Folia
Neuropathologica

MicroRNAs as efficient biomarkers in high-grade gliomas

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Abstract

High-grade gliomas are the most aggressive and devastating brain neoplasms. Therefore much effort is put on understanding their background as well as development of new effective diagnostic and therapeutic methods. However, until now the genetic only approach has not provided a satisfactory answer. Recently, it has been shown that the epigenetic issue is important for high-grade gliomas' development and progression. Out of many epigenetic mechanisms, as DNA methylation, histone methylation and acetylation, especially microRNAs showed to be deeply involved in the carcinogenesis process.

MicroRNAs are short non-coding RNAs. They are new candidates for human disease biomarkers due to their simple identification. MicroRNAs are stable in tissue and body fluids, what makes them very prospective non-invasive, blood-based biomarkers. There is a lot of data showing that various profiles of serum microRNAs are linked to numerous neoplastic processes, indicating that microRNAs can be really a new class of biomarkers for human diseases.

Key words: *microRNA, miRNA, miR, biomarker, high-grade glioma, HGG, glioblastoma, GBM.*

Introduction

Histopathology of tumour specimens gained by microsurgical resection or stereotactic biopsy is a standard diagnostic procedure for patients with high-grade gliomas (HGG). The neuropathological system classifies gliomas according to their morphological resemblance to the corresponding glial cells, cytoarchitecture and immunohistological properties [37]. However, the clinical course of the tumours comprising the same histopathological entity varies significantly. Magnetic resonance imaging is current-ly the best instrumental method for staging of the disease and follow up, but it reveals tumours only at a macroscopic level. It is clear that the effective management of any malignant neoplasm, and brain tumour particularly, requires a diagnosis at an earlier stage, and that states the need for specific and sensitive biomarkers. Although many potentially useful probes have already been suggested, no such markers have been established for brain tumours. In this review several specific microRNA biomarkers will be presented and discussed, including those identified by our group [47].

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Brain gliomas

Human brain cancers form a family of approximately 120 heterogeneous tumour entities and variants [37]. Their annual incidence accounts for 1.8% of all new cancer cases and the mortality reaches 2.3% of cancer deaths worldwide [26]. Gliomas account for over 70% of all primary brain tumours in adults, and they are divided into astrocytic, oligodendroglial, mixed, ependymal and embryonal types [37]. The astrocytic type occurs mainly in adults, including the most common (65%) and most malignant type – glioblastoma (GBM). That typically infiltrates into the adjacent cortex and through the corpus callosum into the contralateral hemisphere making itself surgically untreatable. From a histopathological point of view gliomas are classified by the cell of origin and grade. Grades I to IV are based on histology and clinical criteria [37]. Grade I is assigned to tumours with low proliferative potential, grade II covers diffusely infiltrative tumours with cytological atypia, grade III shows anaplasia and mitotic activity and in grade IV tumours additionally microvascular proliferation and/or necrosis is seen.

Malignant gliomas (HGGs, grade III and IV) are the most common type of primary brain tumours. Each year in more than 22,000 people in the United States a malignant glioma is detected [63], and most of that group will die within the first two years from diagnosis despite aggressive chemo- and radiotherapy. However, many of these relatively uniformly treated patients advance more quickly than others to recurrence and death. It is very rare for GBM patients to survive longer than 3 years [9]. Better prognosis is associated with several clinical and histopathological features, including young age, good performance status, gross total resection, adjuvant treatments, giant-cell subtype and oligodendroglial differentiation [25,37,56]. Glioblastoma patients differ in the clinical presentation and response to treatment because of a strong inter-tumoural heterogeneity coming from different gene mutations affecting signalling networks. Several molecular GBM subtypes have been identified [5]. The proneural subtype localizes typically in the frontal cortex, usually has *IDH1/IDH2* and *TP53* mutations, and demonstrates better prognosis and sensitivity to Notch inhibition [46] than the mesenchymal subtype, which is more aggressive, with greater vascularity, displaying more *NF1* lesions, and depending on TGF-β and NF-κB activity. The classical subtype is aggressive and frequently has *EGFR* lesions [46]. A fourth subtype, neural glioblastoma, is less well characterized.

The neuro-oncological practice depends strictly on tumour classification. Therefore therapies are applied in a relatively uniform way to all patients with a given histopathological diagnosis. Furthermore, classification constrains the scientific approach to brain neoplasia studies, with biological understanding based on presumptions about specific tumour types [24]. However, it is known that histologically identical tumours may have a very different outcome and response to treatment. Therefore cancer heterogeneity, both on a genetic and epigenetic level, has implications for therapy and shows challenges for the rational design of effective treatment rules [43]. Molecular markers with both diagnostic and prognostic potential contribute valuable tools by redefining tumour subtypes within each grade.

Biomarkers

A biomarker is a chemical compound specifically relevant to the disease, which can be applied to monitor the current neoplasm's state. The marker, despite having high diagnostic and prognostic values, may also suggest targeted therapies. Selective biomarkers can identify susceptibility risks and would be critical for establishing a proper time point for effective treatment. It is obvious that patients with early detected tumours have better chances for recovery and survival than those with advanced neoplasms at the time of diagnosis.

Molecular markers are changing the traditional classification by redefining tumour subtypes within each grade of malignancy and providing diagnostic and prognostic information. They become more and more an integral part of neurooncological practice. Biomarker status is already critical for clinical decisions in some gliomas' subtypes. For example, *IDH1/2* mutations show favourable prognosis across all glioma grades [58,62]. Chromosomal co-deletion of 1p/19q is positively predictive for chemotherapeutic response in anaplastic oligodendrogliomas [57]. O6-methylguanine-DNA methyltransferase (*MGMT*) promoter hypermethylation is a favourable prognostic marker in astrocytic high-grade gliomas [48]. It is also predictive for chemotherapeutic response in anaplastic gliomas and GBM [22,27,56].

MicroRNAs

MicroRNAs (miRNAs, miRs) form a family of small non-coding RNA molecules of 18-25 nucleotides that function in post-transcriptional gene regulation [54,55]. To date there have been ca. 2000 different human miRNAs referenced in the miRBase (release 21; June 2014). They are involved in a number of biological processes including cell proliferation, differentiation, developmental timing control, apoptosis and stress responses, as well as pathological states such as cancer [15,23].

miRNAs regulate gene expression through both translational repression and degradation of target messenger RNAs (mRNAs). The biogenesis of miRNAs involves two processes. The first one occurs in the nucleus, where the primary transcript (pri-miRNA) is processed into a precursor (pre-miRNA) by a nuclear RNase III (DROSHA). The second event takes place in the cytoplasm. The pre-miRNA is exported by exportin V from the nucleus and is cleaved by Dicer into a short-lived dsRNA of about 20-25 nucleotides. Then the double-strand is unwound and one strand forms the mature miRNA, which is incorporated into an Argonaut (Ago)-protein containing complex called the RNA induced silencing complex (RISC). The mature miRNA within the RISC recognises complementary sites in the 3'-UTR of target genes, what results in translational inhibition or destabilisation of the target mRNAs and downregulation of the encoded protein expression [19].

It is estimated that up to 50% of all human protein-coding genes are regulated by miRNAs. Each miRNA regulates up to hundreds of different mRNAs, and each mRNA is regulated by tents of miRNAs [55]. Their expression is population-, race-, and gender-dependent, as well as related to tissue state (healthy or diseased), and disease subtype [55]. MiRNAs are considered to be fundamental to normal cellular function in eukaryotes, and the alteration of microRNA expression and activity has been implicated in a variety of pathological processes. The greatest challenge for molecular medicine is while miRNAs regulate many mRNAs, they impact many proteins.

MiRNAs can be considered as cancer biomarkers when variations in their expression identify the cancerous state. Until now almost all analysed tumours have shown distinct miRNA profiles compared to normal tissues [38]. These profiles can be further associated with prognostic factors and disease progression [6,66].

MicroRNAs as biomarkers of gliomas

It has been shown that miRNAs are integrally involved in brain gliomas' development and progression [1,12]. They are essential regulators of many key pathways implicated in tumour pathogenesis [10,18]. Because miRNAs have been shown to play crucial roles in glioblastoma progression, invasion, tumour growth, and therapy responses, it is very likely that some miRNAs could be useful biomarkers in brain tumour patients.

MiRNAs expression can be altered in brain tumour through a variety of mechanisms including chromosomal changes, epigenetic defects and mutations in the machinery of their biogenesis [21,36]. There are many data showing that miRNA signatures can refine glioblastoma classification, differentiate GBM subclasses, as well as provide regulatory links to disrupted signalling pathways such as those facilitating cell growth [29]. Some studies show lower miRNA expression in tumour samples, what can be a function of cellular differentiation status [38]. Microarray studies of glioma tissue have implicated a number of miRNAs involved in glioma formation and propagation [47]. However, a comprehensive set of these differentially expressed RNA species has not been produced. Our group, based on miRNA microarray data analysis and deep RNA sequencing of miRNAs in normal human brain and tumour tissue, has recently found several RNA signatures, which were complemented with meta-analysis [47].

First we selected miRNAs that were most frequently deregulated in glioblastoma tissues as well as in peritumoral areas and compared with normal human brain. There were 22 differentially expressed miRNAs when comparing normal brain and brain tumour adjacent tissue [47]. The analysis revealed 6 miRNAs with elevated expression and 16 miRNAs with low expression within the tumour borders. 21 miRNAs found in the borders of gliomas were also identified in GBM. miR-625 was present within the border tissue, but not in GBM. Five overexpressed miRNAs, including the most abundant miR-21, are involved in the increased invasion and migration, but 15 were downregulated (Table I). We identified miRNAs associated with the progression from glioma grade III to grade IV. They are generally upregu-

Table I. Human microRNAs as high-grade glioma drivers, differentially expressed compared to adjacent tissue [7,47]

No.	Overexpressed	No.	Underexpressed
1.	21	1.	7
2.	142 −5p	2.	124
3.	155	3.	128
4.	221/222	4.	129*
5.	542 −5p	5.	129 −3p
6.	630	6.	132
7.	1260	7.	136
		8.	137
		9.	139 −5p
		10.	153
		11.	323 −3p
		12.	330 −3p
		13.	410
		14.	598
		15.	769 −5p
		16.	625

Table II. Potential new microRNA markers for gliomas' progression from grade III to IV [7,47]

No.	Overexpressed	No.	Underexpressed
1.	20a −3p	1.	33a
2.	134	2.	197
3.	144*	3.	340*
4.	150*	4.	381
5.	202	5.	574 −3p
6.	221/222		
7.	301b		
8.	378		
9.	483 −5p		
10.	494		
11.	500*		
12.	502 −3p		
13.	513a −5p		
14.	575		
15.	939		
16.	940		
17.	1202		
18.	1207 −5p		
19.	1224 −5p		
20.	1225 −5p		
21.	1246		
22.	1260		
23.	1275		
24.	1290		
25.	1471		
26.	1915		

Table III. Differentially expressed human microRNAs in glioblastoma versus normal brain [7,47]

No.	Overexpressed	No.	Underexpressed
1.	9 −5p	1.	7 −5p
2.	10a −5p	2.	124 −3p
3.	10b −5p	3.	128 −3p
4.	15b −5p	4.	129 −5p
5.	16 −5p	5.	132 −3p
6.	17 −5p	6.	136 −5p
7.	20a −5p	7.	137
8.	21 −5p	8.	138 −5p
9.	25 −3p	9.	139 −5p
10.	92a −3p	10.	153 −3p
11.	92b −3p	11.	154 −3p
12.	93 −5p	12.	203a
13.	106a −5p	13.	218 −5p
14.	106b −5p	14.	323a −3p
15.	130a −3p	15.	328 −3p
16.	130b −3p		
17.	155 −5p		
18.	182 −5p		
19.	196 −5p		
20.	210		
21.	221/222		

lated (Table II). The most interesting is the panel of 35 differentially expressed miRNAs in glioblastoma versus normal human brain (Table III). There are 20

and 15 miRNAs up- and downregulated, respectively. One can see the highly expressed miRNA-9, as well as miRNA-21 and 155, what is shown in Table I. They can be used as novel biomarkers and potential therapeutic targets for GBM. MiRNA-7, 124, 128, 129, 132, 136, 137, 139, 153 and 323 overlap with those downregulated listed in Table I. Differentially expressed miRNAs in brain tumour and adjacent tissue reflect the crosstalk between cancer cells and their local environment, which is a key feature of establishing and maintaining a malignant state. Future validation studies of these miRNAs, in combination with other brain tumour biomarkers hold a promise for clinical practice.

Candidate microRNAs for high-grade gliomas biomarkers

Regarding the up-to-date literature findings and our laboratory results we can characterize the most

promising of miRNA to be effective biomarkers for high-grade gliomas, especially GBM.

microRNA-21 shows overexpression in GBM and other gliomas in a grade-specific manner [4,7, 31,34,41,59]. It affects the major cancer pathways: TGF-β, p53, and mitochondrial initiated apoptosis pathways. The miR-21 knock down in glioma cell lines leads to upregulation of tumour suppressor proteins including p53, Bax, DAXX, APAF1, p21, TAp63 and TGFBR2 [45]. Other studies showed that miR-21 regulates matrix metalloproteinase inhibitors (RECK and TIMP3) therefore implicates tissue invasion [13].

microRNA-10b seems to be deeply involved in glioblastoma progression because its expression levels clearly correlate with tumour grade [50]. It is overexpressed in GBM [50,65] and enhances GBM invasiveness [50].

microRNA-221/222 were upregulated in gliomas and cell lines, but in HGGs with increased proliferation rates miRNA-221 levels were distinctly higher [7]. The direct link was found between miRNA-221/222 and cell cycle progression [42]. The ability of miRNA-221/222 to negatively regulate the pro-apoptotic gene PUMA is responsible for its anti-apoptotic effect [69]. The overexpression of PUMA changes the phenotypes caused by the overexpression of microRNA-221/222 [42,69], what suggests that miR-221/222 enhances the proliferative potential of tumour cells. The other way of miRNA-221 action in changing apoptotic signalling and altering cell cycle leads through BIRC family of neural cell fate regulators. It was shown that miRNA-221 is selectively upregulated in glioma tissue samples and cell lines, whereas its target, encoding the survivin-1 homolog BIRC1, a neuronal inhibitor of apoptosis protein (NIAP), is downregulated [40].

microRNA-17~92 cluster is upregulated in glioblastoma cell lines and tumour samples and is composed of miRNA-17-3p, -17-5p, -18a, -19a, -19b, -20a, and -92a [41]. The cluster targets are tumorigenic regulators of DNA-repair and angiogenesis (CTGF, and POLD2), and anti-proliferative transcripts (TGFBRII, SMAD4, and CAMTA1) [11,41].

Compared to non-neoplastic brain tissue **micro-RNA-451** is overexpressed in glioma cells [14,44] and regulates the adaptive response in metabolic stress and low glucose availability [16].

The other upregulated microRNA in highly aggressive GBM cell lines is **microRNA-145** [30]. That fact promotes decreased proliferation and invasion of GBM cell lines [33]. Oct4 and Sox2 are proved to be targets of miR-145 mediating the loss of "stemness".

One of the most commonly downregulated microRNAs in glioblastoma [17] and glioma cell lines, when compared to age-matched controls, is **microRNA-128** [4,34]. The downregulation of miRNA-128 inversely correlates with the WHO tumour grade. While miRNA-128 is downregulated in grade II–IV tumours, the levels in HGGs are significantly lower [69]. When overexpressed, miRNA-128 decreases the proliferative potential of glioblastoma cell lines *in vitro* and in GBM xenografts. miR-128 is also proposed to act by deregulation of self-renewal in glioma stem cells [17]. MiR-128 downregulation promotes an undifferentiated glioma phenotype via increased expression of its targets: angiopoietin-related growth factor protein 5 (ARP5, ANGPTL6), a transcription suppressor promoting stem cell renewal and inhibiting tumour suppressor genes expression involved in senescence and differentiation, Bmi-1, and a transcription factor critical for the control of cell-cycle progression, E2F-3a [8]. Addition of exogenous miRNA-128 to GBM cell lines restored the correct expression of those factors, and decreased the proliferation. Our data suggest that downregulation of miRNA-128 may contribute to glioma and GBM, in part, by co-ordinately upregulating ARP5 (ANGPTL6), Bmi-1 and E2F-3a, resulting in the proliferation of undifferentiated GBM cells [8].

The other commonly downregulated microRNA in GBM cell lines and tissues is **microRNA-34a**, which shows a significant reduction in p53-mutant cells compared to wild-type p53 cells [35,39]. miR-34a interacts e.g. with MYC, CCND1, CDK6, SIRT1 and c-Met oncogenes within the transcriptome [20] and plays an important role in gliomas by inhibiting glioma tumorigenesis as a tumour suppressor with silent information regulator 1 (Sirt1) as a negative target of miR-34a in glioma cell lines. Sirt1 is an regulating apoptosis oncogene in response to oxidative stress and genomic insults [39].

EGFR and Akt activated pathways are the most common genetic alterations in glioblastoma and act together in gliomagenesis. **microRNA-7**, downregulated in GBM [32,65], has been shown to inhibit EGFR expression, what leads to reduction in Akt phosphorylation [28]. The other targets of miRNA-7 are p21 activating kinase (PAK1) and focal adhesion kinase (FAK) microRNA-7. Therefore overexpression of microRNA-7 reduces GBM invasion and migration [3].

These data show the potential of miR-7 in the area of gliomas' therapeutics.

Compared to non-neoplastic brain tissue **micro-RNA-124/137** are downregulated in anaplastic astrocytomas and glioblastomas [53]. In tumour derived and neural stem cells they lead to G1 arrest and reduction in expression levels of CDK6, which is a regulator of the cell cycle and known target of miRNA-124 and -137. Moreover, miRNA-137, but not miRNA-124, is activated by addition of DNA demethylating agents to glioma cell lines and that suggests methylation of CpG islands based on miRNA-137 promoter regulation [53].

miR-181a and **miR-181b** are downregulated in glioma samples and cell lines [7,52] showing 20-30% reduction in glioblastomas. MiRNA-181b is a potential prognostic marker and helps in selection of patients who may benefit from adjuvant therapy.

microRNA-100 reduces proliferation and increases apoptosis of GBM lines by inhibiting the silencing mediator of retinoid or thyroid hormone receptor-2 (SMRT/NCOR2). Compared to normal neural cell controls it is downregulated in multiple GBM cell lines. MicroRNA-100 decreases proliferation in orthotopic GBM xenografts and extends survival [2].

Peripheral blood glioblastoma biomarkers

Monitoring of glioma development during or after completed treatment requires a reliable and quick test for biomarker detection from an easily accessible source, allowing a less extensive and more accurate disease monitoring in shorter time periods (as compared to neuroimaging) [64]. Furthermore, there could be a huge benefit from developing biomarkers for glioblastoma confirmation in order to avoid biopsy for patients with a high risk of surgery-associated mortality or small tumours in eloquent brain areas. The specific miRNA signature in plasma samples derived from GBM patients before tumour resection would be very useful for planning the necessary degree of resection and adjuvant therapies [64]. Circulating microRNAs and exosomal microRNAs may serve as non-invasive biomarkers for various diseases, also in different cancer types [60,64]. Circulating miRNAs are appealing biomolecules to be considered as cancer biomarkers for several reasons including their stability, which sustain a high temperature or extreme pH conditions that would damage other cell components.

A significant difference in serum miRNA profile was found between untreated high-grade astrocytomas (grade III-IV) and controls in a genome-wide miRNA analysis. Seven miRNAs (miR-15b*, miR-23a, miR-133a, miR-150*, miR-197, miR-497, miR-548b-5p) were markedly decreased in grade II-IV patients, and showed high specificity (97.87%) and sensitivity (88.00%) for prediction of malignant astrocytomas. Furthermore, these miRNAs were also elevated in serum after operation, and some of them could be proposed as non-invasive biomarkers of malignant and benign cases, astrogliosis and other primary brain tumours [67,68].

In comparison to normal controls the plasma levels of miR-21, miR-128 and miR-342-3p were shown to be significantly altered in GBM patients and miR-128 and miR-342-3p positively correlated with glioma' histopathological grade [61]. In blood of glioblastoma patients, compared with controls, miR-128 overexpression has been identified [49]. Unlike, in GBM tissue compared with normal human brain miR-128 was downregulated [17,70]. In the study comparing the blood samples obtained immediately after surgery versus more than 6 months after operation and after radio- and chemotherapy, miR-128 and miR-342-3p were deregulated likewise, what suggests that the detected differences are connected with the disease, not with a particular treatment [49]. Recently it has been shown that miR-454-3p in plasma of glioma patients is markedly overexpressed compared to healthy controls and are lower in LGGs than in HGGs. Also miR-29 shows high diagnostic potential, allowing to differentiate between patients of stage I-II with stage III-IV [68]. Furthermore, the miR-454-3p expression in the post-operative plasmas is markedly downregulated in comparison to the pre-operative plasmas, and a correlation of worsening prognosis of glioma was observed with increasing miR-454-3p expression [51]. Also a huge increase in miR-210 expression was found in GBM patients' serum samples compared to controls, and it was associated with the tumour grade and patient's poor outcome [68].

Conclusions

Altered miRNA biogenesis and expression in glioma plays a vital role in important signalling pathways associated with a range of tumour characteristics including gliomagenesis, invasion, and malignancy.

The progress in our understanding of the potential involvement of miRNAs in malignant gliomas is improving rapidly, hurdles remain high before miRNAs are recognized as valid markers in HGGs. The isolation and characterization of miRNA using cellular and molecular biology techniques from the circulation of glioma patients could potentially be used for improved diagnosis, prognosis, and treatment decisions.

Disclosure

Author reports no conflict of interest.

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