered as a therapeutic candidate while its downregulated expression could afford being a diagnostic biomarker [82, 91].

With its exclusive expression in tumours, the miR-10b is another miRNA that plays a crucial role in medulloblastoma cell proliferation, invasion, and survival by controlling B cell lymphoma 2 (*BCL2*) levels. The *BCL2* regulates apoptosis and is maintained in balanced levels in healthy cells. The miR-10b oncomiR affects the modulated apoptotic function of *BCL2* and promotes cancer cell survival. Thus, miR-10b could serve both as a good diagnostic biomarker and therapeutic target for SHH medulloblastoma subgroup [92].

6.2.3 Group 3 (Gr3) and group 4 (gr 4) subtypes

On one hand, the most aggressive and yet the least understood subtype of medulloblastoma is group 3 MB. Around 45% of group 3 MB cases are metastatic at diagnosis stage and most cases are resistant to adjuvant therapies which results in poor prognosis, and low survival rates. On the other hand, group 4 medulloblastoma has better survival rates, also known as intermediate and better prognosis than group 3 MB, regardless of the 40% of cases that are identified/classified as metastatic at diagnosis [93, 94].

In contrast to WNT and SHH subgroups, both group 3 and 4 MB have no distinguishing altered signalling pathways and no signs on known molecular mutation

MB subtype	miRNA	Expression	Targeted genes
All subtypes	miR-21	Upregulated	*PDCD4*
All subtypes	miR-106b		*PTEN*
SHH	miR-183-96-182 cluster		*SHH*
SHH and Group 3	miR-10b		*BCL2*
WNT	miR-224		*WNT*
WNT	miR-193		*WNT*
SHH	miR-124	Downregulated	*SLC16A1*
SHH	miR-324		*SHH*
Group 3/4 SHH	miR-192		*DHFR, CD47*
SHH	miR-128a		*BMI-1*

Table 1.
Different miRNAs expressed in the four subtypes of medulloblastoma [82, 83, 93–100].

including TP53 mutations are observed in both groups. Therefore, challenges regarding diagnosis and therapeutic targets sets on continuous research to identify the mechanisms of origination and development of these MB subgroups [82, 93, 94]. A very low number of expressed miRNAs associated with MB groups 3 and 4 have been identified. For instance, miR-1253 is a brain-enriched microRNA that plays a key role in regulating bone morphogenic proteins during cerebellar development. The increased expression of the miR-1253 has been associated with the activation of apoptotic pathway and reduction of tumour malignancy. The study that stated the aforementioned tumour suppressive properties of miR-1253 has also stated that it is a good potential diagnostic biomarker for mainly groups 3 and 4 by its low expression in tumour cells. The study has also mentioned the promising therapeutic potentials this miRNA upholds by silencing its oncogenic targets *CDK4* and *CDK6* and restoring its expression by epigenic demethylation to inhibit tumour cell growth and proliferation [82, 92–95].

There are several miRNAs expressed in medulloblastoma (**Table 1**) and they have been investigated over the last two decades aiming to identify novel biomarkers for diagnostic, prognostic and therapeutic purposes. The analysis of miRNAs as potential biomarkers is performed using MB tissue, CSF, and blood samples, in addition to the investigation of the miRNA expression in extracellular vesicles isolated from CSF or blood samples. Several miRNAs including miR-30b/d, miR-128a, miR-124, miR106b, and miR 224were found to be differentially expressed in MBs subgroups (**Table 1**) [82, 93].

7. Nanoparticles and miRNAs

In recent years, a novel approach for diagnosis, therapy and/or theranostics by the encapsulation of miRNAs in nanoparticles has emerged and it has been the centre of focus of several studies [15, 17, 97]. For instance, miR-124 and anti-miR-21 were co-encapsulated in polymeric nanocontainers that had a surface modified with Angiopep-2 peptide and injected in mice model. Results revealed promising outcomes in reducing the tumorigenesis of the glioblastoma in the xenograft mice model.

This approach also offered the protection of the encapsulated miRNA from enzyme degradation and assured the overcome of the BBB and the achievement of targeted dual delivery of miR-124 and anti-miR-21.

Co-encapsulation and delivery of the chemotherapeutic drug doxorubicin and anti-miR-21 showed significant decrease in miR-21 expression levels, reduction in tumour growth, and enhancement of the apoptotic activity *in vivo* [15]. Further examples that demonstrate the distinctive therapeutic potential of miRNAs loaded in nanocontainers are the gold nanoparticles functionalized with miR-182. Their administration intravenously in orthotopic glioblastoma xenografts resulted in significant antitumour activity and reduced tumour growth due to the protection of the miR182 and the targeted delivery approach by the nanoparticles. The studies' findings further showed no inflammatory responses related to the miR-182's systemic introduction and insignificant cytotoxicity levels or side effects [17, 96, 97]. Yet, no recent studies focusing on the delivery of miRNAs by the mean of nanoparticles in paediatric brain tumours and in medulloblastoma specifically have been reported [96].

8. Conclusion

The unsatisfying clinical outcomes associated with primary brain tumour malignancies have led to intensifying the work on understanding miRNAs. Their potential as biomarkers and as therapeutic targets could enable their incorporation within the early detection, prognosis and possible treatment of brain tumours. Despite the advances in the molecular techniques used to analyse the role of miRNAs in several cancers, and the plethora of miRNAs reported as potential biomarkers for cancer diagnosis or treatment, no miRNAs candidates have exceled to the Food and Drug Association approval process [92, 93]. Their clinical adaptation is still far from acceptance, due to uncertainties in the experimental findings. Thus, current research should concentrate on the clinical utilisation of miRNAs as potential novel diagnostic and therapeutic tools.

Conflict of interest

The authors declare no conflict of interest.

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