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Circulating MicroRNA as Novel Potential Biomarkers for the Diagnosis of Highly Malignant Gliomas

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Highly malignant glial tumors (highly malignant gliomas) are the most aggressive primary brain neoplasms. Understanding of the pathogenesis and development of new and effective diagnostic and therapeutic methods is therefore of great interest. MicroRNAs are short noncoding RNA molecule of length 18–22 nucleotides which have been shown to play a direct role in carcinogenesis. Circulating microRNA are released into the extracellular space and can remain stable for long periods of time in most biological fluids, including serum and plasma. Circulating microRNA are potential biomarkers for different expression profiles specific for different human diseases, including oncological diseases. Many data have been obtained showing that different circulating microRNA profiles in human biological fluids particularly in extracellular vesicles, are linked with numerous neoplastic processes, such that microRNA may constitute a new class of biomarkers for early diagnosis and prognosis of highly malignant gliomas.

Keywords: microRNA, circulating, biological fluids, marker, glioma, glioblastoma.

Malignant gliomas are the most dangerous primary brain tumors and are classified into four groups (I–IV) on the basis of histopathological assessment; glioblastomas (GBM), classified as type IV gliomas, are the commonest and most malignant [1]. Even the current standard treatment, consisting of maximal surgical resection and combined chemoradiotherapy, gives a mean survival time for patients with GBM approaching 15 months, and only 3–5% of patients survive more than 36 months [2, 3]. Computed tomography and MRI scanning are the main methods used in the diagnosis of brain tumors and for monitoring growth and responses to treatment. Nonetheless, the diagnosis of glial tumors is often difficult, as various pathological states, such as metastases of tumors in other organs (melanomas or primary lung tumors), can have similar morphological patterns on MRI scans. Furthermore, pseudoprogression associated with the effects of radiotherapy and imitating tumor recurrence can additionally complicate the interpretation of MRI scans [4]. All these problems clearly emphasize the need to create reliable, low-invasive biomarkers for more precise and consistent diagnosis and prognostication in patients with gliomas and their malignant forms.

MicroRNA are short noncoding RNA species of length 18–22 nucleotides which act as powerful posttranslational regulators of gene expression. MicroRNA bind with the 3'-untranslated (3'-UTR) regions of their targets (mRNA), protein-encoding genes, and negatively regulate their translation. They are involved in a number of biological processes, including cell proliferation, differentiation, apoptosis, etc., and also in the pathogenesis of many diseases such as tumor processes [5, 6].

Profiling of microRNA expression has already entered experimental oncology as a method for the diagnosis of the onset of progression and the responses of tumors to treatment. Studies with microRNA have reported increases or decreases in their expression in the blood and cerebrospinal fluid (CSF) in patients with glial tumors, which supports the need for studies of microRNA as biomarkers for this pathology. Study methods were complex and highly productive methods such as microchips and the polymerase chain reaction (PCR) in real time (qRT-PCR) for different circulating microRNA profiles. This review seeks to summarize current

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experimental data on the distribution of microRNA as biomarkers. Questions of developing microRNA as biomarkers for diagnosis and prognosis and for altering expression in response to treatment are addressed.

MicroRNA and Glial Tumors. Apart from histology, the 2016 update of the World Health Organization central nervous system (CNS) tumor classification provided molecular parameters including detection of microRNA for improving both diagnostic precision and monitoring of patients [7]. Large studies assessing microRNA have reported changes in the expression of a number of microRNA in tumor cells and tissues of patients with GBM as compared with normal brain tissue [8]. However, inconsistency with the reported subsets of microRNA whose expression is increased or decreased in GBM has the result that the role of particular families of microRNA remains to be determined. It is of note that miR-21 is the only microRNA which has been demonstrated in all studies to date to be overexpressed in GBM tumor cells, while the aberrant microRNA miR-132 is the most consistent, though this is identified in only 60% of cases [8]. The quality of tumor tissues, sample size, the relevance of the "control" brain tissue, and the analytical methods used can explain inconsistent results obtained in published studies. Thus, the potential for microRNA as biomarkers for gliomas requires systematic analysis of existing data. A recent metaanalysis of five studies published from 2012 to 2015 found six microRNA with high sensitivity, specificity, and statistical significance for comparison of glial tumor tissues with control tissue from normal brain [9]. The microRNA miR-15a, miR-16, miR-21, miR-23a, miR-9,and miR-124 found in this study may be useful diagnostic and prognostic markers for gliomas.

Circulating Biomarkers for the Diagnosis of Gliomas. Circulating biomarkers ca be used for diagnostic purposes in brain tumors, especially in those cases in which surgery is contraindicated or biopsy results are unconvincing. The best studied circulating biomarkers at that time were proteins which could be secreted by tumor cells and/or the microenvironment such that they could be detected in the blood, urine, and CSF. Many markers for gliomas, such as mutant EGFRvIII or glial fibrillary acidic protein, have already been observed in blood and CSF, though their clinical value remains to be confirmed [10]. As proteins lack the sensitivity and specificity required for successful use as biomarkers, significant efforts have been made to find more informative and less invasive biomarkers – and microRNA could be candidates for this.

The detection of endogenous microRNA in cells, as well as circulating microRNA, in body fluids, along with altered profiles in various pathological states opens up new perspectives for the use of extracellular or exogenous microRNA as informative biomarkers for human disease. In recent years, circulating microRNA have been at the center of attention of many researchers working on the identification of markers for tumor processes, though most microRNA reported in the literature have not been able to enter clinical use because of inconsistent and irreproducible results. Circulating microRNA can be used reliably as biomarkers in a variety of human pathological states, though analytical factors which might influence their detection must be considered, so there is a need to resolve questions including those of the type of set, the measurement platform, or the normalization strategy. The main preanalytical steps affecting detection of circulating microRNA have been characterized [5]. Mechanical hemolysis induced by incorrect selection or preparation of study samples, especially blood, can affect the specificity of the expression of circulating microRNA due to contamination by other intracellular microRNA, as exemplified by miR-451, which is present in erythrocytes. Samples with hemolysis, even minor and at the subvisible level, should be excluded [5]. Widely used techniques for measuring microRNA expression in biological samples include microchips and quantitative real-time PCR (qRT-PCR). Microchips as microRNA measuring platforms provide determination of the overall microRNA expression profile at reasonable cost and throughput capacity. Nonetheless, qRT-PCR is more expensive and has lower sample throughout capacity, though on the other hand it has much higher sensitivity than microchips [11].

Apart from the platform used for measurement of microRNA content, another important problem is normalization of the data for correcting variability during sample preparation and quantitative assessment of microRNA expression. In the absence of consensus, standardization strategies vary from one study to another, which makes results unreproducible and difficult to interpret [12].

Exosomes. Extracellular vesicles are small fragments of lipid membranes released on activation or death of various cells and serving to mediate intercellular communication; they contain soluble materials such as nucleic acids, lipids, and proteins, protecting them from degradation [13]. New data have provided evidence that exosomes, small extracellular vesicles (40–150 nm) with a multivesicular endosomal origin, are secreted by both normal and neoplastic cells and play a decisive role in tumor genesis [14]. To prevent the degradation of circulating microRNA, they are released by cells both in endosomes and in microRNA/protein complexes (miRNA/Argonaute 2). Exosomes synthesized by GBM cells were first demonstrated in the serum of patients with GBM in 2008 by Skog et al. [15]. Both normal and tumor cells release exosomes in the form of a "communications link" and they carry out a multitude of functions depending on their contents, including cellular material. Although it is unknown how microRNA are packed into endosomes, it is a specific and flexible process associated with binding of sumoylated heterogenous nuclear ribonucleoprotein A2/B1 (hnRNPA2B1) with a specific direction present in the sequences of mature microRNA [16]. This means that only particular microRNA can be incorporated into exosomes and, consequently, some aberrant tumor cell

microRNA can be lacking from endosomes. miR-21, which is known to be overexpressed in GBM cells, has also been found to prevail in exosomes in the serum of patients with GBM [15]. Other examples of circulating microRNA are miR-497 and miR-125b, whose expression was increased both in serum and GBM cells in humans [17]. In contrast to these microRNA, no correlations between the levels of expression in the blood and tissues have been reported for miR-128, which was significantly increased in the blood of patients with GBM and decreased in GBM tissues [18]. In addition, miR-210, miR-196b, and miR-1271 were seen in tumor tissue, but were similar to healthy subjects in the blood [19]. This group of circulating microRNA in exosome should therefore be studied independently of those for tumor tissues [20]. Extracellular vesicles can be isolated from biological fluids by various methods based on their physical and chemical properties, ultimately giving samples with different yields, purities, and integrities. Current approaches such as centrifugation, ultracentrifugation, filtration, and affinity purification are very inconvenient and laborious and are not used in standard clinical practice [21]. There are also other methods for isolating total RNA and its fractions (microRNA) which are rapid, simple, and economical, though they do not guarantee sufficient purity. Using these methods as examples, chemical isolation solutions such as Exoquick™ from System Biosciences (Mountain View, CA, USA) and Life Technologies (Carlsbad, CA, USA) are widely used, providing for simple and efficient concentration of exosome-containing vesicles from small quantities of serum or plasma, though they yield a heterogenous vesicular population [21]. Given that exosomes can be prepared from biological fluids using minimally invasive procedures, "liquid biopsies" of exosomes and the microRNA within them may be an alternative to standard tumor biopsy, which may also not reflect the heterogeneity of gliomas. This provides for real-time assessment of the risk of recurrence and treatment responses.

Exosomal MicroRNA as Biomarkers for GBM. Data showing increases in the number of extracellular vesicles, associated with poor prognosis, provide evidence that the kinetics of exosomes may be useful in the treatment of patients with GBM [22]. Nonetheless, a few studies have demonstrated microRNA expression in biological fluids from patients with GBM. miR-21 is an endogenous microRNA in mammalian cells, in which increased expression is associated with numerous tumor types. miR-21 functions as an antiapoptotic factor and survival factor for tumor cells, such that high levels of this microRNA may result from a tumor process [23]. As noted, miR-21 is one of the best studied microRNA in GBM. As regards the expression of microRNA in exosomes, miR-21 has been found to be overexpressed in serum from patients with GBM and decreased after wide resection of tumors [15]. The level of expression of exosomal miR-21 in CSF is greater in patients with GBM than patients of a control group. in another study, miR-21 expression in serum from patients with GBM was decreased on the background of treatment with bevacizumab [19]. Thus, increased miR-21 expression may provide a platform for the development of a new biomarker for GBM. Apart from miR-21, a small group of microRNA was seen in serum from patients with GBM [24]. The levels of expression of small noncoding RNU6-1, miR-320, and miR-574-3p were significantly linked with GBM. In addition, RNU6-1 was a minor predictor for the diagnosis of GBM [24]. Studies of microRNA expression in exosomes as a platform for creating biomarkers for gliomas have shown that validation using larger set of samples and perspective clinical trials are needed as the next step.

Circulating Nonvesicular Forms of MicroRNA as Biomarkers for Gliomas. As described above, the pool of circulating microRNA contains two types of microRNA: vesicle-packaged microRNA and microRNA associated with Argonaute-2 protein, which are side products of cells which accumulate in the extracellular fluids [25]. We have already noted the use of complex high-productivity approaches such as microchips for different microRNA as potential biomarkers for gliomas [20]. Data have been obtained showing changes in the expression of these forms of circulating microRNA and these may serve as biomarkers for diagnosis and prognosis and for detection of changes in the expression profiles in response to treatment in patients with glioma. Some of these results have shown high levels of expression of miR-21 and miR-128 and decreased expression of miR-342-3p in the blood of patients with GBM. It is of note that miR-128 and miR-342-3p correlate positively with histopathological classes of gliomas [26]. Another study also showed decreases in the activity of miR-342-3p, while, conversely, the level of expression of miR-128 was seen to increase in the blood of patients with GBM [18]. Studies using combined analysis of miR-21 and miR-15b expression showed differences between patients with and without gliomas [27]. Zhang et al. [28] reported that the level of expression of the miR-221/222 cluster in plasma increased significantly in patients with glioma. It is interesting that high levels of miR-221/222 in plasma were associated with lower survival. It was suggested that changes in microRNA expression can be used to predict the outcomes of tumors, as demonstrated by increases in the expression of serum miR-20a, miR-106a, and miR-181b, associated with clinical stages of astrocytoma, or miR-210, which is significantly increased in GBM [29, 30]. It is of note that Siegal et al. [19] found that the level of expression of miR-210 in serum differed from that in the control group. Changes in serum miR-497, miR-125b, and miR-29 expression provided the opportunity to distinguish GBM from low-grade gliomas, and expression of these microRNA was decreased in patients with GBM [17, 31]. miR-21 could be used as a biomarker for progression of GBM, as demonstrated by the decrease in its level in serum in patients after surgical resection and its high level of expression associated with tumor recurrence [15, 31].

Conclusions. Circulating biomarkers are a potential area for studies improving the treatment of patients with gliomas. As regards proteins, their low specificity and limited utility in terms of providing information on the molecular profiles and heterogeneity of tumors means they lack diagnostic value. However, microRNA have potential for creating sensitive and specific biomarkers. Their tissue specificity in different pathologies makes them ideal for the diagnosis and prognosis of diseases. Several potential circulating microRNA have been identified in small studies and further assessment and validation in larger prospective studies are needed before they are introduced into routine clinical practice. Among these microRNA, miR-21 has special importance, as one of the most active microRNA in glioblastomas. Significant limitations of microRNA in terms of the efficacy of their use will probably be overcome by strict methodological standardization. Difficulty in identifying microRNA specific for gliomas comes partly from the complex heterogeneous nature of the tumors themselves. Numerous mutations arising during transformation between classes and subtypes of gliomas facilitate this heterogeneity. Use of a group of biomarkers to detect a range of these characteristics will ultimately be more convenient than relying on a single biomarker. Exosomes secreted by tumor cells become the key components of the biogenesis of gliomas and are a potential source of microRNA for the diagnosis and tracking of progression in patients with gliomas. Further efforts are needed for studies oriented to finding microRNA not associated with vesicles to establish clinically useful biomarkers for gliomas.

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