Thioredoxin System and miR-21, miR-23a/b and let-7a as Potential Biomarkers for Brain Tumor Progression: Preliminary Case Data

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■ BACKGROUND: **The thioredoxin system and microRNAs (miRNAs) are potential targets for both cancer progression and treatment. However, the role of miRNAs and their relation with the expression profile of thioredoxin system in brain tumor progression remains unclear.**

■ METHODS: **In this study, we aimed to determine the expression profiles of redox components Trx-1, TrxR-1 and PRDX-1, and oncogenic miR-21, miR-23a/b and let-7a and oncosuppressor miR-125 in different brain tumor tissues and their association with increasing tumor grade. We studied Trx-1, TrxR-1, and PRDX-1 messenger RNA expression levels by quantitative real-time polymerase chain reaction and protein levels by Western blot and miR-23a, miR-23b, miR-125a, miR-21, and let-7a miRNA expression levels by quantitative real-time polymerase chain reaction in 16 glioma, 15 meningioma, 5 metastatic, and 2 benign tumor samples. We also examined Trx-1, TrxR-1, and PRDX-1 protein levels in serum samples of 36 patients with brain tumor and 37 healthy volunteers by enzyme-linked immunosorbent assay.**

■ RESULTS: **We found that Trx-1, TrxR-1, and PRDX-1 presented high messenger RNA expression but low protein expression in low-grade brain tumor tissues, whereas they showed higher protein expression in sera of patients with low-grade brain tumors. miR-23b, miR-21, miR-23a, and let-7a were highly expressed in low-grade brain tumor tissues and positively correlated with the increase in thioredoxin system activity.**

■ CONCLUSIONS: **Our findings showed that Trx-1, TrxR-1, miR-21, miR-23a/b, and let-7a might be used for brain tumor diagnosis in the clinic. Further prospective studies including molecular pathway analyses are required to validate the miRNA/Trx system regulatory axis in brain tumor progression.**

INTRODUCTION

Brain tumors are diagnosed both in adults and in children and lead to high mortality and morbidity worldwide.[1-3] In 2020, 1.6% of new cases and 2.5% deaths of brain tumors globally have been reported.[4] Malignant glioma and meningiomas are the most common types among all brain tumors.[2,5] Recent radiologic methods such as magnetic resonance imaging (MRI), total-body computed tomography scans and various molecular diagnostic markers are used in the clinic for patients with brain tumor.[6,7] However, whole-body scans cannot detect some metastases, especially isolated central nervous system lymphomas.[8,9] Mass lesions with diffuse vasogenic edema showing heterogeneous contrast are accepted as high-grade glial mass,

Key words
- Biomarker
- Brain tumor progression
- MicroRNA (miRNA)
- Thioredoxin system

Abbreviations and Acronyms
AKT: Protein kinase B
ELISA: Enzyme-linked immunosorbent assay
miRNA: microRNA
MRI: Magnetic resonance imaging
mRNA: Messenger RNA
PI3K: Phosphatidylinositol 3-kinase
PRDX: Peroxiredoxin
qRT-PCR: Quantitative real-time polymerase chain reaction
Trx: Thioredoxin
TrxR-1: Thioredoxin reductase 1
WHO: World Health Organization

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metastatic mass, or abscess and cannot be differentiated by MRI.[10,11] Because MRI techniques are nonspecific, they provide limited information about the tumor physiology.[12] Pathologic assessment is the gold standard and essential for cellular diagnosis and grading.[13] Although radiologic methods present accurate tools to facilitate diagnosis of primary and secondary tumors, molecular variances of primary and metastatic tumors, as potential determinants of prevention, prediction, etiopathogenesis, diagnosis, and prognosis of the disease, are challenging and remain unknown.[14] Therefore, novel biomolecular markers and their related pathways providing various new strategies on prediction, diagnosis, prognosis, and molecular subtyping of tumors are open to investigation.

The cytoplasmic thioredoxin (Trx) antioxidant system, which comprises Trx-1, Trx reductase-1 (TrxR-1), and NADPH,[15-18] is highly expressed in human cancers,[15,19-22] including of the brain.[23-27] Peroxiredoxin (PRDX) inhibits apoptosis of human glioma[28] by interacting with the Trx system,[29-31] induces apoptosis, and reduces glioma cell proliferation when knocked down.[32]

Several microRNAs (miRNAs) regulate proliferation, migration, and invasion of different types of tumor cells through Trx and/or PRDX families.[33-42] Tumor suppressor miR-17 levels have been correlated with TrxR-2 downregulation in T98G glioblastoma multiforme cells in vitro.[43] Antioncogenic miR-383 was downregulated in medulloblastoma cells by inhibiting PRDX-3 protein expression.[44] In addition, redox status including ROS accumulation regulates production or inhibition of miR-21, miR-23b, and miR-125a[41,45] through Nrf2/ARE[46] and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)[47] pathways in various tumor cells and miRNAs are known to target transcripts of ROS-related enzymes such as NADPH oxidases and superoxide dismutase.[41] Still, the role of miRNAs and their relation between the expression profile of Trx components in brain tumor progression remains unclear. Thus, in this study, we hypothesized that higher levels of redox components including Trx-1, TrxR-1, and PRDX-1 might be related to the brain tumor grade and might have a relation with oncogenic miR-21, miR-23a/b, and let-7a and tumor suppressor miR-125a levels, which both have a codependent relationship among Nrf2/ARE and PI3K/Akt pathways. As a preliminary study, we aimed to determine the expression profiles of Trx-1, TrxR-1, and PRDX-1 by quantitative real-time polymerase chain reaction (qRT-PCR) and protein levels by Western blot and miR-23a, miR-23b, miR-125a, miR-21, and let-7a miRNA expression levels by qRT-PCR in 16 glioma, 15 meningioma, 5 metastatic, and 2 benign tumor samples. We also examined Trx-1, TrxR-1, and PRDX-1 protein levels in serum samples of 36 patients with brain tumor and 37 healthy volunteers by enzyme-linked immunosorbent assay (ELISA) and their association with increasing tumor grade.

METHODS

Ethics Statement and Sample Collection

This research was carried out with Medicana International Ankara Hospital Research ethics committee approval (21102019/04). Brain tumor samples were surgically resected from 38 patients who underwent surgery at Medicana International Ankara Hospital from January 2020 to October 2020. All patients obtained written informed consents. The brain tumors were classified according to 2021 World Health Organization (WHO) criteria.[48-50] Grade 1 or 2 gliomas referred to lower grade, whereas higher-grade gliomas referred to grade 3 or 4.[49,51,52] Sixteen of these tumors were low-grade and high-grade glioma (WHO grades 1 and 2 and grades 3 and 4, respectively), 15 were low-grade and high-grade meningioma (WHO grade 1 [benign meningioma] and 2 [atypical meningioma]; grade 3 [anaplastic meningioma], respectively), 5 were metastatic tumors, and 2 were other benign tumors. Clinicopathologic features of tumor samples are listed in **Table 1**. Serum samples of 36 patients with brain tumor and of 37 healthy volunteers were also obtained. Thirteen of these volunteers were aged 35 years and younger, 20 were aged between 36 and 56 years, and 4 were aged 57 years and older. The minimum required number of samples and replicates was shown by power analysis using G*Power program version 3.1 (Erdfelder, Faul, & Buchner, 1996, Kiel, Germany).

A randomized observational study including control and experimental groups was carried out. All control and experimental groups were independent variables whereas messenger RNA (mRNA), miRNA, and protein expression results were defined as dependent variables.

qRT-PCR

Trx-1, TrxR-1, and PRDX-1 mRNA expression levels were determined in human brain tumor samples by qRT-PCR.[15,24,28] Samples of 100 mg of brain tissues were homogenized with 1 mL TRIzol (RiboEx, 301-001 [GeneAll, Seoul, South Korea]) by handheld homogenizer (MT-30K [Miulab, Hangzhou, China]). Total RNA was isolated by mRNA isolation kit (305-101 [GeneAll, Seoul, South Korea]) and concentrations and purities of RNA samples were measured via NanoDrop spectrophotometer (NanoDrop 1000 [ThermoScientific, Massachusetts, USA]) at 260–280 nm wavelength. Complementary DNA was synthesized (W2211 [Wizbio Solutions, Seongnam, South Korea]) and qRT-PCR was performed on a Biorad instrument (CFX Connect [Biorad, California, USA]). Relative mRNA expression was determined by WizPure qPCR SYBR Green Master Mix (W1711 [Wizbio Solutions, Seongnam, South Korea]) fluorescent dye.

miR-23a/b, miR-125a, miR-21, and let-7a miRNA expression levels were determined by homogenizing 50 mg of brain tissues with 500 μL TRIzol via handheld homogenizer. Total miRNA was isolated by miRNA isolation kit (325-150 [GeneAll, Seoul, South Korea]) based on glass-fiber membrane technology providing high purification. Complementary DNA synthesis was accomplished using stem-loop transcriptase primers and relative miRNA expression was assessed as performed for relative mRNA expression. All mRNA and miRNA levels were normalized to housekeeping gene GAPDH[53] ($n = 30$ in total). The 8 brain tissue samples that belonged to the patients with low-grade (4 excluded) and high-grade (2 excluded) glioma and low-grade meningioma (2 excluded) were excluded from the evaluation by qRT-PCR because of the lower RNA yields than expected. Relative fold-change was analyzed according to $2^{-\Delta\Delta Ct}$. Primer sequences

Table 1. Clinicopathologic Features of Brain Tumors of Patients

Patient Characteristic	Patients (N = 38) (%)
Age	53.78 ± 14.99
≤35 years	4 (10.53)
36–56 years	15 (39.47)
≥57 years	19 (50.00)
Gender	
Male	21 (55.26)
Female	17 (44.74)
Type of brain tumor with clinical stage	
Glioma (stage I–IV)	16 (42.11)
Astrocytoma	2 (*IDH-mutant*) (5.26)
Oligodendroglioma	4 (*IDH-mutant, 1p/19q-codeletion*) (10.53)
Glioblastoma	10 (*IDH-wildtype*) (26.32)
Meningioma (stage I–III)	15 (39.47)
Metastasis	5 (13.16)
Other benign primary tumors	2 (5.26)

Values are n (%) except where indicated otherwise.

are summarized in Table 2. A cutoff of Trx-1, TrxR-1, and PRDX-1 >1 was applied to extract low-quality data.

Western Blot

Thirty-milligram brain tumor tissues (n = 31) were lysed with RIPA buffer (R0278-50ML [Sigma-Aldrich, Darmstadt, Germany]) containing protease inhibitor (ProBlock Gold Mammalian Protease Inhibitor Cocktail [100x], GB-331-1 [Gold Biotechnology, Missouri, USA]) by handheld homogenizer and total protein content was calculated by BCA test (Pierce BCA Protein Assay Kit, 23225 [ThermoScientific, Massachusetts, USA]) at 562 nm wavelength. Extracted proteins were separated by 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, Mini-PROTEAN Electrophoresis Cell, 1658004 [Biorad, California, USA]), subsequently transferred onto poly(-vinylidene fluoride) membrane via transfer blot system (Trans-Blot Turbo Transfer System, 1704150 [Biorad, California, USA]). After 2 hours of preblocking step (5% nonfat milk), membranes were kept overnight with rabbit anti-human Trx-1 (1:500, bs-50523R [Bioss Antibodies, Massachusetts, USA]), TrxR-1 (1:500, bs-8299R [Bioss Antibodies, Massachusetts, USA]), PRDX-1 (1:500, bs-3875R [Bioss Antibodies, Massachusetts, USA]) and GAPDH (house-keeping gene, 1:500, bs-2188R [Bioss Antibodies, Massachusetts, USA]) primary antibodies, then washed in TBS-t and kept with horseradish peroxidase-conjugated IgG as secondary antibody (BT-AS00010 [Bioassay Technology Laboratory, Shanghai, China]) for 1 hour at room temperature. Enhanced chemiluminescence was applied for visualization of bands via a chemiluminescence imaging system (ChemiDoc Imaging System, 12003153 [Biorad, California, USA]). Bands were analyzed by Image Lab version 6.0 (Biorad, California, USA). The 7 brain tissue samples that belonged to the patients with low-grade (3 excluded) and high-grade (2 excluded) glioma and low-grade meningioma (2 excluded) were excluded from the evaluation by Western blot because of the lower total protein yields.

ELISA

Human Trx-1 (E1452Hu), TrxR-1 (E3953Hu), and PRDX-1 (E2924Hu) (all from Bioassay Technology Laboratory, Shanghai, China) protein concentrations in sera of patients with brain tumor and healthy volunteers were evaluated by ELISA following the manufacturer's guidelines. Standard solutions and samples were added to 96-well plate and biotinylated primary antibodies and streptavidin-HRP were added to wells respectively. Plates were then incubated for 60 minutes at 37°C. Subsequently, plates were washed with wash buffer; incubated with substrate solutions for 10 minutes at 37°C in the dark, as recommended in guidelines. After adding stop solution to wells, a color change from blue to yellow was determined and the optical density of each well was measured by plate reader (SPECTROstar Omega [BMG LABTECH, Ortenberg, Germany]) at 450 nm. Each serum sample was studied in triplicate and protein levels in serum samples of patients with brain tumor (n = 36) were compared with those of healthy volunteers (n = 37). The 2 missing serum samples belonged to the patients with high-grade glial tumor were excluded from the evaluation by ELISA because of the insufficient volume.

Table 2. Primer Sequences Designed for Quantitative Real-Time Polymerase Chain Reaction

Gene	Oligonucleotide Sequence
GAPDH	
Forward	5'-GGTGTGAACCATGAGAAGTATGA-3'
GAPDH	
Reverse	5'-GAGTCCTTCCACGATACCAAG-3'
Trx-1 (TXN)	
Forward	5′-CAACCCTTTCTTTCATTCCCTCT-3′
Trx-1 (TXN)	
Reverse	5′-CACCCACCTTTTGTCCCTTCT-3′
TrxR-1 (TXNRD1)	
Forward	5'-GTTGCCAAGACTGCAAACCAC-3'
TrxR-1 (TXNRD1)	
Reverse	5'-CCCTGCCAAATGTCAGCTTC-3'
PRDX1	
Forward	5'-GCACCATTGCTCAGGATTATG-3'
PRDX1	
Reverse	5'-GCCAACAGGGAGGTCATTTAC-3'
miR-23a	
ST-primer	5'-GAAAGAAGGCGAG…TAGG-3'
miR-23a	
Forward	5'-ATCACATTGCCAGGGATTTCC-3'
miR-23b	
ST-primer	5'-GAAAGAAGGCGAG…ATTA-3'
miR-23b	
Forward	5'-ATCACATTGCCAGGGATTACCAC-3'
miR-125a	
ST-primer	5'-GAAAGAAGGCGAG…TCCA-3'
miR-125a	
Forward	5'-TCCCTGAGACCCTTTAACCTGTGA-3'
miR-21	
ST-primer	5'-GAAAGAAGGCGAG…GTAG-3'
miR-21	
Forward	5'-TAGCTTATCAGACTGATGTTGA-3'
let-7a	
ST-primer	5'-GAAAGAAGGCGAG…TATG-3'
let-7a	
Forward	5'-TCCCTGAGACCCTTTAACCTGTGA-3'
Universal Primer	
Reverse	5'-CGAGGAAGAAGACGGAAGAAT-3'

Statistical Analysis

Data used for qRT-PCR and Western blot data showed normal distribution, whereas ELISA data presented nonnormal distribution by a Shapiro-Wilk test. Pairwise comparison of qRT-PCR results was subjected to a Student t test. Western blot results were analyzed with 1-way analysis of variance and Tukey HSD (honestly significant difference) tests. A 2-sample Kolmogorov-Smirnov test was used for comparison of nonparametric results in ELISA. A Pearson correlation test was conducted for qRT-PCR. Whole data were analyzed within 95% confidence intervals.

RESULTS

Trx System Components Showed Higher mRNA Expression but Low Protein Expression in Low-Grade Brain Tumor Tissues

TrxR-1 mRNA expression was significantly higher in all brain tumor tissues compared with control group (Figure 1A) by qRT-PCR. Trx-1 and TrxR-1 mRNA expressions were higher in low-grade meningioma tissues than in high-grade meningioma (Figure 1B) but vice versa for protein expressions by Western blot (Figure 1C). Similarly, high-grade glioma tissues had higher Trx-1 and TrxR-1 protein expressions than did low-grade glioma by Western blot (Figure 1C). There was no significant difference in Trx-1, TrxR-1, and PRDX-1 mRNA expression levels between high-grade and low-grade glioma tissues (Figure 1B). Trx-1, TrxR-1, and PRDX-1 mRNA expression levels were lower in other benign primary tumors compared with metastatic tumors (Figure 1B). All low-grade and high-grade gliomas and meningiomas showed lower Trx-1 and PRDX-1 mRNA expression levels compared with metastatic tumors (Figure 1B). However, metastatic tumors had lower Trx-1 and TrxR-1 protein expression compared with high-grade gliomas and meningiomas by Western blot.

Trx System Components Showed Higher Protein Expression in Serum Samples of Patients with Low-Grade Brain Tumors

Sera of patients with brain tumor had significantly lower Trx-1 (Figure 1D) and TrxR-1 (Figure 1E) protein expressions compared with serum samples of healthy volunteers by ELISA. No significant difference was observed in PRDX-1 protein expression between the 2 groups (Figure 1F). Serum samples of patients with high-grade glioma had significantly lower Trx-1 (Figure 1G), TrxR-1 (Figure 1H), and PRDX-1 (Figure 1I) protein expressions than did those of patients with low-grade glioma. Serum samples of patients with high-grade meningioma and other benign primary tumors had significantly lower Trx-1 (Figure 1G) and TrxR-1 (Figure 1H) protein expressions compared with patients with low-grade meningioma and metastatic tumors, respectively. Sera of healthy volunteers had significantly higher Trx-1 and TrxR-1 protein expressions than all groups by ELISA (Figure 1G and H). PRDX-1 protein expression level was significantly higher in healthy volunteers compared with sera of patients with high-grade meningioma, metastatic tumor, or other benign primary tumors (Figure 1I).

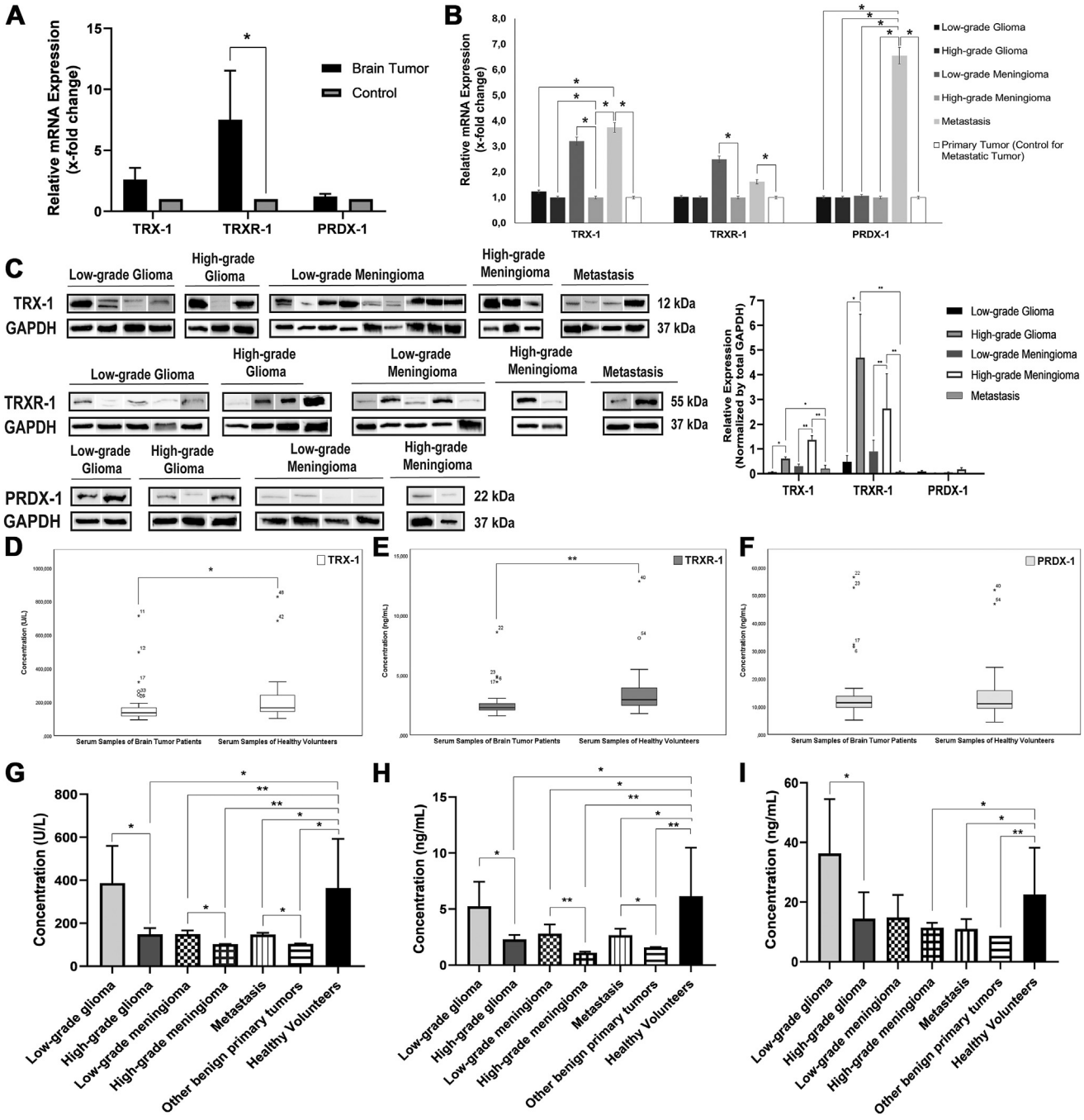

Figure 1. Messenger RNA (mRNA) and protein expressions of brain tumor samples of patients. (**A**) Relative mRNA fold-change of Trx-1, TrxR-1, and PRDX-1 of brain tumor samples of patients and control group by quantitative real-time polymerase chain reaction ($n = 30$, $*P < 0.05$ by Student t test); (**B**) relative mRNA fold-change of Trx-1, TrxR-1, and PRDX-1 of low-grade and high-grade glioma, low-grade and high-grade meningioma and metastatic and primary tumor samples by quantitative real-time polymerase chain reaction ($*P < 0.05$ by Student t test); (**C**) relative protein expressions of Trx-1, TrxR-1, and PRDX-1 for low-grade and high-grade glioma, low-grade and high-grade meningioma and metastatic tumor samples ($*P < 0.05$; $**P < 0.001$ by 1-way analysis of variance and post hoc Tukey HSD tests); and serum (**D**) Trx-1, (**E**) TrxR-1, and (**F**) PRDX-1 protein concentrations for patients with brain tumor ($n = 36$) and healthy volunteers ($n = 37$) by enzyme-linked immunosorbent assay test ($*P < 0.05$ and $**P < 0.001$ by 2-sample Kolmogorov-Smirnov test; and bar graphs indicating serum (**G**) Trx-1 (U/L), (**H**) TrxR-1 (ng/mL), and (**I**) PRDX-1 (ng/mL) protein concentrations for low-grade and high-grade glioma, low-grade and high-grade meningioma and metastatic and primary tumor samples by enzyme-linked immunosorbent assay test ($*P < 0.05$ and $**P < 0.001$ by 2-sample Kolmogorov-Smirnov test).

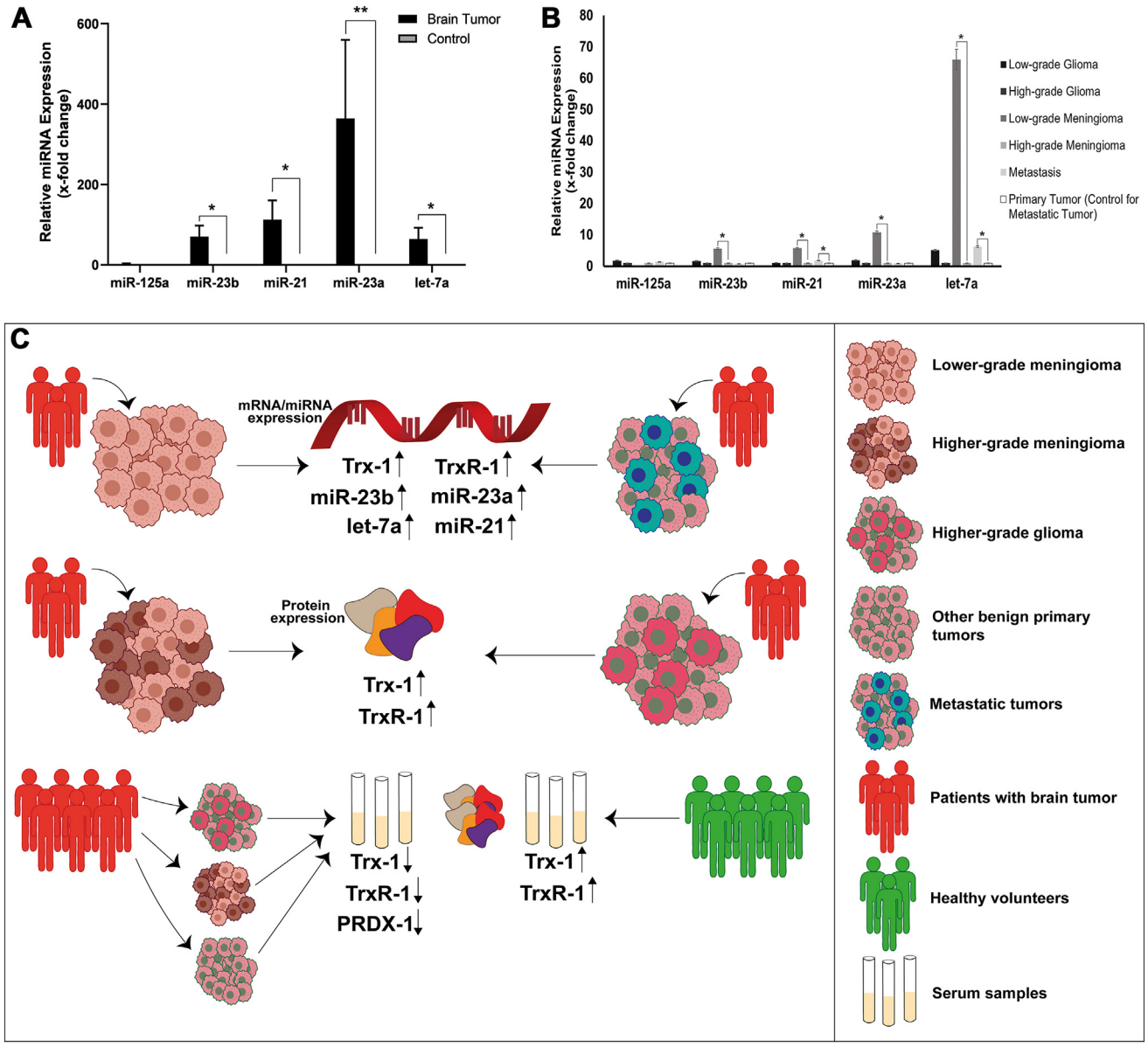

Figure 2. MicroRNA (miRNA) expressions of brain tumor samples of patients and correlation analysis between miRNAs and thioredoxin system. (**A**) Relative miRNA fold-change of miR-125a, miR-23a/b, miR-21, and let-7a for brain tumor samples of patients and control group by quantitative real-time polymerase chain reaction ($n = 30$, $^{*}P < 0.05$ and $^{**}P < 0.001$ by Student t test); (**B**) relative miRNA fold-change of miR-125a, miR-23a/b, miR-21, and let-7a of low-grade and high-grade glioma, low-grade and high-grade meningioma and metastatic and primary tumor samples by quantitative real-time polymerase chain reaction ($^{*}P < 0.05$ by Student t test) (**C**) A summary of the messenger RNA (mRNA) and protein expressions of Trx system components (Trx-1, TrxR-1, and PRDX-1) and miRNAs (miR-23a, miR-23b, miR-21, and let-7a) in tissues and/or serum samples of patients with brain tumors and healthy volunteers.

Table 3. Pearson Correlation Analysis for Trx-1, TrxR-1, and PRDX-1 and miRNAs Involving miR-125a, miR-23a/b, miR-21, and let-7a, 2-Tailed Significant Values

	Trx-1	TrxR-1	PRDX-1	miR-125	miR-23b	miR-21	miR-23a	Let-7a
Trx-1								
Pearson correlation	1.000	0.960	0.959	0.753	0.945	0.968	0.966	0.967
Significance (2-tailed)	—	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
TrxR-1								
Pearson correlation	0.960	1.000	0.889	0.601	0.873	0.907	0.879	0.885
Significance (2-tailed)	<0.001	—	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
PRDX-1								
Pearson correlation	0.959	0.889	1.000	0.827	0.954	0.956	0.922	0.958
Significance (2-tailed)	<0.001	<0.001	—	<0.001	<0.001	<0.001	<0.001	<0.001
miR-125								
Pearson correlation	0.753	0.601	0.827	1.000	0.908	0.870	0.825	0.885
Significance (2-tailed)	<0.001	0.001	<0.001	—	<0.001	<0.001	<0.001	<0.001
miR-23b								
Pearson correlation	0.945	0.873	0.954	0.908	1.000	0.995	0.938	0.981
Significance (2-tailed)	<0.001	<0.001	<0.001	<0.001	—	<0.001	<0.001	<0.001
miR-21								
Pearson correlation	0.968	0.907	0.956	0.870	0.995	1.000	0.952	0.982
Significance (2-tailed)	<0.001	<0.001	<0.001	<0.001	<0.001	—	<0.001	<0.001
miR-23a								
Pearson correlation	0.966	0.879	0.922	0.825	0.938	0.952	1.000	0.980
Significance (2-tailed)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	—	<0.001
Let-7a								
Pearson correlation	0.967	0.885	0.958	0.885	0.981	0.982	0.980	1.000
Significance (2-tailed)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	—

miR-23a/b, miR-21, and let-7a Are Highly Expressed in Low-Grade Brain Tumor Tissues and Positively Correlated with the Increase in Trx System Activity

miR-23a/b, miR-21, and let-7a expression levels were greater in all brain tumor tissues compared with the control group (**Figure 2A**) by qRT-PCR. No significant difference was noticed between the groups for miR-125a expression by qRT-PCR. Low-grade meningioma tissues had significantly higher miR-23b, miR-21, miR-23a, and let-7a miRNA expression levels than did high-grade meningioma (**Figure 2B**). miR-21 and let-7a expressions were lower in other primary benign tumors compared with metastatic tumors (**Figure 2B**). There was no significant alteration in miR-125a miRNA expression levels among all groups by qRT-PCR.

Trx-1, TrxR-1, and PRDX-1 mRNA expression levels are positively correlated with miR-125, miR-23b, miR-21, miR-23a, and let-7a miRNA expressions in brain tumor tissues (**Table 3**) by Pearson correlation analysis.

DISCUSSION

In this study, we showed that Trx components had higher expression in low-grade brain tumor samples and had a strong positive correlation with oncogenic miR-21, miR-23a/b, and let-7a and tumor suppressor miR-125a levels. We examined high mRNA and low protein expressions of Trx-1 and TrxR-1 in low-grade meningioma and benign primary tumor tissues compared with high-grade meningioma and metastatic tumor tissues, respectively, by qRT-PCR and Western blot and high Trx-1 and TrxR-1 protein expressions in high-grade glioma compared with low-grade glioma. Our findings regarding the increase in Trx-1[15,25,27,54] and TrxR-1[23,25,27,55] protein expression with the increasing tumor grade are consistent with previous studies showing poor clinical outcome for patients with brain tumor. High mRNA and low protein concentration in low-grade meningioma might occur as a result of the shorter half-life of the protein or an insufficient translation with a low level of mRNA.[56] The discrepancy between gene and protein expression might cause unreliable data, which needs to be further evaluated in

a large sample size of patients.[27,56] Trx-1 and TrxR-1 protein expressions were significantly higher in serum samples of patients with low-grade glioma and meningioma compared with high-grade glioma and meningioma, respectively, by ELISA. High serum levels of Trx-1[57,58] and TrxR-1[59,60] were reported in patients with early cancer by ELISA. Thus, our findings regarding Trx-1 and TrxR-1 in human sera might be important for future studies investigating their biomarker potential. In our study, metastatic tumor samples had significantly higher PRDX-1 mRNA expression compared with benign primary tumors; however, serum samples of all patients with brain tumor had lower PRDX-1 expression than did that of healthy volunteers. PRDX-1 was upregulated in esophageal[61] and pancreatic[62] cancer cells and glioma[63] cells to regulate cell growth and apoptosis through the ROS-dependent pathway.[64] Thus, our results regarding qRT-PCR were coherent with the literature.[28,64] PRDX-1 may induce apoptosis of tumor cells.[64-66] A lower level of PRDX-1 protein in sera of patients with brain tumor might show that decrease in PRDX-1 promotes the proliferation and invasion of tumor cells.[65,66] Our finding on PRDX-1 showed that it may act as either oncogenic or tumor suppressor protein.

We report that miR-23a/b, miR-21, and let-7a expression levels were greater in low-grade meningioma compared with high-grade meningioma. Findings regarding miR-21,[67-73] miR-23a,[70,74-77] miR-23b,[74,75] and let-7a[69,78,79] show that they have oncogenic capacity in brain tumor progression.[80-82] Moreover, miRNAs have tissue-specific expression patterns in cancerous cells.[83,84] Meningiomas can largely be diagnosed by MRI. However, preoperative evaluation of grade of meningiomas is not possible.[85,86] If distinguishing between low-grade and high-grade meningiomas might be achieved with easier assays such as the evaluation of miRNA expressions in the preoperative assessments and postoperative follow-ups of the patient to detect recurrence, expressions of miRNAs might be evaluated as an easier and cost-effective method compared with routine MRI.[87,88] Using miRNAs as biomarkers might facilitate diagnosis, prediction, and prognosis of meningiomas and also contribute to understanding their relation with the pathogenesis of meningioma.

We also found that miR-21 and let-7a expressions were greater in metastatic tumors compared with other primary benign tumor tissues showing a positive correlation between miRNA expressions and tumor grade. Trx-1, TrxR-1, and PRDX-1 mRNA expression levels were positively correlated with miR-21, miR-23a/b, miR-125, and let-7a expressions in brain tumor tissues by Pearson correlation analysis. Kalinina et al.[89] reported high correlation between the antioxidant protection including Trxs and PRDXs and miRNAs, which also supports our results. PRDX-3 was linked with miR-23b for human prostate cancer progression.[37] Similarly, miR-23a and miR-23b regulate TrxR-1 expression during skeletal muscle differentiation.[90] Our key findings may implicate the diagnostic value of miR-21, miR-23a/b, let-7a, and Trx system components for brain tumors, which also improves the understanding in determining the levels of biomarkers in different brain tumor grades.

The current study has some limitations. A correlation analysis was performed for the relation between Trx components and various miRNAs as oncogenes in brain tumor tissues and serum samples of patients; however, in vitro and in vivo functional studies including the association between miRNAs and redox system through Nrf2/ARE[46] and PI3K/Akt[47] pathways must be performed for the miRNA/Trx system regulatory axis in brain tumor progression, which generates a crucial limitation for this study. However, this limitation does not hinder further studies because the expression and correlation profiles in the Trx system and miRNAs would facilitate in-depth studies for better understanding of miRNA and redox system component functions as novel biomarkers. Because this research was conducted with human participants, brain tissue samples could not be obtained from healthy volunteers. To cope with this limitation, we compared levels of Trx system components with metastatic and/or other benign tumors for qRT-PCR and Western blot and serum samples of healthy volunteers for ELISA. We did not evaluate the diagnostic values of those comparisons by receiver operating characteristic curve, because we could not obtain larger sample sizes.[91] The current study consists of preliminary case data, and still gives a perspective of Trx mRNA and protein expression and miRNA expression profiles in different grades of brain tumor. In the context of the study, we evaluated the levels of miRNAs in brain tissue samples. MiRNAs are known to be stable in body fluids with a higher specificity in the detection by qRT-PCR.[92] For further evaluation, profiling of miRNAs should be analyzed in body fluids of patients with brain tumor.[93-99] Nevertheless, our findings for brain tumor contribute to the literature.

Because radiologic methods have challenges in detecting molecular variances of primary and metastatic tumors[14] or pathologic grading, distinguishing low-grade brain tumors from high-grade brain tumors based on those potential bloodstream biomarkers as further supplementary tools might be useful in diagnosing progression or transformation of glial or other brain tumors. Our preliminary findings show that Trx components including Trx-1/TrxR-1 and miR-21, miR-23a/b, and let-7a could be potential biomarkers together for brain tumor diagnosis and prognosis especially for patients with meningioma. Further prospective studies are needed to validate their relation and its projection to brain tumor treatment in the clinic.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Nedret Kılıç: Conceptualization, Methodology, Formal analysis, Investigation, Writing — original draft, Writing — review & editing. **Ozge Boyacioglu:** Conceptualization, Methodology, Formal analysis, Investigation, Writing — original draft, Writing — review & editing. **Gamze Turna Saltoglu:** Conceptualization, Methodology, Formal analysis, Investigation. **Erkut Baha Bulduk:** Formal analysis, Investigation. **Gokhan Kurt:** Formal analysis, Investigation. **Petek Korkusuz:** Writing — original draft, Writing — review & editing.

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