A promising light for an impossible disease: miRNAs in malignant gliomas

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“The benefits of miRNAs’ small size and stability are now being used as developmental tools for novel miRNA-based treatments.”

Malignant glioma remains an elusive tumor to manage despite advances in the understanding of its biology. The past few decades has shed much light into the disease’s mechanisms since the days of Bailey and Cushing’s insightful classification of these tumors – glioblastoma multiforme (GBM) – in 1926 [1], and attempts at radical hemispheric resection for affected patients by Dandy in 1928 [2]. In 2005, the landmark paper by Stupp *et al.* altered the course of history of malignant gliomas with the establishment of the ‘Stupp regimen’ as standard of care for newly diagnosed patients [3]. Although the combination of surgical resection, followed by concurrent radiation therapy and temozolomide (TMZ) has conferred better survival, the overall prognosis for these patients remains poor. Tumor recurrence is an inevitable reality. At present, median survival is between 12 and 18 months [3].

Recently, there has been growing evidence for the role of miRNAs in various tumors, including gliomas. miRNAs are small, highly-conserved, nonprotein-coding RNAs that function as gene expression regulators at the translational level [4].

The discovery of miRNAs has provided us with a deeper insight into gene expression regulation. They have been found to play important roles in normal mammalian cell development, proliferation and death [5,6]. Conversely, anomalous miRNA expression has been found to be associated with cancer initiation, progression and resistance to therapeutics. Even more significantly, evidence so far has demonstrated that miRNA profiles appear to be cell type- and disease-specific key factors for disease identification, prognostication and response to treatment monitoring [6].

In the context of malignant gliomas, profiling work has demonstrated consistency in miRNAs whose expression differs from surrounding brain tissue [7]. Furthermore, Lages *et al.* show that there are specific miRNAs responsible for different malignant glioma subtypes [8] – a leap forward from the current WHO histopathological classification [9]. Work achieved in the field of glioma molecular biology has established that GBM tumors are known to possess aberrant cell signaling and networking pathways due to mutated growth receptors, silenced tumor suppressor genes or

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upregulated oncogenes. All these add layers of complexity for the use of effective chemotherapeutics. However, certain miRNAs have been identified in such entities, for example, miR-7 in EGF receptor expression [10], miR-21 in matrix metalloproteinase inhibition [11] and miR-296 in proangiogenic VEGF receptor-2 expression [12]. Hence, miRNAs can provide further insight into the disease, beyond current understanding.

At present, there is no screening tool for GBM patients treated with adjuvant chemoradiotherapy. Most centers still rely on follow-up interval MRI with gadolinium contrast in order to monitor response to treatment. This is a costly modality with some limitations, such as the possibility of pseudoprogression [13], which is seen on the scans and may cast doubt on the diagnosis. miRNAs have been effectively detected in blood and tissue samples for other diseases; hence, similar techniques may be applied to GBM patients. There are advantages of using miRNAs as biomarkers in this context. Firstly, owing to their small size and stem-loop structure, miRNAs are more stable and less susceptible to degradation during fixation and sample processing. Next, the probability of discovering reliable biomarkers from approximately 1000 miRNA candidates will be higher than from over 40,000 genes. In their study, Yu *et al.* report plasma levels of miR-21, miR-128 and miR-342-3p being altered in GBM patients compared with normal, healthy controls [14]. In addition, the observations of Slaby *et al.* illustrate that there is a trend of downregulation of miRNA-181b and miRNA-181c in patients with better response to concomitant radiation therapy and TMZ, in comparison to those with disease progression [15]. Overall, there is much potential for the future use of miRNAs to augment current patient monitoring.

A key hallmark of GBM is its notoriety for recurrence, owing to an innate resistance to chemo- and radio-therapy. It is an accepted fact that acquired TMZ resistance occurs in more than 90% of recurrent malignant gliomas [16]. The topic of TMZ resistance is synonymous with the DNA repair enzyme, *O*⁶-methylguanine-methyltransferase (MGMT). To date, MGMT is the best characterized modulator of chemoresistance in GBM. Interestingly, the bioinformatic analysis by Zhang *et al.* of potential genes regulated by miR-181d, suggested MGMT to be a downstream target. They showed that transfection of miR-181d in a glioma cell model effected downregulation of MGMT mRNA

and protein expression [17]. Furthermore, there have been published reports of certain miRNAs implicated in TMZ resistance by various groups. For example, *in vitro* work by Ujifuku *et al.* found miR-195, miR-455 and miR-10a* to be upregulated in TMZ-resistant glioma cells [18]. Radioresistance adds another barrier to treatment efficiency in GBM. Not surprisingly, Niemoeller *et al.*'s analysis of human glioma cells demonstrated upregulation of miRNA clusters after treatment with clinically relevant radiation doses [19]. These results are somewhat expected as miRNAs can also be targets of molecular therapy, and those involved in apoptosis or regulating the expression of specific genes may induce the apoptosis of tumor cells exhibiting radioresistance [20].

Malignant gliomas are composed of a heterogeneous population of differentiated and non-differentiated cells. Compared with other primary tumors, they display less sensitivity to adjuvant treatment, resulting in disease recurrence. We are now aware of a subpopulation of glioma stem cells (GSCs) with higher tumorigenic potential that contributes significantly to this inevitability [21]. These GSCs exist in a tumor microenvironment where they are often unaffected by standard therapeutics. Research in the field of GSCs have found certain miRNAs to be associated with their maintenance and propagation [16]. Therefore, by using these miRNAs as modulators, strategies targeting GSCs within their protected niche may be clinically relevant. For years, we have known the blood–brain barrier as an infallible wall, preventing many chemotherapeutics from effectively reaching the brain tumor of interest. The benefits of miRNAs' small size and stability are now being used as developmental tools for novel miRNA-based treatments [22].

Conclusion & future perspective

Taken together, especially considering the complexities of GBM, miRNA approaches show great promise to improve current clinical management in this difficult disease. By improving our understanding of this heterogeneous tumor at a genomic level, miRNAs may hold the answers toward targeted therapy in this era of personalized medicine. In 2006, Rutka *et al.* wrote an inspiring review entitled 'Gliomas: *quo vadis*' [23]. Today, nearly a decade later, with the versatile input of miRNAs, we can perhaps, optimistically, look forward to 'Gliomas: *ad locum meliorem*'.

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