Tissue and Serum Thioredoxin System and miR-21, miR-23a/b and let-7a as Potential Biomarkers for Brain Tumor Progression

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Abstract

Thioredoxin system and miRNAs are potential targets for both cancer progression and treatment. However, role of miRNAs and their relation between the expression profile of thioredoxin system in brain tumor progression remains unclear. Thus, 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 mRNA expression levels 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 brain tumor patients and 37 healthy volunteers by ELISA. We found that Trx-1, TrxR-1 and PRDX-1 presented high mRNA 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. Our findings showed that Trx-1, TrxR-1 and PRDX-1 and 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/thioredoxin system regulatory axis in brain tumor progression.

Introduction

Brain tumors are diagnosed both in adults and children which lead to high mortality and morbidity worldwide [1–3]. 1.6% new cases and 2.5% deaths of brain tumors globally have been reported in 2020 [4]. Malignant glioma and meningiomas are the most common types among all brain tumors [2]. Magnetic resonance imaging and various molecular diagnostic markers are in use for patients with brain tumor in the clinic [5]. However, distinguishing primary and metastatic tumors is challenging with these methods [6]. Therefore, novel diagnostic biomarkers remain under investigation.

Oxidative stress is a key feature of cancer progression which involves the increased reactive oxygen species (ROS) and antioxidant response [7–9]. Thioredoxin (Trx) system is a crucial cytoplasmic antioxidant system which comprises Trx-1, thioredoxin reductase-1 (TrxR-1) and NADPH [9]. Trx-1 and/or TrxR-1 levels are highly expressed in various human cancers[9–13] including brain tumors [14–18] when compared to healthy ones which are also associated with cancer cell growth, proliferation, invasion, metastasis and prognosis [19, 20]. Peroxiredoxin (PRDX) family is involved in cellular homeostasis and redox system which is responsible for antioxidant and cell death mechanism activation by interacting with Trx system [21–23]. High PRDX-1 expression has been shown in human glioma cells by inhibiting apoptosis of U87MG cells [24] and 293T cells by preventing PTEN oxidation and Akt activation [25] in vitro. PRDX-1 knockdown induces apoptosis and reduces glioma cell proliferation in vitro [26].
MicroRNAs (miRNAs) are small noncoding RNAs regulating mRNA expression levels and functions of target genes as protooncogenes or oncosuppressor genes [27–29]. MiRNAs are linked with cell metabolism, proliferation, apoptosis and survival [27, 28]. In brain tumors, expression of protooncogenes are elevated whereas expression of oncosuppressor miRNAs are reduced [30]. Recent studies showed that expression of several miRNAs including miR-21 [31, 27], miR-23a [31–33, 22], miR-23b [33] increased whereas miR-101 [34], miR-204 [35], miR-128 [36] and miR-135a [37] expressions decreased in brain tumor tissues mostly constituting glioma or meningioma cases. Relation between miRNAs and brain tumors have been implicated in numerous studies [38–43, 31, 44, 32, 35, 34]. Moreover, a large number of studies shows that several miRNAs regulate proliferation, migration and invasion of different types of tumor cells through thioredoxin and/or peroxiredoxin families[45–54]. However, limited number of studies regarding the relation between miRNAs and thioredoxin system components in brain tumors take place in the literature [38, 39]. Tumor suppressor miR-17 levels have been correlated with TrxR-2 downregulation in T98G glioblastoma multiforme cells in vitro [38]. Anti-oncogenic miR-383 was downregulated in medulloblastoma cells by inhibiting PRDX-3 protein expression [39]. In addition, redox status including ROS accumulation regulates production or inhibition of miR-21, miR-23b and miR-125a [53] through Nrf2/ARE [55] and Phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) [56] pathways in various tumor cells. These studies show that thioredoxin system components and miRNAs are potential targets for both cancer progression and treatment. Still, role of miRNAs and their relation between the expression profile of thioredoxin system components in brain tumor progression remains unclear. Thus, in this study we hypothesized that redox components including Trx-1, TrxR-1 and PRDX-1 might have a relation with oncogenic miR-21, miR-23a/b and let-7a and tumor suppressor miR-125a levels which both having 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, PRDX-1, 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.

Herein, we studied Trx-1, TrxR-1 and PRDX-1 mRNA expression levels 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 brain tumor patients and 37 healthy volunteers by ELISA. We found that Trx-1, TrxR-1 and PRDX-1 presented high mRNA 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. Our findings enhanced the understanding of the relation between miRNAs and thioredoxin system activity. Moreover, miR-23a/b, miR-21 and let-7a might be utilized as diagnostic and prognostic markers for brain tumor patients and potential therapeutic targets for its treatment in the clinic.

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 World Health Organization (WHO) criteria [57]. 16 of these tumors were low- and high-grade glioma (WHO grade I and II; grade III and IV, respectively), 15 were low- and high-grade meningioma (WHO grade I and II; grade III and IV, respectively), 5 were metastatic tumors and 2 were other benign tumors. Clinicopathological features of tumor samples are listed in Table 1. Serum samples of 36 brain tumor patients and of 37 healthy volunteers were also obtained. 13 of these volunteers were at the age 35 and below, 20 were ages between 36-56 and 4 were at the age of 57 and over. Minimum required number of samples and replicates were revealed by power analysis using G-Power.

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

**Quantitative Real-time Polymerase Chain Reaction (qRT-PCR)**
Trx-1, TrxR-1 and PRDX-1 mRNA expression levels were determined in human brain tumor samples by qRT-PCR [15, 24, 9]. 100 mg of brain tissues were homogenized with 1 ml TRLzol (RiboEx, #301-001, GeneAll, South Korea) by hand-held homogenizer (#MT-30K, Miulab, China). Total RNA was isolated by mRNA isolation kit (#305-101, GeneAll, South Korea) and concentrations and purities of RNA samples were measured via NanoDrop spectrophotometer (NanoDrop 1000, ThermoScientific, USA) at 260-280 nm wavelength. cDNA was synthesized (#W2211, Wizbio Solutions, South Korea) and qRT-PCR was done on Biorad instrument (CFX Connect, Biorad, USA). Relative mRNA expression was determined by WizPure qPCR SYBR Green Master Mix (#W1711, Wizbio Solutions, 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 TRLzol via hand-held homogenizer. Total miRNA was isolated by miRNA isolation kit (#325-150, GeneAll, South Korea) and concentration and purity of miRNA were measured. cDNA 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 house-keeping gene GAPDH[58] (n=30 in total) and relative fold change was analyzed according to $2^{-\Delta\Delta Ct}$. Primer sequences were summed-up in Table 2.
### Table 2
Primer sequences designed for qRT-PCR.

| Gene          | Oligonucleotide Sequence                  |
|---------------|------------------------------------------|
| GAPDH         | 5'-GGTGTGAACCATGAGAAGTATGA-3'             |
| GAPDH         | 5'-GAGTCTTTCCACGATACCAAG-3'               |
| Trx-1 (TXN)   | 5'-CAACCCTTTTCTTCATTCCCTCT-3'            |
| Trx-1 (TXN)   | 5'-CACCCACCTTTTGTCCCTTCT-3'              |
| TrxR-1 (TXNRD1)| 5'-GTTGCCAAGACTGCAAACCAC-3'             |
| TrxR-1 (TXNRD1)| 5'-CCCTGCAAATGTCAGCTTC-3'               |
| PRDX1         | 5'-GCACCATTGCTCAGATTATG-3'               |
| PRDX1         | 5'-GCCAACAGGGAGTCTATTAC-3'               |
| miR-23a       | 5'-GAAAGAAGGGCGAG...TAGG-3'              |
| miR-23a       | 5'-ATCACATTGCCAGGGATTCC-3'               |
| miR-23b       | 5'-GAAAGAAGGGCGAG...ATTA-3'              |
| miR-23b       | 5'-ATCACATTGCCAGGGATTACCAC-3'            |
| miR-125a      | 5'-GAAAGAAGGCGAG...TCCA-3'               |
| miR-125a      | 5'-TCCCTGAGACCCTTTAACCTGTGA-3'           |
| miR-21        | 5'-GAAAGAAGGCGAG...GTAG-3'               |
| miR-21        | 5'-TAGCTTTACAGACTGTGGTGA-3'              |
| let-7a        | 5'-GAAAGAAGGCGAG...TATG-3'               |
| let-7a        | 5'-TCCCTGAGACCCTTTAACCTGTGA-3'           |
| Universal Primer | 5'-CGAGGAAGAGACGGAAGAAT-3'            |

### Western blot

30 mg brain tumor tissues (n=31 in total) were lysed with RIPA buffer (#R0278-50ML, Sigma-Aldrich, Germany) containing protease inhibitor (ProBlock™ Gold Mammalian Protease Inhibitor Cocktail [100x], #GB-331-1, Gold Biotechnology, USA) by hand-held homogenizer and total protein content was calculated by BCA test (Pierce™ BCA Protein Assay Kit, #23225, ThermoScientific, 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, USA), subsequently transferred onto poly(vinylidene fluoride) membrane via transfer blot system (Trans-Blot Turbo Transfer System, #1704150, Biorad, USA). Following 2 hours of pre-blocking step (5% non-fat milk), membranes were kept...
overnight with rabbit anti-human Trx-1 (1:500, #bs-50523R, Bioss Antibodies, USA), TrxR-1 (1:500, #bs-8299R, Bioss Antibodies, USA), PRDX-1 (1:500, #bs-3875R, Bioss Antibodies, USA) and GAPDH (housekeeping gene, 1:500, #bs-2188R, Bioss Antibodies, USA) primary antibodies, then washed in TBS-t and kept with horseradish peroxidase-conjugated IgG as secondary antibody (#BT-AS00010, Bioassay Technology Laboratory, China) for 1 hour at room temperature. Enhanced chemiluminescent (ECL) was applied for visualization of bands via chemiluminescence imaging system (ChemiDoc Imaging System, #12003153, Biorad, USA). Bands were analyzed by Image Lab Software (v6.0, Biorad).

Enzyme-Linked ImmunoSorbent Assay (ELISA)

Human Trx-1 (#E1452Hu), TrxR-1 (#E3953Hu) and PRDX-1 (#E2924Hu) (all from Bioassay Technology Laboratory, China) protein concentrations in sera of brain tumor patients and healthy volunteers were evaluated by ELISA following manufacturer’s guideline. Briefly, 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 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 recommended in guideline. After adding stop solution to wells, color change from blue to yellow was determined and optical density of each well was measured by plate reader (SPECTROstar® Omega, BMG LABTECH, Germany) at 450 nm. Each serum sample was studied triplicate and protein levels in serum samples of brain tumor patients (n=36) were compared with that of healthy volunteers (n=37).

Statistical Analysis

Data used for qRT-PCR and Western blot data exhibited normal distribution whereas ELISA data presented non-normal distribution by Shapiro-Wilk test. Pairwise comparison of qRT-PCR results was subjected to Student’s t-test. Western blot results were analyzed with one-way analysis of variance (ANOVA) and Tukey’s HSD tests. Two-sample Kolmogorov-Smirnov test was used for comparison of nonparametric results in ELISA. Pearson correlation test was conducted for qRT-PCR. Whole data were analyzed within 95% confidence interval.

Results

Thioredoxin system components showed higher mRNA expression but low protein expression in low-grade brain tumor tissues

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

**Thioredoxin system components showed higher protein expression in serum samples of patients with low-grade brain tumors**

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

**miR-23a/b, miR-21 and let-7a are highly expressed in low-grade brain tumor tissues and positively correlated with the increase in thioredoxin system activity**

miR-23a/b, miR-21 and let-7a expression levels were greater in all brain tumor tissues when compared to control group (Fig. 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 that of high-grade meningioma (Fig. 2B). miR-21 and let-7a expressions were lower in other primary benign tumors when compared to metastatic tumors (Fig. 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 (Fig. 1C) by Pearson correlation analysis.

**Discussion**
In this study, we showed that thioredoxin system components had higher expression in low-grade brain tumor samples also having a strong positive correlation with oncogenic miR-21, miR-23a/b and let-7a and tumor suppressor miR-125a levels for the first time. We examined high mRNA and low protein expressions of Trx-1 and TrxR-1 in low-grade meningioma and benign primary tumor tissues when compared to high-grade meningioma and metastatic tumor tissues, respectively by qRT-PCR and high Trx-1 and TrxR-1 protein expressions in high-grade glioma comparing to low-grade glioma by Western blot. Our findings regarding the increase in Trx-1[9, 16, 18, 59] and TrxR-1 [14, 16, 18] protein expression with the increasing tumor grade are consistent with the other findings of prior studies showing poor clinical outcome for patients with brain tumor. Metastatic tumor samples had significantly higher PRDX-1 mRNA expression compared to benign primary tumors, however, serum samples of all brain tumor patients had lower PRDX-1 expression than that of healthy volunteers. PRDX-1 was up-regulated in various cancer cells [60–62] to regulate cell growth and apoptosis through ROS-dependent pathway [63]. Thus, our results regarding qRT-PCR were coherent with the literature [24, 63]. However, various studies revealed that PRDX-1 may induce apoptosis of tumor cells [63–65]. Herein, lower level of PRDX-1 protein in sera of brain tumor patients might show that decrease in PRDX-1 promotes the proliferation and invasion of tumor cells [64, 65]. Our finding on PRDX-1 revealed that it may act as either oncogenic or tumor suppressor protein.

Here we report that miR-23a/b, miR-21 and let-7a expression levels were greater in low-grade meningioma comparing to high-grade meningioma. miR-21 [66, 28, 67, 27, 42, 41, 43], miR-23a [68, 69, 27, 70, 32], miR-23b [68, 69] and let-7a [67, 71, 72] is have oncogenic capability in various tumor cells including brain tumors [73, 74, 44]. Thus, our findings imply that those miRNAs might be potential biomarkers for diagnosis of meningioma. Herein, we also found that miR-21 and let-7a expressions were greater in metastatic tumors when compared to 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 demonstrated high correlation between the antioxidant protection including thioredoxins and peroxiredoxins and miRNAs [75] which also support our results. PRDX-3 was linked with miR-23b for human prostate cancer progression [49]. Similarly, miR-23a and miR-23b regulate TrxR-1 expression during skeletal muscle differentiation [76]. Our key findings may implicate the diagnostic value of miR-21, miR-23a/b, let-7a and thioredoxin system components for brain tumors which also improve the understanding in determining the levels of biomarkers in different brain tumor grades.

In the current study, a correlation analysis has been done for the relation between thioredoxin system 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 [55] and PI3K/Akt [56] pathways must be performed for the miRNA/thioredoxin system regulatory axis in brain tumor progression which generates a crucial limitation for this study. This limitation, still, does not hinder our further studies since the expression and correlation profiles in thioredoxin system and miRNAs would facilitate in-depth studies for better understanding of miRNA and
redox system component functions as novel biomarkers. Since this research has been conducted with human subjects, brain tissue samples could not be obtained from healthy volunteers. To cope with this limitation, we compared levels of thioredoxin system components with metastatic and/or other benign tumors for qRT-PCR and Western blot and serum samples of healthy volunteers for ELISA.

Taken together, our findings show that thioredoxin system components including Trx-1, TrxR-1 and PRDX-1 and miR-21, miR-23a/b, and let-7a could be potential biomarkers together for brain tumor diagnosis especially for meningioma cases. Further prospective studies are needed to validate their relation and its projection to brain tumor treatment in the clinic.

**Declarations**

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**Author Contributions**

Kilic N., Boyacioglu O. and Tuma Saltoglu G. contributed to the design of the work and conducted the experiments. Kurt G. and Bulduk E.B. obtained the human subjects. Kilic N., Boyacioglu O. and Korkusuz P. analyzed and interpreted of whole data and completed drafting and revising the manuscript. All authors agreed on the accuracy of any part of the work which have been appropriately investigated and finally approved the manuscript to be published.

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**Conflict of Interest**

Authors declare that they have no relevant or material financial interests relating to the research.

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Figures
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 qRT-PCR (n=30, *p<0.05 by Student’s t-test); b Relative mRNA fold-change of Trx-1, TrxR-1 and PRDX-1 of low- and high-grade glioma, low- and high-grade meningioma and metastatic and primary tumor samples by qRT-PCR (*p<0.05 by Student’s t-test); c Relative protein expressions of Trx-1, TrxR-1 and PRDX-1 for low- and high-grade glioma, low- and high-grade meningioma and metastatic tumor samples (*p<0.05; **p<0.001 by One-way
analysis of variance (ANOVA) and post-hoc Tukey’s HSD tests; and serum d Trx-1, e TrxR-1 and f PRDX-1 protein concentrations for brain tumor patients (n=36) and healthy volunteers (n=37) by ELISA test (*p<0.05 and **p<0.001 by Two-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- and high-grade glioma, low- and high-grade meningioma and metastatic and primary tumor samples by ELISA test (*p<0.05 and **p<0.001 by Two-sample Kolmogorov-Smirnov test)

|                      | Trx-1 | TrxR-1 | PRDX-1 | miR-125 | miR-23a/b | miR-21 | miR-23a | Let-7a |
|----------------------|-------|--------|--------|---------|-----------|--------|---------|--------|
| Correlation          |       |        |        |         |           |        |         |        |
| Sig. (2-tailed)      |       |        |        |         |           |        |         |        |
| Trx-1                | 1.000 | 0.960  | 0.959  | 0.753   | 0.945     | 0.968  | 0.966   | 0.967  |
| TrxR-1               |       |        |        |         |           |        |         |        |
| PRDX-1               | 0.960 | 1.000  | 0.889  | 0.601   | 0.873     | 0.907  | 0.879   | 0.885  |
| miR-125              |       |        |        |         |           |        |         |        |
| Correlation          | <0.001| -      |        | <0.001  | <0.001    | <0.001 | <0.001  | <0.001 |
| Sig. (2-tailed)      |       |        |        |         |           |        |         |        |
| miR-23a/b            | 0.959 | 0.889  | 1.000  | 0.827   | 0.954     | 0.956  | 0.922   | 0.958  |
| miR-21               | <0.001| <0.001 | <0.001 | <0.001  | <0.001    | <0.001 | <0.001  | <0.001 |
| miR-23a              | 0.753 | 0.601  | 0.827  | 1.000   | 0.908     | 0.870  | 0.825   | 0.885  |
| Let-7a               |       |        |        |         |           |        |         |        |
| Correlation          | <0.001| 0.001  | <0.001 | <0.001  | <0.001    | <0.001 | <0.001  | <0.001 |
| Sig. (2-tailed)      |       |        |        |         |           |        |         |        |
| miR-23a/b            | 0.945 | 0.873  | 0.954  | 0.908   | 1.000     | 0.955  | 0.938   | 0.981  |
| miR-21               | <0.001| <0.001 | <0.001 | <0.001  | <0.001    | <0.001 | <0.001  | <0.001 |
| miR-23a              | 0.968 | 0.907  | 0.956  | 0.870   | 0.995     | 1.000  | 0.952   | 0.982  |
| Let-7a               | <0.001| <0.001 | <0.001 | <0.001  | <0.001    | <0.001 | <0.001  | <0.001 |

Figure 2

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 qRT-PCR (n=30, *p<0.05 and **p<0.001 by Student’s t-test); b Relative miRNA fold-change of miR-125a, miR-23a/b, miR-21 and let-7a of low- and high-grade glioma, low- and high-grade meningioma and metastatic and primary tumor samples by qRT-PCR.
(*p<0.05 by Student's t-test); c Pearson correlation analysis for thioredoxin system including Trx-1, TrxR-1 and PRDX-1 and miRNAs involving miR-125a, miR-23a/b, miR-21 and let-7a, two-tailed significant values are shown in table