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Unraveling the Impact of *miRNA-17* in Glial Tumors and Cerebral Metastases: A Step Towards Enhanced Diagnosis and Prognosis

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Abstract. *Background/Aim: MicroRNAs (miRNAs) have been identified as key regulators in various cancer types, including brain tumors. This study aimed to investigate the differential expression of miRNA-17 in glial tumors, cerebral metastases, and normal glial tissues. Materials and Methods: A total of 42 patients were included in this cross-sectional study. Tissue samples were obtained from patients with glial tumors or cerebral metastases and from normal glial tissues. miRNA-17 expression levels were computed by using real-time polymerase chain reaction. Receiver operating characteristics analysis was used to determine the predictive potential of miRNA-17. Results: In this study, we demonstrated a statistically significant difference in miRNA-17 expression levels between glial tumors and the control group (p=0.001), with higher miRNA-17 expression observed in glial tumors. Similarly, there was statistically higher miRNA-17 expression in metastatic cases compared with the control group (p=0.007). Conclusion: These findings suggest miRNA-17 might be a potential biomarker for differentiating glial tumors and cerebral metastases from normal glial tissue, although further research is necessary to validate these findings and investigate the potential role of miRNA-17 in the pathogenesis of these brain tumors.*

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MicroRNAs (miRNAs) are non-coding RNAs that participate in various biological processes, including cell proliferation, differentiation, and apoptosis. Among these, *miRNA-17* has drawn significant attention in the context of neural cell biology and cancer (1-6).

In the central nervous system (CNS), *miRNA-17* has been found to regulate neural stem cell expansion and their transition to intermediate progenitors, thus influencing neural development (1). Moreover, *miRNA-17* has been implicated in regulating oligodendroglial cell numbers, underscoring its relevance in maintaining CNS homeostasis (7). Interestingly, *miRNA-17* also exhibits neuroprotective effects, as evidenced by its role in protecting neonatal rats from hypoxic-ischemic brain damage (8).

Despite these crucial roles in normal CNS functioning, aberrant expression of *miRNA-17* has been associated with pathological conditions, including glial tumors and cerebral metastases (4, 5). Glial tumors, including gliomas and glioblastomas, are the most common primary brain tumors with high morbidity and mortality rates. Cerebral metastases, on the other hand, represent the most common brain tumors and result

from the spread of cancer from different parts of the body to the brain. In both these conditions, altered miRNA profiles, including dysregulation of *miRNA-17*, have been reported (4, 5).

Yet the precise role and potential of *miRNA-17* in glial tumors and cerebral metastases still need to be understood. Previous studies have presented a complex picture, with *miRNA-17* functioning as a tumor promoter and suppressor, depending on the context (2, 5). Such contradictory roles raise intriguing questions about the underlying mechanisms and potential therapeutic implications of *miRNA-17* in glial tumors and cerebral metastases.

In light of these considerations, our study aimed to compare the expression *miRNA-17* in glial tumors, cerebral metastases and normal glial tissue. Our findings may shed light on the role *miRNA-17* in these conditions and pave the way for future research on potential therapeutic strategies.

Materials and Methods

Study design and participants. This cross-sectional study was conducted with samples collected from patients diagnosed with glial tumors or cerebral metastases and from normal glial tissue. In our study, the samples analyzed comprised 22 cases of metastasis, 20 cases of glial tumors, and 10 samples of normal tissue, providing a diverse range of specimens for a comprehensive analysis. The study received ethical approval from the Institutional Review Board (B10.1.TKH.4.34.H.GP.0.01/367), and all patients provided informed consent.

Tissue collection and storage. The tissue samples from patients diagnosed with glial tumors or cerebral metastases were obtained during neurosurgical procedures. Control samples (normal glial tissue) were obtained from patients with epilepsy undergoing surgery for seizure control, where the tissue had no histopathological abnormalities. The collected samples were immediately snap-frozen in liquid nitrogen and stored at −80˚C until further use.

miRNA extraction and real-time polymerase chain reaction (PCR) analysis. miRNA was extracted from the tissue samples homogenized in QIAzol Lysis Reagent (Qiagen, Hilden, Germany) using steel beads in TissueLyser LT (Qiagen). Subsequently, miRNeasy Tissue/Cells Advanced Kit (Qiagen) was used for miRNA extraction. The concentration and purity of the extracted RNA were determined by using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). The cDNA was synthesized from the miRNA using miScript II RT Kit (Qiagen). The protocol for real-time PCR was performed using miScript SYBR Green PCR Kit (Qiagen) on a StepOnePlus RT-PCR instrument (Applied Biosystems, Foster City, CA, USA). The RNA *U6* was used as an endogenous control to compute relative expression levels of *miRNA-17*.

Data analysis. The relative expression levels of *miRNA-17* in glial tumors, cerebral metastases and normal glial tissue were compared using the $2^{-\Delta\Delta Ct}$ method. The data were then analyzed to identify significant differences in *miRNA-17* expression across the three groups.

Statistical analysis. For the statistical analyses, The Number Cruncher Statistical System 2007 (Kaysville, UT, USA) was used.

Table I. *Information on descriptive features of the patients with tumors (n=42).*

Sex, n (%)	Female	16 (38.1)
	Male	26 (61.9)
Age, years	Min-Max	25-72
	Average±SD	51.62±11.54
Preoperative complaints, n (%)	Headache	26 (61.9)
	Seizure	10 (23.8)
	Dizziness vomiting	8 (19)
	Motor weakness	6 (14.3)
	Loss of consciousness	4 (9.5)
Tumor localization, n (%)	Frontal	9 (21.4)
	Frontoinsular	1 (2.4)
	Frontoparietal	5 (11.9)
	Occipital	4 (9.5)
	Parietal	6 (14.3)
	Parietooccipital	3 (7.1)
	Cerebellum	6 (14.3)
	Temporal	4 (9.5)
	Temporoparietal	4 (9.5)
Side, n (%)	Midline	1 (2.4)
	Right	29 (69)
	Left	12 (28.6)
Preoperative examination findings, n (%)	No symptoms	25 (59.5)
	Motor weakness	13 (31)
	Cerebellar deficit	2 (4.8)
	Visual problems	1 (2.4)
	Speech problems	2 (4.8)
	Other	2 (4.8)

SD: Standard deviation.

The normality of data distribution was tested with the Shapiro-Wilk test and graphical inspections for suitability of quantitative data. Normally distributed values were evaluated with Student's *t*-test and Mann-Whitney *U*-test was used to compare two groups of quantitative variables that did not show normal distribution. Analysis of variance was used to compare more than two groups. Receiver operating characteristic (ROC) curve analysis was conducted with MedCalc software (MedCalc Software Ltd, Ostend, Belgium). Statistical significance was accepted at values of $p<0.05$.

Results

Our study revealed notable findings regarding *miRNA-17* expression in glial tumors and cerebral metastases. The expression levels *miRNA-17* were significantly elevated in both glial tumors and cerebral metastases compared to the control group. This elevation suggests a potential role for *miRNA-17* as a biomarker in these conditions.

Demographic and clinical characteristics. In our study, the patient cohort exhibited a diverse age range and a balanced sex distribution. The demographic details of the patients, along with their surgical information, have been comprehensively summarized in Table I and Table II. Specifically:

Table II. *Surgical information about the patients.*

	Subgroup	Frequency, n (%)
Number of surgeries (n=42)	1	31 (73.8)
	2	8 (19)
	3	3 (7.1)
Glial tumor grade (n=42)	2	3 (7.1)
	3	5 (11.9)
	4	12 (28.6)
Pathology of primary surgery (n=42)	Glial tumor	20 (47.6)
	Metastasis	22 (52.4)
Resection (n=42)	Subtotal	8 (19)
	Total	34 (81)
Additional disease (n=42)	Lung cancer	14 (33.3)
	Malign mesenchymal tm	1 (2.4)
	Breast cancer	5 (11.9)
	Thyroid cancer, breast cancer	1 (2.4)
	None	21 (50)
Pathology of 2nd surgery (n=10)	Anaplastic oligodendroglioma, WHO grade 3	2 (20)
	Glioblastoma multiforme	4 (40)
	Carcinoma metastasis	2 (20)
	Oligodendroglioma, WHO grade 2	1 (10)
	Squamous cell carcinoma metastasis	1 (10)
Pathology of 3rd surgery (n=3)	Anaplastic oligodendroglioma, WHO grade 3	1 (33.3)
	Glioblastoma multiforme, WHO grade 4	2 (66.7)

WHO: World Health Organization.

- Sex distribution: Out of the total cases, 38.1% (n=16) were female. This representation provides insights into the sex-related aspects of the disease under study.
- Age range and average: The ages of the patients varied significantly, ranging from 25 to 72 years. The mean age was calculated to be 51.62 years.

Expression in glial tumors. The *miRNA-17* levels in the glial tumor group were markedly higher than in the control group (p=0.001). This finding aligns with the hypothesis that *miRNA-17* plays a role in tumorigenesis, potentially influencing tumor growth and progression (Figure 1).

Expression in cerebral metastases. The cerebral metastasis group exhibited significantly higher *miRNA-17* levels than controls (p=0.007). This suggests a possible role of *miRNA-17* in the metastatic process, which warrants further investigation (Figure 1).

ROC analysis. The ROC analysis provided a cut-off value for *miRNA-17* expression, distinguishing between glial tumor and control groups with a sensitivity of 76.472% and specificity of 76.92%. For cerebral metastases, the sensitivity was 72.22% and specificity 76.92%. These values indicate a promising diagnostic potential for *miRNA-17* (Figure 2).

No significant difference among glial tumor grades. Interestingly, *miRNA-17* values did not significantly vary across tumor grades (p>0.05). This finding suggests that while *miRNA-17* is elevated in glial tumors, its expression does not correlate with tumor grade.

Clinical correlations. The clinical implications of these findings are profound. Elevated *miRNA-17* levels in patients might be an early indicator of glial tumor or cerebral metastases, aiding in timely diagnosis and treatment planning.

Discussion

This study presents the differential expression patterns of *miRNA-17* in glial tumors, cerebral metastases, and normal glial tissues, suggesting a potential role of *miRNA-17* in the development and progression of these tumors. These findings are consistent with prior studies that have pointed to the role of *miRNA-17* in neural stem cell expansion, development of the mouse neocortex (1), and regulation of oligodendroglial cell number (9). It has been established that *miRNA-17* can silence P21, a key player in cell-cycle regulation, to maintain the neural progenitor pool in the developing cerebral cortex (10).

Many studies have documented the active role of various miRNAs in metastatic processes. For instance, the work by Zhao *et al*. focused on the effects of celastrol, an anti-inflammatory drug, on oxygen-induced retinopathy and demonstrated that celastrol inhibits pathological neovascularization by targeting the *miRNA-17*-5p/hypoxia-inducible factor 1-alpha/vascular endothelial growth factor pathway (11). Likewise, the research conducted by Zhang *et al*. established that the long non-coding RNA *MIR17HG* promotes neuronal damage and microglial activation in Parkinson's disease by targeting the miRNA-153-3p/alpha-synuclein axis (12). These findings underscore the significant roles of various miRNAs in regulating cell proliferation, apoptosis, and angiogenesis, which are critical aspects of the metastatic process.

Similarly, several miRNAs have been implicated in glial tumorigenesis. For instance, the study by Li *et al*. showed that astrocyte elevated gene-1 (*AEG1*) serves as a target of *miR542* to promote glioblastoma proliferation and invasion (13). In another study by Yuan *et al*., a 4-miRNA signature, including *miR-17*, was found to predict survival in glioblastoma multiforme, highlighting the potential role of miRNAs as biomarkers in patients with glial tumors (5). Moreover, studies such as those conducted by Billur *et al*. (6) and Ozdogan *et al*. (6, 7) have shed light on the potential of miRNAs, such as *miR-582-5p* and *miR-221*, respectively, as potential non-invasive

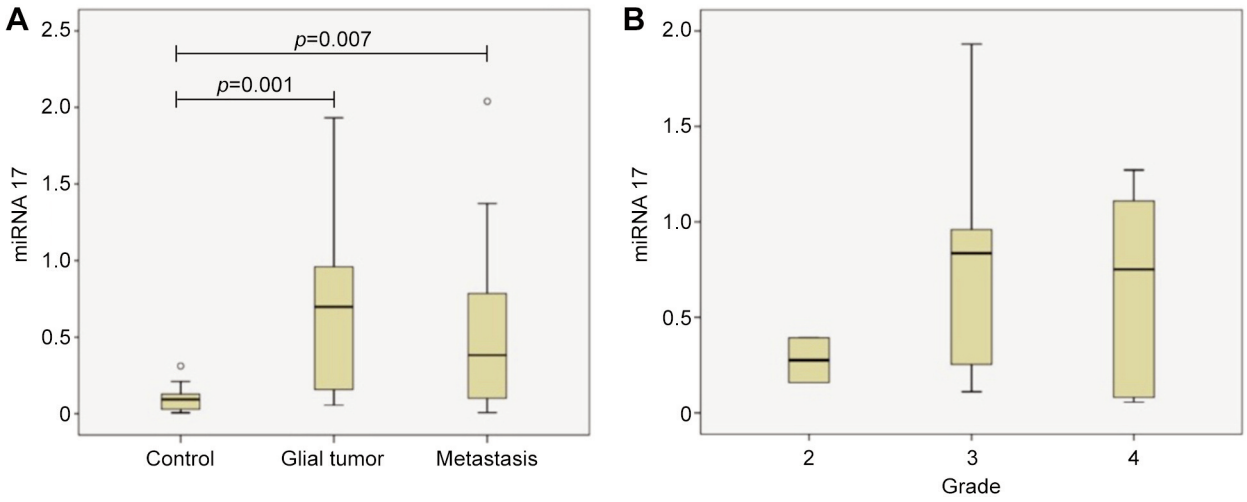

Figure 1. *miRNA-17 expression in normal tissue (control) glial tumor and cerebral metastases (A) and according to grade of glial tumor (B). p-Values are from Mann-Whitney U-test. The boxplots show the median (line), interquartile range (box) and minimum-maximum (whiskers) values.*

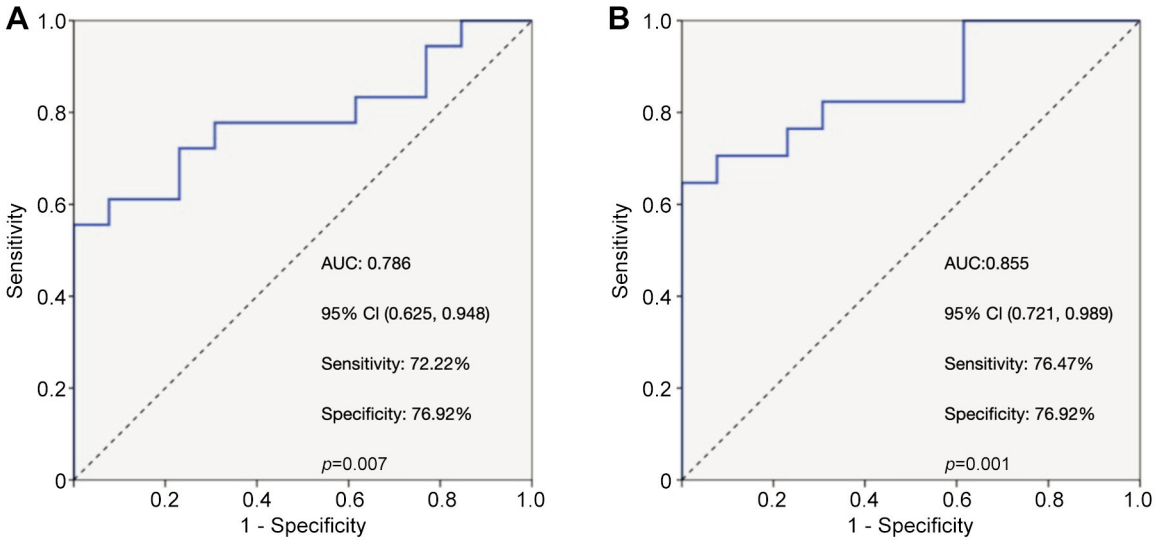

Figure 2. *Receiver operating characteristics curve for miRNA-17 expression in cerebral metastases (A) and glial tumor (B) vs. normal tissue (control).*

biomarkers for glioblastoma multiforme. These findings emphasize the relevance of miRNAs in the pathogenesis, diagnosis, and prognosis of glial tumors.

Notably, *miRNA-17*-5p was found to play a crucial role in protecting neonatal rats from hypoxic-ischemic brain damage, further signifying its neuroprotective function (8). In contrast, other studies have reported its role in promoting cell growth and chemoresistance, implying its oncogenic nature (2).

Our results showing higher expression of *miRNA-17* in glial tumors and cerebral metastases are consistent with studies suggesting that *miRNA-17*-5p may contribute to glial scar formation after spinal cord injuries (14), astrocyte proliferation after spinal cord injury (15), and increased survival of astrocytes under hypoxic conditions (3). In glioblastoma multiforme, a complex interplay between *miRNA-17-5p* and other miRNAs has been demonstrated, leading to survival prediction (5).

The elevated expression of *miRNA-17* in cerebral metastases is supported by evidence from studies indicating its involvement in axon-myelin remodeling and functional recovery after stroke (4, 16). Other studies demonstrated that *miRNA-17-5p* facilitated neuronal differentiation of transplanted neural stem/precursor cells under neuroinflammatory conditions (17).

The limitations of our study are primarily related to the sample size and the sole focus on the expression of *miRNA-17*. Further studies should explore the mechanistic pathways

of how *miRNA-17* contributes to the development and progression of glial tumors and cerebral metastases while considering the influence of other miRNAs (6, 7, 18-20).

In conclusion, the significantly higher expression of *miRNA-17* in glial tumors and cerebral metastases, compared to normal glial tissue, highlights its potential role as a biomarker for these conditions. Our findings underline the need for further research to investigate the precise role and therapeutic potential of *miRNA-17* in glial tumors and cerebral metastases.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

Okan Türk, Nail Demirel: Organized and coordinated the research. Cumhur Kaan Yaltirik: Led the article writing. Seda Güleç Yılmaz, Fatma Tuba Akdeniz: Carried out the laboratory work. Mustafa Kaya: Contributed to the design and implementation of the research methodology. Ömer Faruk Şahin: Assisted in data collection and preliminary data analysis. Turgay İsbir: Supervised the study and contributed to data analysis.

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