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Circulating and Tissue MicroRNA Expression Profiles for the Diagnosis and Grading of Central Nervous System Tumors

Amit Aiwale[1], Syed Paspala[2], Sandeep Kumar Vishwakarma[3] and Abhay Morajkar[4,*]

[1]*DNB Neurosurgeon, Solaris Hospital, Kasarvadavali, Thane – 400615, India*
[2]*MCH Neurosurgery, Care Hospital, Banjara Hills, Hyderabad, Telangana – 500034, India*
[3]*National Institutes of Health, Bethesda, United States*
[4]*VPM's B. N. Bandodkar College of Science, Autonomous, Thane – 400601, India*

Abstract:

Introduction: Central nervous system (CNS) tumors remain difficult to diagnose and monitor due to the invasive nature of tissue biopsy and the limited sensitivity of current imaging-based approaches. MicroRNAs (miRNAs) have emerged as promising molecular biomarkers due to their stability and regulatory roles in tumor progression. The present study aimed to evaluate the expression profiles of selected miRNAs in tissue, cerebrospinal fluid (CSF), and serum samples from patients with CNS tumors to assess their diagnostic and prognostic significance across different tumor grades.

Methods: A total of 27 patients with histologically confirmed CNS tumors were included in this study, following institutional ethical approval and informed consent. Tumor tissue, CSF, and serum samples were collected during surgical intervention. Expression levels of miRNA-7, miRNA-21, miRNA-33, miRNA-34, and miRNA-200 were quantified using reverse transcription quantitative polymerase chain reaction (RT-qPCR). Relative expression analysis was performed using the Livak and Pfaffl methods, and statistical significance was assessed using one- and two-way ANOVAs, t-tests, and chi-square tests, with $p < 0.05$ considered significant.

Results: Distinct expression patterns of miRNAs were observed among tissue, CSF, and serum samples. miRNA-21 expression increased progressively with higher tumor grades and showed maximum expression in grade IV tumors, suggesting its association with aggressive malignancy. In contrast, miRNA-7, miRNA-34, and miRNA-200 demonstrated higher expression in low-grade tumors and decreased expression with increasing tumor grade. miRNA-33 exhibited comparatively higher expression in CSF than in serum and tissue samples. CSF-derived miRNA profiles showed superior sensitivity and more consistent tumor-associated expression patterns compared with serum samples. No statistically significant gender-based differences were observed.

Discussion: The findings support the growing evidence that circulating and tissue-derived miRNAs play a significant role in CNS tumor biology and may serve as minimally invasive biomarkers for diagnosis, grading, and disease monitoring. The strong association of miRNA-21 with high-grade tumors and the inverse relationship of miRNA-7, miRNA-34, and miRNA-200 with tumor progression indicate their potential utility in molecular stratification of CNS malignancies. Furthermore, CSF-based miRNA analysis demonstrated greater disease specificity than serum profiling, highlighting its clinical relevance in neuro-oncology.

Conclusion: The present study demonstrates that miRNA profiling in tissue, CSF, and serum can effectively reflect grade-specific molecular alterations in CNS tumors. miRNA-21 may serve as a potential biomarker for high-grade malignancy, whereas miRNA-7, miRNA-34, and miRNA-200 are associated with lower-grade tumors. CSF-derived miRNA analysis represents a promising minimally invasive approach for improving CNS tumor diagnosis, prognostication, and longitudinal disease monitoring.

Keywords: Circulating microRNAs, CNS Tumors, Glioblastoma, Glioma Diagnosis, MicroRNA Biomarkers, RT-qPCR.

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*Address correspondence to this author at the VPM's B. N. Bandodkar College of Science, Autonomous, Thane – 400601, India; E-mail: asmorajkar@vpmthane.org

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1. INTRODUCTION

Current diagnostic approaches for brain cancer, such as clinical evaluation, neuroimaging techniques including magnetic resonance imaging (MRI) and computed tomography (CT), along with histopathological examination, remain essential for diagnosis; however, these methods possess certain limitations and are often effective only during the later stages of disease progression [1, 2]. Clinical signs and symptoms are often subtle and nonspecific, while sensitivity and specificity of neuroimaging differ widely with the type of cancer and location [3]. Histopathological evaluation continues to be the most reliable standard for confirming diagnosis; however, obtaining tumour tissue through biopsy is an invasive procedure that may lead to complications such as intracranial bleeding and neurological impairment [4]. Furthermore, certain brain lesions are located in regions that are difficult to access surgically, making tissue biopsy impractical or unsafe [5]. Consequently, there is a growing clinical need to identify highly sensitive biological markers to facilitate the early detection of brain malignancies [6].

In the field of blood, there is growing interest in identifying circulating tumor cells (CTCs) using antibody and molecular tagging [7]. These methods, including the FDA-approved Cell-Search technology, should be investigated in CSF, particularly in primary CNS tumors, where CSF may be seeded before blood [8]. Oncogenic miRNAs, which are frequently upregulated in various cancers, promote cellular proliferation by enhancing cell cycle initiation and progression, whereas the loss or reduced expression of tumor-suppressor miRNAs in malignant cells contributes to dysregulation of mechanisms responsible for cell cycle arrest [9].

Tumor cells may enter the cerebrospinal fluid (CSF) either by direct extension from primary CNS malignancies located adjacent to CSF spaces or by crossing the blood–brain barrier [10]. Examination of CSF through cytological analysis remains the standard diagnostic approach for identifying neoplastic cells. Growing evidence suggests that detecting molecular alterations in CSF may provide earlier indications of malignancy than relying solely on tumor mass detection [3]. Nevertheless, tissue-based diagnostic methods are limited by the need for invasive surgical sampling. Consequently, current biomarker research in brain tumors has increasingly focused on circulating miRNAs in biological fluids such as CSF and serum, as these samples can be obtained via minimally invasive techniques and may facilitate long-term disease monitoring [11]. Furthermore, circulating miRNAs identified in the CSF of brain tumor patients, particularly those released from tumor cells, are believed to possess potential pro-tumorigenic properties [12]. These miRNAs may convey oncogenic signals to recipient cells, thereby altering their biological behavior by modulating molecular pathways associated with tumor growth, invasion, and resistance to therapeutic agents [13]. The transfer of miRNAs between primary tumor cells and recipient target cells represents an emerging mechanism that modulates cellular phenotype and may play a critical role in cancers with high metastatic or disseminating potential [14]. Primary brain tumors exhibiting intracranial spread, as well as secondary metastatic tumors originating from distant organs, may release miRNAs into the CSF that carry clinically significant oncogenic signatures [15]. Therefore, profiling circulating CSF miRNAs in patients with brain tumors could provide valuable insights into the molecular characteristics of the primary malignancy and its metastatic behavior.

Improved detection of CSF malignancy is a clinical imperative as well, since current diagnostic techniques are insensitive, resulting in delays until disease-related symptoms are profound and irreversible, and therapeutic options are limited or nonexistent [16]. Our study will focus on investigating more specific and predictive miRNA biomarkers with high sensitivity and specificity in blood, CSF, and tumor tissue from patients with CNS tumors to develop next-generation clinical detection strategies for improved diagnosis or prognosis of CNS tumors using less-invasive procedures.

2. MATERIALS AND METHODS

2.1. Patients and Sample Collection

An exploratory, single-center study was conducted over one year at the Department of Neurosurgery, CARE Hospital, Hyderabad. No formal a priori sample-size or power calculation was performed; all eligible patients available during the predefined study period were considered for enrollment. Of 37 patients screened, 27 met the predefined inclusion and exclusion criteria and had samples suitable for molecular analysis. The final cohort comprised 18 males (66.7%) and 9 females (33.3%), aged 3–88 years (mean 45.96 ± 24.11 years; age available

for 26 patients), with histologically confirmed CNS tumors of WHO Grades I–IV, including glioblastoma, astrocytoma, oligodendroglioma, ependymoma, medulloblastoma, meningioma, schwannoma, and other tumor types. Tissue, CSF, and serum samples were collected during surgery according to clinical accessibility and the approved study protocol. Written informed consent was obtained from all patients or their legal guardians, as applicable. Clinical information, including previous surgery, radiotherapy, chemotherapy, or treatment-naïve status, was obtained from the medical records.

2.2. Inclusion/Exclusion Criteria

Patients with a histologically confirmed CNS tumor who underwent surgical intervention during the study period and whose patient or legally authorized representative provided informed consent were eligible for inclusion. Patients were excluded if the final diagnosis was not consistent with a CNS tumor, the collected sample was contaminated, the sample volume was inadequate, there was prior treatment, clinical data were incomplete, or RNA quality was poor.

2.3. Quantification of miRNA Expression using RT-qPCR

For miRNA isolation and subsequent analysis, samples were sent to the Centre for Liver Research and Diagnostics in Hyderabad. All the extracted miRNAs were converted immediately into complementary DNA using U6 universal miRNA primers by using qPCR (Applied Biosystems, Thermo Fisher Scientific, USA) (Catalog number: 4427975)

2.4. Statistical Analysis

Statistical analysis was performed using one-way and two-way Analysis of Variance in GraphPad Prism. Wherever required, analysis values were represented as mean ± SD. RT–qPCR efficiency was calculated using StepOne software (Version 2.2). RT-qPCR for all the markers was performed in triplicate. The expression for each target gene was calculated as relative fold change and was normalized against GAPDH/β-actin expression according to the Livak method for relative quantification [17]. Fold difference values for each transcript were further validated using the Pfaffl method of relative quantification [18]. A regression value (R2) ≥ 0.99 was considered significant at 100% PCR efficiency. The t-test and chi-square test were used, and differences with p-values < 0.05 were considered statistically significant.

3. RESULT

The study population demonstrated a heterogeneous distribution of CNS tumors, with cases spanning across low- and high-grade lesions. CSF samples were collected from multiple intracranial compartments depending on clinical access and tumor location

The expression levels of miRNA-7, 21, 34, and 200 were significantly higher in tissue samples, except for miRNA-33, which was higher in CSF in both males and females compared with tissue and Serum samples, as shown in Fig. (**1**). Whereas no significant difference was observed, gender-wise, in any sample analysed.

The expression levels of miRNAs 7, 21, 34, and 200 were significantly higher in tissue samples than in serum and CSF samples. No statistically significant difference was observed in serum vs CSF, except for miRNA 33, which was higher in CSF. The fold changes in each type of sample may be variable, but the pattern of expression was relatively similar, as can be seen in Fig. (**2**).

The expression levels of miRNA-7 were consistently low, with fold changes increasing with higher CNS tumor grade across all three sample types, as shown in Fig. (**3**). They showed greater fold changes in patients with grade 1 CNS tumors.

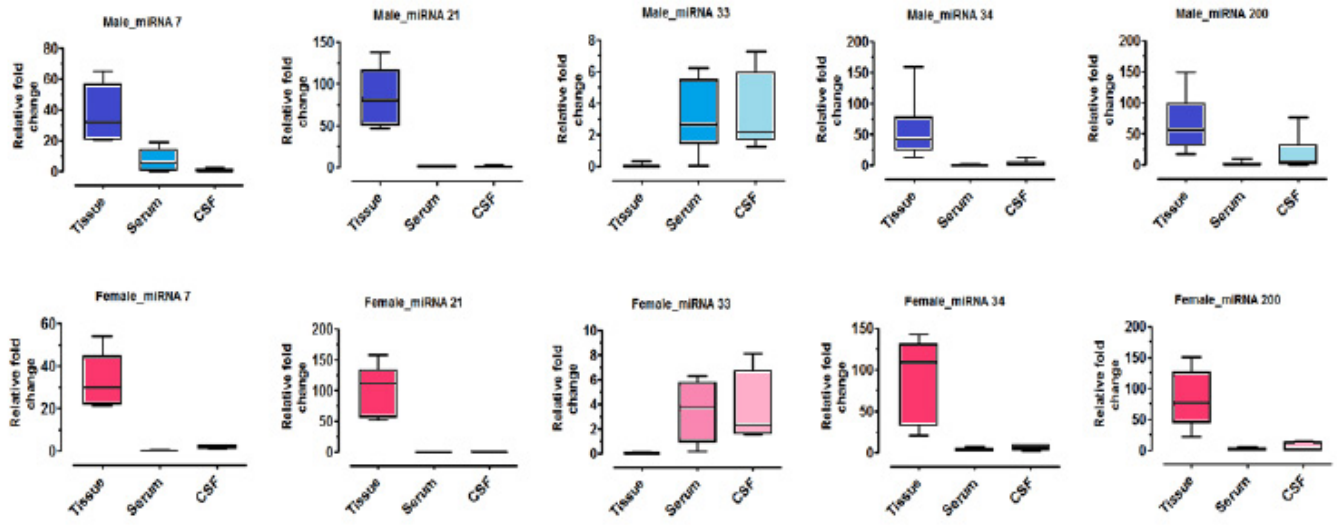

Fig. (1). Gender wise miRNA Expression in Tissue, Serum and CSF.

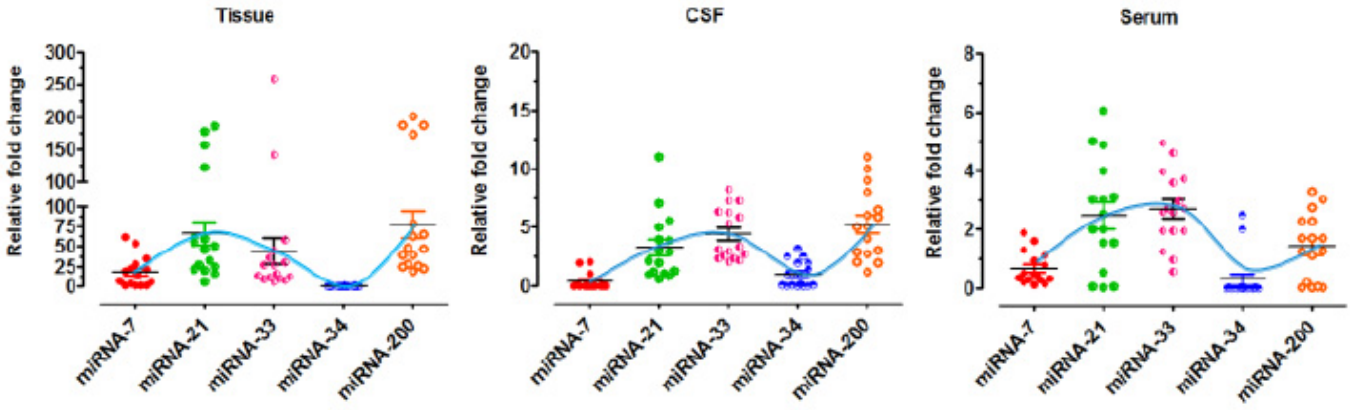

Fig. (2). Levels of Different miRNAs in Tumor Tissue, Serum and CSF.

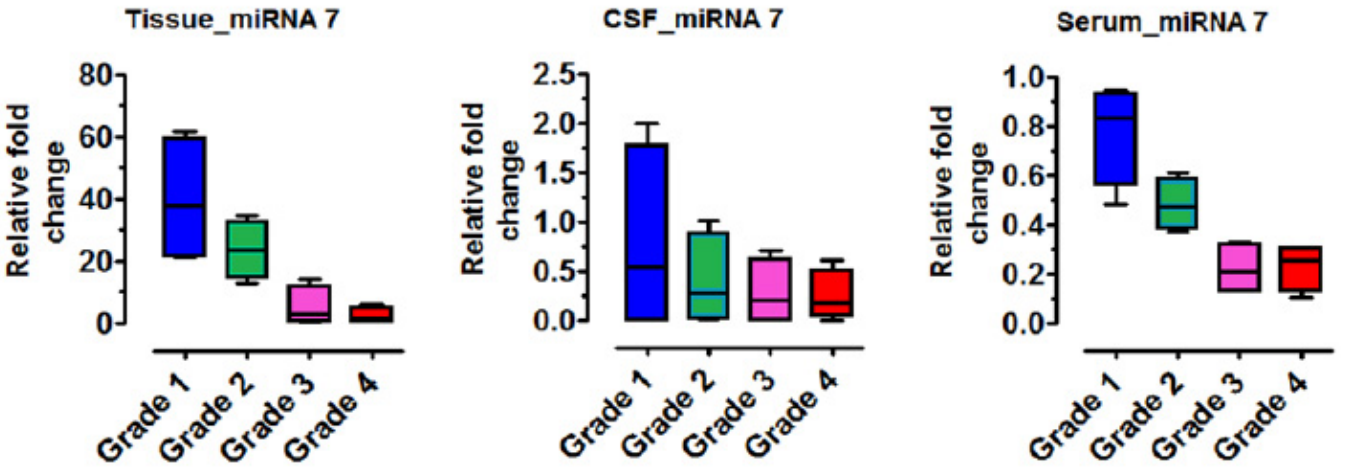

Fig. (3). miRNA-7 Expression in different grades of CNS tumor in Tissue, Serum and CSF.

The expression levels of miRNA-21 were found significantly higher in tissue samples with increasing Tumor Grades. It was also consistently higher with increasing grade of CNS Tumor in serum and CSF samples. However, no significant change was observed between serum and CSF for miRNA-21 expression levels in each grade. Tumours are shown in Fig. (**4**). Grade 4 tumours had the highest fold changes in miRNA-21 compared with lower-grade tumours.

Consistent down-regulation was observed for the expression of miRNA-33 in tissue and CSF samples from grade 1 to grade 4 of human CNS tumor patients. However, no significant variation was observed in serum; Fig, (**5**) shows the same patient's results.

The expression levels of miRNA-34 were significantly higher in tissue, serum, and CSF samples from grade 1 CNS tumors, whereas no significant change was observed in other grades of CNS tumors, as shown in Fig. (**6**).

The expression levels of miRNA-200 were significantly higher in tissue and CSF samples from grade 1 and significantly lower in grades 2, 3, and 4. Whereas no significant difference was observed in serum samples with respect to CNS Tumor grades, as can be seen in Fig. (**7**).

4. DISCUSSION

Brain tumors are among the most aggressive and difficult malignancies to manage, largely because the blood–brain barrier limits therapeutic effectiveness and contributes to treatment resistance. Advances in genomic classification have improved diagnostic accuracy; however, tumor heterogeneity and the limited accessibility of tissue biopsies continue to pose significant clinical challenges [19]. Altered miRNA expression is recognized as an early event during tumor development, suggesting that miRNAs present in CSF may serve as promising biomarkers for the early detection of brain cancers and potentially improve therapeutic outcomes [20]. Moreover,

distinct circulating miRNA expression patterns observed between cancer patients and healthy individuals, along with their correlation with disease progression, indicate that c-miRNAs could provide a sensitive, minimally invasive strategy for early cancer diagnosis [21].

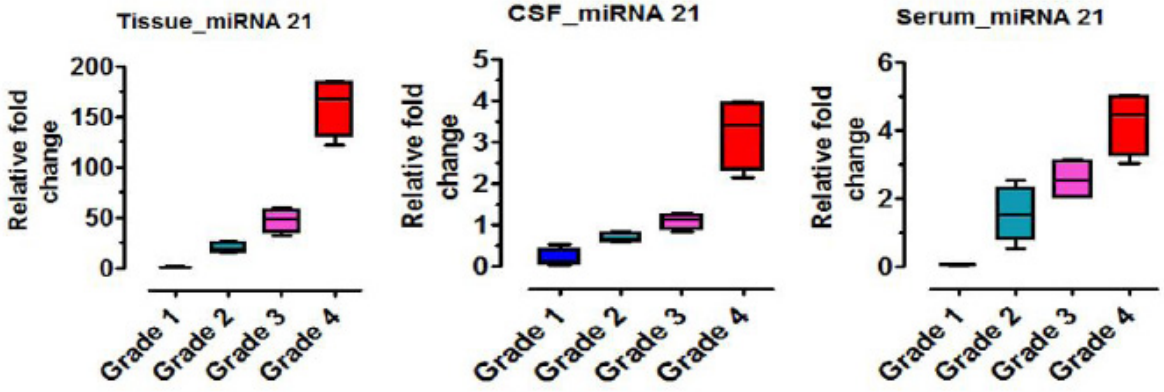

Fig. (4). miRNA-21 Expression in different grades of CNS tumor in Tissue, Serum and CSF.

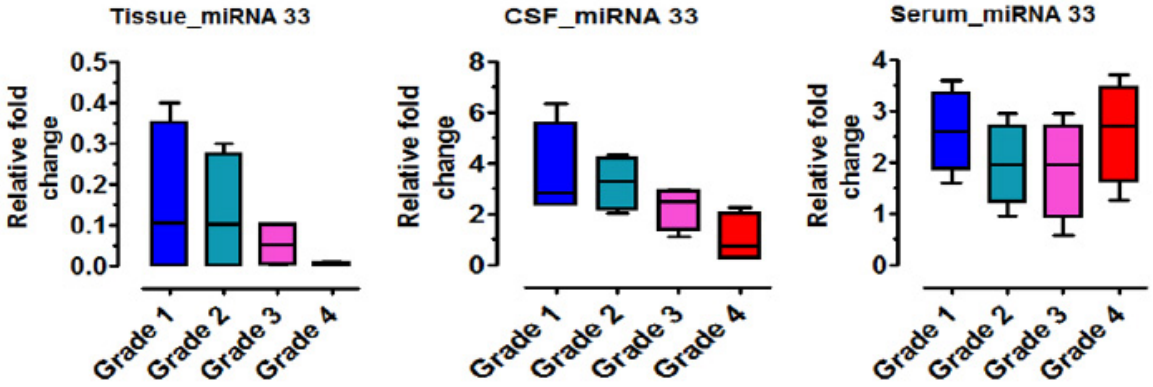

Fig. (5). miRNA-33 Expression in different grades of CNS tumor in Tissue, Serum and CSF.

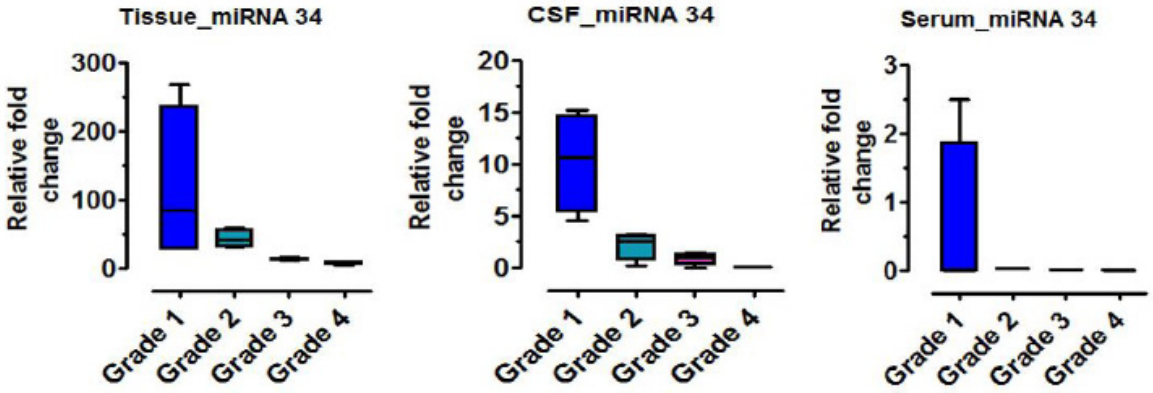

Fig. (6). miRNA-34 Expression in different grades of CNS tumor in Tissue, Serum and CSF.

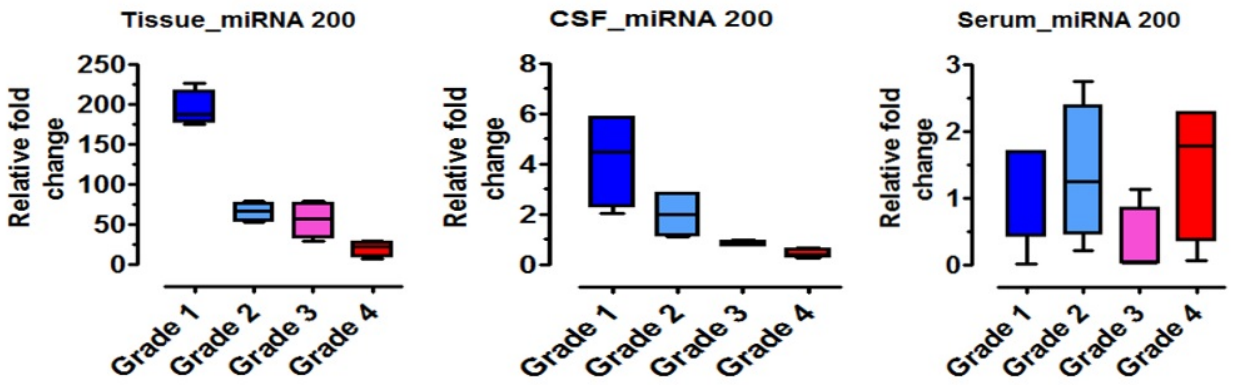

Fig. (7). miRNA-200 Expression in different grades of CNS tumor in Tissue, Serum and CSF.

Our recent investigation confirmed the upregulation of these specific miRNAs (miR-7, 21, 34, 200) in Central Nervous System (CNS) tumors, particularly gliomas and glioblastoma multiforme (GBM), in both males and females. Similar observations are noted in miR-7, which is heavily involved in brain development and whose regulation is complex [22]. miR-21 is consistently increased in glioma tissues compared to non-neoplastic brain tissue, regardless of sex [23]. Specific studies have shown that miR-34 regulates the Notch and Numb proteins in gliomas [24]. miR-200 family is involved in regulating epithelial-mesenchymal transition (EMT) in gliomas. Specifically, miR-200a is associated with glioma malignancy and negatively regulates DNA repair mechanisms [25]. In neurological diseases, cerebrospinal fluid (CSF) is generally considered to provide more reliable and disease-specific molecular information compared with peripheral blood samples [26]. A meta-analysis of more than 20 independent studies on CNS malignancies demonstrated that CSF-derived miRNA profiles have greater diagnostic sensitivity and specificity than blood-based miRNA profiles. The study reported that the sensitivity for identifying CNS tumors improved from 83% with blood-derived miRNAs to 86% with CSF miRNAs, while specificity increased from 81% to 88% [27]. Since the blood compartment has a substantially larger volume than CSF, miRNA molecules originating from the CNS may become diluted after traversing the blood–brain barrier (BBB) into the systemic circulation. Our study also shows the presence of c-miRNA in both CSF and Serum of patients with CNS tumors [28]. The fold change varied across CSF and Serum for different miRNAs, as shown in the figure.

Let-7 family members are direct and strong regulators of the RAS family. The expression levels of let-7 miRNA family are not reduced in brain tumours (with few exceptions); growing evidence shows that let-7 miRNA inhibits the malignant behaviour - proliferation, migration, and invasion - of glioma cells and glioma stem-like cells as well as the tumour size in nude mice xenograft-transplanted glioblastoma (GBM) via *KRAS* inhibition [29]. More recently, the genetic loss of let-7 is implicated in neuroblastoma oncogenesis, placing *let*-7 disruption at the centre of neuroblastoma pathogenesis [30]. In addition, loss of let-7 increases resistance to certain chemotherapeutic drugs and to radiation therapy in GBM. In our study, the fold changes in let-7 were inversely related to tumour grade [22].

A miRNA profiling analysis conducted in glioblastoma multiforme (GBM) identified a distinct pattern of candidate miRNA gene expression, with miR-221 showing marked upregulation in GBM samples [31]. In contrast, several other miRNAs, including miR-128, miR-181a, miR-181b, and miR-181c, exhibited reduced expression levels. Interestingly, the same investigation did not recognize miR-21 among the major overexpressed miRNAs in GBMs. Additional studies evaluating GBM-associated miRNA signatures have employed reverse transcription with stem-loop primers, followed by real-time PCR analysis [32]. In that investigation, the expression profiles of 180 human miRNAs were compared between normal brain tissue and GBM samples to characterize disease-specific miRNA patterns. Pronounced alterations exceeding 10-fold were identified in approximately 14% of the analyzed miRNAs, among which nearly 2% demonstrated increased expression in GBM tissues, including miR-10a, miR-10b, and miR-96. Elevated expression levels of miRNA-21 and miR-10b have been reported specifically in brain tumor lesions, including glioblastoma multiforme (GBM) and metastatic brain tumors, when compared with non-neoplastic conditions. In contrast, members of the miR-200 family were detected exclusively in the CSF of patients with brain metastases, suggesting their potential utility in distinguishing glioblastoma from metastatic brain tumors, which is clinically relevant for therapeutic decision-making [33]. In addition, longitudinal assessment of CSF miRNA profiles, particularly miR-10b, miR-21, and miR-200, demonstrated their usefulness in monitoring disease progression and treatment response over extended periods in patients with brain tumors. The authors further indicated that circulating CSF miR-21 levels may serve as a valuable biomarker for glioma diagnosis and prognosis, with potential utility in predicting tumor recurrence and metastatic progression [23]. Their

findings also revealed a strong association between elevated CSF miRNA-21 expression and poor clinical outcomes and recurrence in glioma patients. Similar to primary central nervous system lymphoma (PCNSL), gliomas frequently exhibit elevated miR-21 expression, with reported diagnostic sensitivity ranging from 85–87% and specificity between 93–100% for detecting glioblastoma [34]. Furthermore, previous studies have documented a reduction of more than 50-fold in miR-21 levels following surgical intervention when compared with pre-operative measurements, indicating that miR-21 may also serve as a useful biomarker for evaluating therapeutic response and treatment effectiveness [23].

While certain miRNAs are present in both blood and CSF, they are often poorly correlated. Furthermore, other miRNA subtypes have been exclusively identified in CSF or blood. Condrat *et al.* [21] also compared microRNAs present in serum and CSF from the study, which revealed that profiles of microRNAs are more similar from different subjects for either CSF or serum and are less similar to one another, even within the same subject. In our study, all miRNAs except miRNA-33 were found in higher folds in tumor tissue compared to CSF and Serum samples. miR-33a is recognized as an important regulator of glucose and cholesterol homeostasis and has been implicated in the development and progression of several human malignancies, including pancreatic, lung, colon, and bone cancers, as well as gliomas. In addition, evidence suggests that miR-33a may contribute to the maintenance and self-renewal capacity of glioma-initiating cells [35]

Several authors have studied miRNA-34a and considered it a potential prognostic marker for glioma, as its expression negatively correlates with patient survival in grade III and IV glial tumors. They showed that miR-34a expression decreased in a graded manner in glioma and glioma stem cell lines compared to normal brain tissues [36-38]. claimed to have found that microRNA-34a (miR-34a) is down-regulated in human glioma tumors as compared to normal brain, and that miR-34a levels in mutant-p53 gliomas were lower than inwildtype-p53 tumors. The new data show that miR-34a expression inhibits various malignancy endpoints in glioma stem cells. They also show for the first time that miR-34a expression induces glioma stem cell differentiation. Altogether, the data suggest that miR-34a is a tumor suppressor and a potentially potent therapeutic agent that acts by targeting multiple oncogenic pathways in brain tumors and by inducing the differentiation of cancer stem cells [39]. In our present investigation, we also found that miRNA-34 was negatively associated with CNS tumor grade; i.e., the lower the grade, the higher the fold increase in miRNA levels in CSF and Serum.

A meta-analytical study identified miR-21 as one of the most effective individual CSF miRNA biomarkers for glioma detection, demonstrating a sensitivity of 82% and specificity of 94%. In contrast, combining multiple miRNAs into a diagnostic panel improved overall diagnostic performance, yielding 91% sensitivity and 94% specificity [40]. Because miRNAs are involved in a broad range of biological pathways, the use of combined miRNA signatures may be necessary to distinguish among different CNS disorders. Elevated expression of miR-21 and miR-10b has been associated with several forms of brain malignancies, whereas comparatively higher levels of miR-200 are more suggestive of metastatic brain disease than primary tumors. Furthermore, analysis of a seven-miRNA panel comprising miR-10b, miR-21, miR-125b, miR-141, and multiple members of the miR-200 family achieved approximately 99% diagnostic accuracy, outperforming individual biomarkers [23]. The expression of miRNA-21 and miR-10b is markedly elevated in brain tumor tissues, particularly in patients with glioblastoma multiforme (GBM) and metastatic brain lesions, when compared with non-neoplastic conditions. Conversely, members of the miR-200 family were detected exclusively in the CSF of patients with metastatic brain tumors, suggesting that CSF-derived miRNAs may help differentiate glioblastoma from metastatic brain malignancies, with important implications for treatment planning [37].

Circulating miRNAs present in the CSF of brain cancer patients represent a promising and dynamic tool for investigating brain tumor progression across different stages of the disease. Analysis of these miRNAs may provide valuable information regarding tumor biology and genetic alterations, thereby helping to characterise the molecular profile of the underlying malignancy [41]. CSF miRNAs' specificity and chemical stability are clinically appealing, as they are readily accessible via standard, minimally invasive clinical methods. Hence, the use of these techniques in clinical practice may offer advantages over currently available modalities for diagnosing and prognosticating CNS tumours [27]. However, despite the promise, the available data on the suitability of the CSF miRNAs as diagnostic biomarkers for brain cancer are still very limited, and critical issues need to be clarified before making recommendations for their clinical implementation.

5. STUDY LIMITATIONS

The present study has several limitations that should be considered while interpreting the findings. First, the study was conducted with a relatively small sample size comprising 27 CNS tumor patients from a single clinical centre, which may limit the generalizability of the results across broader populations and different tumor subtypes. Second, the heterogeneous distribution of CNS tumors and unequal representation among tumor grades may have influenced the observed miRNA expression patterns. Third, healthy control subjects and non-neoplastic neurological disease controls were not included for comparative analysis, thereby restricting the evaluation of the specificity of the investigated miRNAs as diagnostic biomarkers.

In addition, the study relied primarily on RT-qPCR-based expression analysis without functional validation of the biological roles of the investigated miRNAs in CNS tumor progression. Variability in CSF collection sites,

sample handling, and pre-analytical processing may also have introduced experimental bias affecting circulating miRNA quantification. Furthermore, longitudinal follow-up data were unavailable to assess changes in miRNA expression during treatment response, recurrence, or survival outcomes. Although the present findings support the diagnostic and prognostic potential of circulating and tissue-derived miRNAs in CNS tumors, larger multicentric studies with standardized methodologies, inclusion of appropriate control groups, and long-term clinical follow-up are required before these biomarkers can be translated into routine clinical practice.

CONCLUSION

Circulating and tissue miRNA profiling reveals reproducible grade-specific expression patterns in CNS tumors. miRNA-21 serves as a marker of high-grade malignancy, whereas miRNA-7, miRNA-34, and miRNA-200 are associated with low-grade tumors. CSF-based miRNA analysis provides superior diagnostic sensitivity and may support early diagnosis and longitudinal monitoring.

AUTHORS' CONTRIBUTIONS

AA contributed to study conception and design, patient recruitment, clinical data and sample collection, experimental work, interpretation of results, and manuscript preparation. SP contributed to methodology development, RT-qPCR analysis, validation of experimental findings, interpretation of results, and manuscript revision. SV contributed to study design, statistical analysis, data interpretation, literature review, and manuscript preparation. AM contributed to study conception, scientific supervision, and interpretation of findings, critical manuscript revision, and final approval of the manuscript.

All authors confirm responsibility for study conception and design, data collection, analysis and interpretation of results, and manuscript preparation, and have approved the final version of the manuscript for publication.

LIST OF ABBREVIATIONS

BBB	=	Blood–Brain Barrier
CNS	=	Central Nervous System
CSF	=	Cerebrospinal Fluid
CT	=	Computed Tomography
DNB	=	Diplomate of National Board
GBM	=	Glioblastoma Multiforme
HGG	=	High-Grade Glioma
IEC	=	Institutional Ethics Committee
miRNA	=	MicroRNA
MRI	=	Magnetic Resonance Imaging
mRNA	=	Messenger Ribonucleic Acid
PCNSL	=	Primary Central Nervous System Lymphoma
PCR	=	Polymerase Chain Reaction
qPCR	=	Quantitative Polymerase Chain Reaction
RT-qPCR	=	Reverse Transcription Quantitative Polymerase Chain Reaction
RNA	=	Ribonucleic Acid
SD	=	Standard Deviation
WHO	=	World Health Organization

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted at the Department of Neurosurgery, Care Institute of Medical Sciences, Banjara Hills, Hyderabad, India. Institutional Ethics Committee approval was obtained (CIMS/IEC/07/2025) prior to the commencement of the study following submission of the research protocol.

HUMAN AND ANIMAL RIGHTS

All procedures involving human participants were conducted in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Written informed consent was obtained from all patients or their legal guardians, as applicable.

STANDARDS OF REPORTING

STROBE guidelines were followed.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of this article may be obtained from the corresponding author [A.M.] upon reasonable request.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Martucci, M.; Russo, R.; Schimperna, F.; D'Apolito, G.; Panfili, M.; Grimaldi, A.; Perna, A.; Ferranti, A.M.; Varcasia, G.; Giordano, C.; Gaudino, S. Magnetic resonance imaging of primary adult brain tumors: State of the art and future perspectives. *Biomedicines*, **2023**, *11*(2), 364. http://dx.doi.org/10.3390/biomedicines11020364 PMID: 36830900

[2] Kandimalla, S.Y.; Vamsi, D.M.; Bhavani, S. Recent methods and challenges in brain tumor detection using medical image

processing. *Recent Pat. Eng.,* **2023**, *17*(5), 8-23.
[3] Hickman, R.A.; Miller, A.M.; Arcila, M.E. Cerebrospinal fluid: A unique source of circulating tumor DNA with broad clinical applications. *Transl. Oncol.,* **2023**, *33*, 101688. http://dx.doi.org/10.1016/j.tranon.2023.101688 PMID: 37196447
[4] Li, M.; Ren, X.; Dong, G.; Wang, J.; Jiang, H.; Yang, C.; Zhao, X.; Zhu, Q.; Cui, Y.; Yu, K.; Lin, S. Distinguishing pseudoprogression from true early progression in isocitrate dehydrogenase wild-type glioblastoma by interrogating clinical, radiological, and molecular features. *Front. Oncol.,* **2021**, *11*, 627325. http://dx.doi.org/10.3389/fonc.2021.627325 PMID: 33959496
[5] An, Y.; Fan, F.; Jiang, X.; Sun, K. Recent advances in liquid biopsy of brain cancers. *Front. Genet.,* **2021**, *12*, 720270. http://dx.doi.org/10.3389/fgene.2021.720270 PMID: 34603383
[6] de Lima, B.P.; Ferraz, L.S.; Devalle, S.; Borges, H.L. Liquid biopsy-derived tumor biomarkers for clinical applications in glioblastoma. *Biomolecules,* **2025**, *15*(5), 658. http://dx.doi.org/10.3390/biom15050658 PMID: 40427551
[7] Vidlarova, M.; Rehulkova, A.; Stejskal, P.; Prokopova, A.; Slavik, H.; Hajduch, M.; Srovnal, J. Recent advances in methods for circulating tumor cell detection. *Int. J. Mol. Sci.,* **2023**, *24*(4), 3902. http://dx.doi.org/10.3390/ijms24043902 PMID: 36835311
[8] Stoecklein, N.H.; Oles, J.; Franken, A.; Neubauer, H.; Terstappen, L.W.M.M.; Neves, R.P.L. Clinical application of circulating tumor cells. *Med. Genetik,* **2023**, *35*(4), 237-250. http://dx.doi.org/10.1515/medgen-2023-2056 PMID: 38835741
[9] Sharma, P.C.; Gupta, A. MicroRNAs: Potential biomarkers for diagnosis and prognosis of different cancers. *Transl. Cancer Res.,* **2020**, *9*(9), 5798-5818. http://dx.doi.org/10.21037/tcr-20-1294 PMID: 35117940
[10] Shalaby, T.; Achini, F.; Grotzer, M.A. Targeting cerebrospinal fluid for discovery of brain cancer biomarkers. *J. Cancer Metastasis Treat.,* **2016**, *2*(5), 176-187. http://dx.doi.org/10.20517/2394-4722.2016.12
[11] Sandau, U.S.; Wiedrick, J.T.; McFarland, T.J.; Galasko, D.R.; Fanning, Z.; Quinn, J.F.; Saugstad, J.A. Analysis of the longitudinal stability of human plasma miRNAs and implications for disease biomarkers. *Sci. Rep.,* **2024**, *14*(1), 2148. http://dx.doi.org/10.1038/s41598-024-52681-5 PMID: 38272952
[12] Latowska, J.; Grabowska, A.; Zarębska, Ż.; Kuczyński, K.; Kuczyńska, B.; Rolle, K. Non-coding RNAs in brain tumors, the contribution of lncRNAs, circRNAs, and snoRNAs to cancer development—their diagnostic and therapeutic potential. *Int. J. Mol. Sci.,* **2020**, *21*(19), 7001. http://dx.doi.org/10.3390/ijms21197001 PMID: 32977537
[13] Attathikhun, M.; Jurj, A.; Calin, G.A. MicroRNAs as key regulators of cancer drug resistance: Insights and future directions in chemotherapy, targeted-therapy, radiotherapy, and immunotherapy. *Cancer Drug Resist.,* **2025**, *8*, 64. http://dx.doi.org/10.20517/cdr.2025.146 PMID: 41425255
[14] Si, W.; Shen, J.; Zheng, H.; Fan, W. The role and mechanisms of action of microRNAs in cancer drug resistance. *Clin. Epigenetics,* **2019**, *11*(1), 25. http://dx.doi.org/10.1186/s13148-018-0587-8 PMID: 30744689
[15] Gareev, I.; Beylerli, O.; Liang, Y.; Xiang, H.; Liu, C.; Xu, X.; Yuan, C.; Ahmad, A.; Yang, G. The role of MicroRNAs in therapeutic resistance of malignant primary brain tumors. *Front. Cell Dev. Biol.,* **2021**, *9*, 740303. http://dx.doi.org/10.3389/fcell.2021.740303 PMID: 34692698
[16] Bae, Y.S.; Cheong, J.W.; Chang, W.S.; Kim, S.; Oh, E.J.; Kim, S.H. Diagnostic accuracy of cerebrospinal fluid (CSF) cytology in metastatic tumors: An analysis of consecutive CSF samples. *Korean J. Pathol.,* **2013**, *47*(6), 563-568. http://dx.doi.org/10.4132/KoreanJPathol.2013.47.6.563 PMID: 24421850
[17] Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods,* **2001**, *25*(4), 402-408. http://dx.doi.org/10.1006/meth.2001.1262 PMID: 11846609
[18] Pfaffl, M.W. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.,* **2001**, *29*(9), e45. http://dx.doi.org/10.1093/nar/29.9.e45 PMID: 11328886
[19] Pilotto Heming, C.; Niemeyer Filho, P.; Moura-Neto, V.; Aran, V. Recent advances in the use of liquid biopsy to fight central nervous system tumors. *Cancer Treat. Res. Commun.,* **2023**, *35*(April), 100709. http://dx.doi.org/10.1016/j.ctarc.2023.100709
[20] Chadda, K.R.; Blakey, E.E.; Coleman, N.; Murray, M.J. The clinical utility of dysregulated microRNA expression in paediatric solid tumours. *Eur. J. Cancer,* **2022**, *176*, 133-154. http://dx.doi.org/10.1016/j.ejca.2022.09.010 PMID: 36215946
[21] Condrat, C.E.; Thompson, D.C.; Barbu, M.G.; Bugnar, O.L.; Boboc, A.; Cretoiu, D.; Suciu, N.; Cretoiu, S.M.; Voinea, S.C. miRNAs as biomarkers in disease: Latest findings regarding their role in diagnosis and prognosis. *Cells,* **2020**, *9*(2), 276. http://dx.doi.org/10.3390/cells9020276 PMID: 31979244
[22] Visani, M.; de Biase, D.; Marucci, G.; Cerasoli, S.; Nigrisoli, E.; Bacchi Reggiani, M.L.; Albani, F.; Baruzzi, A.; Pession, A. Expression of 19 microRNAs in glioblastoma and comparison with other brain neoplasia of grades I–III. *Mol. Oncol.,* **2014**, *8*(2), 417-430. http://dx.doi.org/10.1016/j.molonc.2013.12.010 PMID: 24412053
[23] Aloizou, A.M.; Pateraki, G.; Siokas, V.; Mentis, A.F.A.; Liampas, I.; Lazopoulos, G.; Kovatsi, L.; Mitsias, P.D.; Bogdanos, D.P.; Paterakis, K.; Dardiotis, E. The role of MiRNA-21 in gliomas: Hope for a novel therapeutic intervention? *Toxicol. Rep.,* **2020**, *7*, 1514-1530. http://dx.doi.org/10.1016/j.toxrep.2020.11.001 PMID: 33251119
[24] Jegathesan, Y.; Stephen, P.P.; Sati, I.S.E.E.; Narayanan, P.; Monif, M.; Kamarudin, M.N.A. MicroRNAs in adult high-grade gliomas: Mechanisms of chemotherapeutic resistance and their clinical relevance. *Biomed. Pharmacother.,* **2024**, *172*, 116277. http://dx.doi.org/10.1016/j.biopha.2024.116277 PMID: 38377734
[25] Peng, L.; Fu, J.; Ming, Y. The miR-200 family: Multiple effects on gliomas. *Cancer Manag. Res.,* **2018**, *10*, 1987-1992. http://dx.doi.org/10.2147/CMAR.S160945 PMID: 30034253
[26] Shahan, B.; Choi, E.Y.; Nieves, G. Cerebrospinal fluid analysis. *Am. Fam. Physician,* **2021**, *103*(7), 422-428. PMID: 33788511
[27] Beylerli, O.; Encarnacion Ramirez, M.J.; Shumadalova, A.; Ilyasova, T.; Zemlyanskiy, M.; Beilerli, A.; Montemurro, N. Cell-free miRNAs as non-invasive biomarkers in brain tumors. *Diagnostics,* **2023**, *13*(18), 2888. http://dx.doi.org/10.3390/diagnostics13182888 PMID: 37761255
[28] Archie, S.R.; Al Shoyaib, A.; Cucullo, L. Blood-brain barrier dysfunction in cns disorders and putative therapeutic targets: An overview. *Pharmaceutics,* **2021**, *13*(11), 1779. http://dx.doi.org/10.3390/pharmaceutics13111779 PMID: 34834200
[29] Messina, S. The RAS oncogene in brain tumors and the involvement of let-7 microRNA. *Mol. Biol. Rep.,* **2024**, *51*(1), 531. http://dx.doi.org/10.1007/s11033-024-09439-z PMID: 38637419
[30] Powers, J.T.; Tsanov, K.M.; Pearson, D.S.; Roels, F.; Spina, C.S.; Ebright, R.; Seligson, M.; de Soysa, Y.; Cahan, P.; Theißen, J.; Tu, H.C.; Han, A.; Kurek, K.C.; LaPier, G.S.; Osborne, J.K.; Ross, S.J.; Cesana, M.; Collins, J.J.; Berthold, F.; Daley, G.Q. Multiple mechanisms disrupt the let-7 microRNA family in neuroblastoma. *Nature,* **2016**, *535*(7611), 246-251. http://dx.doi.org/10.1038/nature18632 PMID: 27383785
[31] Bassot, A.; Dragic, H.; Haddad, S.A.; Moindrot, L.; Odouard, S.; Corlazzoli, F.; Marinari, E.; Bomane, A.; Brassens, A.; Marteyn, A.; Hibaoui, Y.; Petty, T.J.; Chalabi-Dchar, M.; Larrouquere, L.; Zdobnov, E.M.; Legrand, N.; Tamburini, J.; Lincet, H.; Castets, M.; Yebra, M.; Migliorini, D.; Dutoit, V.; Walker, P.R.; Preynat-Seauve, O.; Dietrich, P.Y.; Cosset, É. Identification of a miRNA multi-targeting therapeutic strategy in glioblastoma. *Cell Death Dis.,* **2023**, *14*(9), 630.

http://dx.doi.org/10.1038/s41419-023-06117-z PMID: 37749143
[32] Khan, R.B.; Tiwari, S.; Jarkharya, A.; Tiwari, A.; Chowdhary, R.; Shrivastava, A. Glioblastoma multiforme mirna based comprehensive study to validate phytochemicals for effective treatment against deadly tumour through *in silico* evaluation. *MicroRNA,* **2024**, *13*(3), 240-250. http://dx.doi.org/10.2174/0122115366302365240618122812 PMID: 38982916
[33] Makowska, M.; Smolarz, B.; Romanowicz, H. microRNAs (miRNAs) in glioblastoma multiforme (GBM) — Recent literature review. *Int. J. Mol. Sci.,* **2023**, *24*(4), 3521. http://dx.doi.org/10.3390/ijms24043521 PMID: 36834933
[34] Gatto, L.; Franceschi, E.; Di Nunno, V.; Tosoni, A.; Lodi, R.; Brandes, A.A. Liquid biopsy in glioblastoma management: From current research to future perspectives. *Oncologist,* **2021**, *26*(10), 865-878. http://dx.doi.org/10.1002/onco.13858 PMID: 34105205
[35] Rencelj, A.; Gvozdenovic, N.; Cemazar, M. MitomiRs: Their roles in mitochondria and importance in cancer cell metabolism. *Radiol. Oncol.,* **2021**, *55*(4), 379-392. http://dx.doi.org/10.2478/raon-2021-0042 PMID: 34821131
[36] Nikolova, E.; Laleva, L.; Milev, M.; Spiriev, T.; Stoyanov, S.; Ferdinandov, D.; Mitev, V.; Todorova, A. miRNAs and related genetic biomarkers according to the WHO glioma classification: From diagnosis to future therapeutic targets. *Noncoding RNA Res.,* **2024**, *9*(1), 141-152. http://dx.doi.org/10.1016/j.ncrna.2023.10.003 PMID: 38035044
[37] Li, W.J.; Wang, Y.; Liu, R.; Kasinski, A.L.; Shen, H.; Slack, F.J.; Tang, D.G. MicroRNA-34a: Potent tumor suppressor, cancer stem cell inhibitor, and potential anticancer therapeutic. *Front. Cell Dev. Biol.,* **2021**, *9*(March), 640587. http://dx.doi.org/10.3389/fcell.2021.640587 PMID: 33763422
[38] Raucci, A.; Macrì, F.; Castiglione, S.; Badi, I.; Vinci, M.C.; Zuccolo, E. MicroRNA-34a: The bad guy in age-related vascular diseases. *Cell. Mol. Life Sci.,* **2021**, *78*(23), 7355-7378. http://dx.doi.org/10.1007/s00018-021-03979-4 PMID: 34698884
[39] Zhou, Q.; Liu, J.; Quan, J.; Liu, W.; Tan, H.; Li, W. MicroRNAs as potential biomarkers for the diagnosis of glioma: A systematic review and meta-analysis. *Cancer Sci.,* **2018**, *109*(9), 2651-2659. http://dx.doi.org/10.1111/cas.13714 PMID: 29949235
[40] Mafi, A.; Rahmati, A.; Babaei Aghdam, Z.; Salami, R.; Salami, M.; Vakili, O.; Aghadavod, E. Recent insights into the microRNA-dependent modulation of gliomas from pathogenesis to diagnosis and treatment. *Cell. Mol. Biol. Lett.,* **2022**, *27*(1), 65. http://dx.doi.org/10.1186/s11658-022-00354-4 PMID: 35922753
[41] Felekkis, K.; Papaneophytou, C. The circulating biomarkers league: Combining miRNAs with cell-free DNAs and proteins. *Int. J. Mol. Sci.,* **2024**, *25*(6), 3403. http://dx.doi.org/10.3390/ijms25063403 PMID: 38542382

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