

Evaluation of miR-362 Expression in Astrocytoma of Human Brain Tumors

Abstract

Background: Patients affected by gliomas have a poor prognosis. Astrocytoma is a subtype of glioma. Identification of biomarkers could be an effective way to an early diagnosis of tumor or to distinguish more aggressive tumors that need more intensive therapy. In this study, we investigated whether the expression of miR-362 was increased or decreased in patients with different grades of astrocytoma. **Materials and Methods:** miR-362 expression was compared in 25 patients with astrocytoma with that of 4 normal nonneoplastic brain tissues. **Results:** In all tumor tissues, the expression of miR-362 was significantly decreased relative to its expression in normal brain tissues. However, there was no significant difference between miR-362 expressions in high and low grades of astrocytoma. **Conclusions:** In conclusion, miR-362 showed a down-regulation pattern in astrocytoma tissues that was different from the pattern obtained from previously published microarray studies.

Keywords: Astrocytoma, brain tumors, expression, miR-362

Introduction

Glioma is the most prevalent type of tumors in the primary central nervous system and accounts for 2% of cancers. Despite advancements in treating these type of tumors, there is still a high rate of morbidity and mortality associated with gliomas.^[1] The word “glioma” includes all tumors that are originated from glial cells; that is to say, astrocytomas, oligodendrogliomas, ependymomas, mixed gliomas, and malignant gliomas.^[2] The most common type of glioma is astrocytoma (60–70%) which is mainly forms from inseparable astrocytes and is responsible for 75% of neuroepithelial cases.^[3] Astrocytoma is classified using the most common grading system, World Health Organization classification system, based on the growth rate of the tumor. According to this system, astrocytomas are classified as astrocytoma grade I (pilocytic astrocytoma, low-grade, slow growth, biologically least aggressive, and benign), II (diffuse astrocytoma, low-grade, tendency to recurrence), III (anaplastic astrocytoma, mid-grade), and IV (glioblastoma [GBM], high-grade, rapidly growing, and highly malignant).^[4,5]

Like other lesions that occupy space in the central nervous system, a patient affected by an astrocytic tumor may present symptoms because of intracranial

pressure posed by a tumor or general dysfunction of the brain. These symptoms include a headache, vomiting, papilledema, fluctuations in the level of consciousness and focal or generalized convulsions. The most malignant part of the tumor is the basis of diagnosis.^[6] Hence, sampling a tumor in adequate amount is determinant in identifying its type and malignancy. Considering the facts that grading of tumor based on sampling would be a minimum grading because the more malignant regions may be in areas which has not been sampled and that chemo- or radiation therapy makes it very difficult to type and grade the tumor, to reach a precise, definite, and easy-to-do diagnosis, it is more beneficial to use biomarkers such as cell proliferation, cell death and genetic factors.

Genetic factors play significant roles in the pathogenesis of tumors. In this regard, the biologists are trying to understand the genetic abnormality and cellular mechanisms which influence the features of tumors.^[7] Nowadays, it is recognized that the classification of gliomas based on genetic changes and profiles serves as a diagnostic or predictive tool.^[8] In this respect, there have been some attempts in identifying involved genes in astrocytomas. Many engaged genes are recognized in some grades. For example, in a study, it was observed that

Majid Kheirollahi^{1,2},
Mahdiye Moodi^{1,2},
Saeideh Ashouri^{1,2},
Parvaneh Nikpour^{2,3},
Mohammad
Kazemi^{1,2}

From the ¹Pediatric Inherited Diseases Research Center, Research Institute for Primordial Prevention of Non-communicable Disease, ²Department of Genetics and Molecular Biology, School of Medicine, ³Applied Physiology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Address for correspondence:

Dr. Majid Kheirollahi,
Pediatric Inherited Diseases Research Center, Research Institute for Primordial Prevention of Non-communicable Disease, Isfahan University of Medical Sciences, Isfahan, Iran;
Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: mkheirollahi@med.mui.ac.ir

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inactivation of Retinoblastoma susceptibility gene by mutations contributes to the occurrence of high grade astrocytomas.^[9] In another study, it was shown that in patients affected by primary GBM, loss of heterozygosity of 10q, amplification of epidermal growth factor receptor, deletion of p16^{INK4a}, and PTEN mutations has occurred.^[10] Overexpression of platelet-derived growth factor ligand and receptor has also been reported in astrocytoma.^[11] In addition, the involvement of another class of genes, that is, micro RNA (miRNA) coding genes in the pathogenesis of astrocytomas has been recently observed.^[12,13]

miRNAs are small, noncoding, and single-stranded RNA molecules which are of 19–25 nucleotides length and regulate different genes' expressions and protein production via matching to mRNA 3'UTR.^[14] Broad research over last years showed that miRNAs are more common than previously thought, and they play many underlying roles in the regulation of biological processes.^[15] According to available evidence, dysregulation of miRNAs causes diseases especially cancers by acting as oncogenes or tumor suppressors.^[16,17] In different studies, the involvement of different miRNAs in astrocytoma pathogenesis is reported. For instance, in a study by Huse *et al.*, they observed that the expression of 29 miRNAs including miR-362 increased by at least 2 folds.^[18] In some other studies deregulated miRNAs were identified in malignant astrocytoma using genome-wide expression profiling.^[12,13,19]

The aim of this study was identifying the miR-362 expression pattern in tumor samples obtained from patients affected by astrocytoma of different grades and determining whether there is any difference in its expression between different grades of astrocytoma.

Materials and Methods

Patients and sampling

Tumor samples were collected from patients affected by any types of brain tumors who were operated at Alzahra Hospital, Isfahan, Iran. Tissues were received freshly from neurosurgical operating rooms and stored at -70°C for subsequent RNA extraction. After being histopathologically confirmed the diagnosis of astrocytoma by using part of the tissue samples, 25 specimens were finally selected. Fresh nonneoplastic brain tissues were taken from 4 donors who died 2 h before surgery. Informed consents were taken from all donors before sampling. The study was approved by the Ethics Committee of Isfahan University of Medical Sciences. A total of 25 samples including 5 samples with astrocytoma grade II, 2 samples with astrocytoma Grade III, and 18 samples with astrocytoma grade IV were used in the current study.

RNA extraction from samples and complementary DNA synthesis

First, total RNA was extracted from tissue samples using solvent TRIzol (Invitrogen, USA) according to

manufacturer's protocol and solved in RNase-free water. Then, RNA quality and quantity was examined by ultraviolet spectrophotometry and gel electrophoresis. Finally, 2 μl of extracted RNA was converted to complementary DNA (cDNA) using miRCURY LNATM miRNA polymerase chain reaction (PCR), polyadenylation and cDNA synthesis kit II (Exiqon, Denmark).

Real-time-polymerase chain reaction

Following synthesis of cDNA, real-time PCR was accomplished with ExiLent SYBR[®] Green master mix kit (Exiqon, Denmark) using StepOne PlusTM quantitative real-time PCR detection system (Applied Biosystems, Thermo Fisher Scientific corporation, USA). Ready-to-use primers were from Exiqon. U6 small nuclear RNA primers were used for normalizing the data. All reactions were performed in triplicates for each sample.

Statistical analysis

For expression analysis, the experiment applied the nontumor tissue as the control, so the relative quantification of miR-362 in tumor tissue was calculated using the equation:

$$\text{Amount of target} = 2^{-\Delta\Delta\text{Ct}}, \Delta\Delta\text{Ct} = (\text{Ct}_{\text{miR-362}} - \text{Ct}_{\text{U6}})_{\text{tumor}} - (\text{Ct}_{\text{miR-362}} - \text{Ct}_{\text{U6}})_{\text{normal}}$$

For the nontumor tissue control sample, $\Delta\Delta\text{Ct}$ was considered zero and $2^{-\Delta\Delta\text{Ct}}$ was set as 1. After finishing the reaction, using SPSS software version 20.0 (SPSS, Chicago, IL, USA), the expression of miR-362 was quantitatively analyzed to compare the mean of relative miR-362 expression in two groups of astrocytoma tumor and normal tissues and also in different grades.

Results

The expression of miR-362 was evaluated in 25 astrocytoma samples and 4 normal nonneoplastic tissues using real-time PCR method. miR-362 expression was significantly decreased in astrocytoma samples compared to normal samples ($P = 0.043$) [Figure 1a]. Moreover, the expression of miR-362 was assessed in high-grade and low-grade samples relative to the normal tumor ($P = 0.01$) [Figure 1b]. A brief description of patients' characteristics is given in Table 1.

In addition, to determine the association of miR-362 with cancer progression, we compared miR-362 expression in low-grade astrocytoma samples compared with high-grade ones. Our results revealed that there was no significant difference between two groups ($P > 0.05$) [Figure 2].

Discussion

Nowadays, evaluation of miRNA expression profiles in different types of cancers is performed for finding diagnostic and prognostic tools as well as for developing targeted avenues for intervening therapies.^[20] Alternations

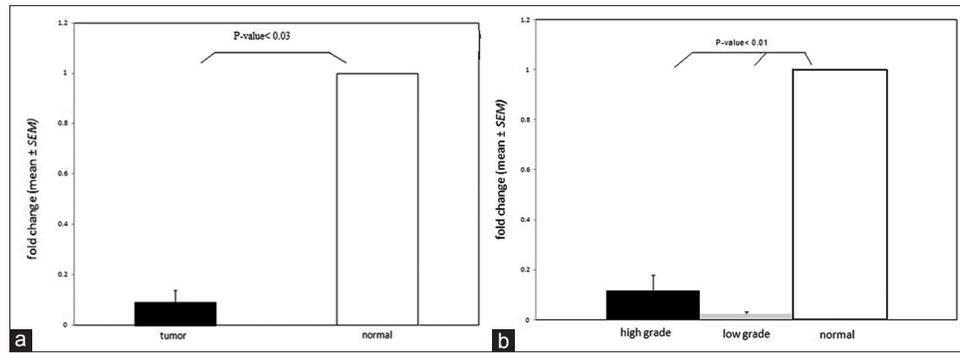


Figure 1: miR-362 expression in astrocytoma. (a) miR-362 expression also decreased in all of 25 astrocytoma samples compared with 4 normal tissues ($P < 0.03$). (b) miR-362 expression decreased between 7 low grades and 18 high grades of astrocytoma samples relative to 4 normal tissues ($P < 0.01$)

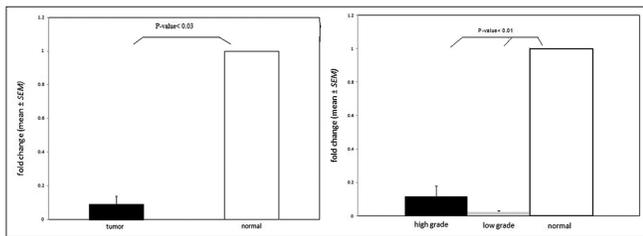


Figure 2: Comparison of miR-362 expression in low and high grades of astrocytoma. miR-362 expression decreased nonsignificantly in low grades relative to high grades ($P > 0.05$)

of miRNAs expression have been studied by different methods including microarray and real-time reverse transcription-PCR.^[21] For the first time, Chan *et al.* in 2005, showed that miR-21 was strongly overexpressed in GBM.^[22] Then, several other studies were conducted on this issue.

In a study by Xia *et al.* it was observed that miR-362 overexpression in gastric cancer increases NF- κ B activity and expression of NF- κ B-regulated genes.^[23] NF- κ B signaling pathway is more active in GBM tissues than in non-GBM ones.^[24]

Rao *et al.* profiled the expression of miRNAs in 26 GBM and 13 anaplastic astrocytoma and 7 normal brain samples using microarray technique and observed that 55 miRNAs including miR-362 were up-regulated, and 29 miRNAs were down-regulated in 39 astrocytoma samples in comparison with 7 normal tissues. Then, they assessed expression of miRNAs to see if they can identify the minimal number of miRNAs which can be used to distinguish anaplastic astrocytoma and GBM and they observed that there is a 23 miRNA signature discriminating these two kinds of astrocytoma.^[12] Furthermore, in another study, a 29 miRNA signature was identified in 3 GBM samples using microarray, and it was observed that expression of 29 miRNAs including miR-362 is elevated relative to normal brain tissues by at least 2 folds.^[18] Lang MF *et al.* profiled the expression of miRNAs using microarray technique in GBM multiforme (GBM) and identified a miRNA expression signatures including miR-362 that, in was up-regulated.^[13]

Table 1: A brief description of patients' characteristics

Characteristics	Values
Mean age (years)	44.95±0.87
Age range (years)	5-82
Sex	
Male	17
Female	4
Grade	
I	0
II	5
III	2
IV	19
Total	25

In our data analysis, inconsistent with previous microarray data, miR-362 expression decreased significantly in 25 astrocytoma tumor tissues compared with normal tissues ($P = 0.043$). In other word, in contrast to the prior observation that reported its 1–3 fold overexpression in GBM,^[12,13,18,19] our analysis showed that miR-362 was significantly under expressed both in high grades and in low grades of astrocytoma samples relative to normal samples.

In the same way, diverse expression patterns of miR-362 have been reported in other cancers. For example, Christensen *et al.* showed that in patients affected by colorectal cancer, miR-362 expression was higher in patients with no recurrence compared with patients with recurrence. In addition, they observed that there was a significant association between miR-362 and increased free disease survival.^[25] While, Shen *et al.* observed that induced expression of miR-362-3p in human hepatocellular cells and tissues increased proliferation and anchorage-independent soft agar growth.^[26] These observations may be an indicator of the possible different expression patterns and effects of miR-362 according to different tissue contexts.

In addition, to investigate whether miR-362 is differentially expressed in astrocytomas with different grades, we compared its expression pattern between low (II) and high (III and IV) grades astrocytomas and determined that

miR-362 expression did not change significantly among different grades of astrocytoma [Figure 2].

In conclusion, previous microarray data showed that miR-362 expression was mostly overexpressed in astrocytoma samples specifically, GBM, but our data analysis could not confirm previous microarray data and showed that its expression has decreased significantly in astrocytoma tissues.

Conclusion

Previous microarray data showed that miR-362 expression was mostly overexpressed in astrocytoma samples specifically, GBM, but our data analysis could not confirm previous microarray data and showed that its expression has decreased significantly in astrocytoma tissues.

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Conflicts of interest

There are no conflicts of interest.

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