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RESEARCH ARTICLE

Long non-coding RNA Panel on Prognosis of Glioma in Iranian Patients

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Abstract

Objective: Glioma is one of the most malignant brain tumors, accounting for about half of the gliomas that occur in central nervous system (CNS), originates from the glial tissue of the brain. The aim of the present study was to determine the expression levels of 3 lncRNAs (AGAP2-AS1, LINC01446 and HOTAIRM1) in patients with high grade glioma in comparison with low grade glioma. **Methods:** Case group consisted of 70 high grade glioma patients control group consisted of 70 patients affected with low grade glioma. RNA extraction was performed using a RNA extraction kit. **Result:** Our results showed that the expression of AGAP2-AS1 and LINC01446 genes significantly increased with increasing tumor grade (with fold-change ratio of 2.1 and 3.8 respectively). Also the expression of HOTAIRM1 gene increased with increasing tumor grade but this increase was not statistically significant (P value=0.6). **Conclusion:** We concluded that AGAP2-AS1 and LINC01446 are promising lncRNA markers in prognosis of glioma.

Keywords: Giloma- cancer- lncRNA

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Introduction

Glioma is a type of tumor that originates from the glial cells of the brain or the spine. Gliomas comprise about 30% of all brain and central nervous system tumors, and 80% of all malignant brain tumors. High-grade gliomas show extensive areas of necrosis and hypoxia and often, tumor growth causes a breakdown of the blood–brain barrier. As a rule, high-grade gliomas almost always grow back even after complete surgical excision, so are commonly called recurrent cancer of the brain [1-3]. Conversely, low-grade gliomas grow slowly, often over many years, and can be followed without treatment unless they grow and cause symptoms [4]. Glioma often occurs in the fourth to sixth decade of life, although some types are more common in children. Brain tumors are slightly more likely to occur in men. History of exposure to radiation is a risk factor for malignant glioma. Certain genetic disorders also increase the risk of developing these tumors in children, and rarely in adults. Several lifestyle risk factors have been investigated in relation to malignant glioma, including smoking or using mobile phones. The symptoms, prognosis and treatment of malignant glioma depend on the age of the diagnosis, the exact type of tumor and the location of the tumor in the brain. These tumors grow and invade normal brain tissue, which makes surgical removal very difficult, or sometimes impossible, and complicates treatment [5-7]. Several acquired genetic mutations have been found in gliomas. Tumor suppressor protein 53 (p53) is mutated

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early in the disease. p53 is the "guardian of the genome", which, during DNA and cell duplication, makes sure the DNA is copied correctly and destroys the cell (apoptosis) if the DNA is mutated and cannot be fixed. When p53 itself is mutated, other mutations can survive. Phosphatase and tensin homolog (PTEN), another tumor suppressor gene, is itself lost or mutated. Epidermal growth factor receptor, a growth factor that normally stimulates cells to divide, is amplified and stimulates cells to divide too much. Together, these mutations lead to cells dividing uncontrollably, a hallmark of cancer. In 2009, mutations in IDH1 and IDH2 were found to be part of the mechanism and associated with a less favorable prognosis [8-10].

One of the factors that can help to increase the effectiveness of treatment is to better understand the molecular pathogenesis and genetic basis of gliomas. Previous studies have shown that long non-coding RNAs (lncRNA) are dysregulated in many malignant human tumors such as colorectal, prostate, bladder, hepatic, as well as cerebral. Recent evidence indicates that lncRNAs probably play an important role in the pathogenesis of gliomas. For example, these molecules can modulate cellular proliferation and apoptosis, leading to tumorigenesis. Studies also show that the abnormal expression of lncRNAs leads to a poor prognosis aggravates patients' clinical condition, especially in GBMs. Therefore, lncRNAs seem to be applicable as potential diagnostic markers or therapeutic targets [11-13].

According to the aforementioned and the important role of lncRNAs (as potential diagnostic and therapeutic markers) in the pathogenesis of gliomas (regulating cellular proliferation and apoptosis, tumorigenesis) and their importance in determining GBM patients' clinical outcomes and prognosis and risk of the disease, the aim of the present study was to investigate the expression profile of three lncRNAs including AGAP2-AS1, LINC01446 and HOTAIRM1 and their potential role as prognostic biomarkers in GBM.

Materials and Methods

In this study, 70 grade II or lower (as the control group) and 70 grade III or IV GBM tumors (as the case group) were collected from the patients who underwent surgery. All patients signed an informed consent form before surgery and agreed to their tissue samples being used in the research project. Tumor tissues were transferred into 1.5 mL sterile DNAse/RNAse free microtubes, and stored in -70 °C. RNA extraction was performed using a RNA extraction kit (MN Co, Germany, Cat No: 740304). Selected lncRNAs for the present study was AGAP2-AS1, LINC01446 and HOTAIRM1. Primer sequences used in this study to amplify target genes by Real-time PCR method showed in Table 1. TaqMan probe real time PCR method used for specific amplification of lncRNAs and expression levels of these genes were compared using U6 as reference gene. PCR thermal program was as below: 95 °C for 5 minutes (Initial Denaturation), 95 °C for 30 seconds, 59 °C for 45 seconds, 72 °C for 30 seconds (repeat for 35 cycles) and 72 °C for 5 minutes (final extension).

Results

Demographic features of the studied population

Demographic information of assessed patients has been mentioned in Table 2. The mean age of patients was 44.70 ±12.44 years.

Gene expression findings

Changes in the expression of assessed genes in tumor tissues have been shown in Table 3.

AGAP2-AS1 gene expression

There was a statistically significant difference in AGAP2-AS1 gene expression between low- and high-grade tumors (p=0.001). The relative expression of this gene (fold change) was 2.1 in high grade (III and IV) respective to low grade (I and II) tumor tissues.

LINC01446 gene expression

Our findings showed that the expression of LINC01446 gene significantly increased in high grade tumors (p=0.001). The relative expression of this gene in grade III and IV samples was 3.8 folds higher compared to grade I and II tumors.

HOTAIRM1 gene expression

There was no association between the expression of HOTAIRM1 gene and tumor grade (p=0.45). The relative expression of this gene was 1.2-fold higher in high grade (III and IV) compared to low grade (I and II) samples (Figure 1).

Table 1. Primers Used in this Study to Amplify Target Genes

Name of lncRNA	Primer sequence
AGAP2-AS1	Forward: 5′-TACCTTGACCTTGCTGCTCTC-3′
	Reverse: 5′-TGTCCCTTAATGACCCCAT CC-3
LINC01446	Forward: 5′-AGAGCATACGGGAGAGATGAA-3′
	Reverse: 5′-AATTCTCCGAACGTGTCACGT-3′
HOTAIRM1	Forward:5′-GAAAGGCGAGCTTGGTTACGCTTAA-3′
	Reverse: 5′-GACTTCGAAGCATTAACGATC-3′

Table 2. Demographic and Clinical Information of Studied Patients

Variables		Mean ±SD (Range)
Age (years)		44.70 ±12.44 (42-77) (%)
Grade	I	5.30
	II	31.90
	III	16.70
	IV	46.10

Table 3. The Expression of Target Genes in Low- and High-grade Glioma Tumor Tissues

Name of lncRNA	Expression Change	Mean of fold change (ratio of Grade III&IV/Grade I&II)	P value
AGAP2-AS1	Overexpressed	2.1	0.001
LINC01446	Overexpressed	3.8	0.001
HOTAIRM1	Overexpressed	1.2	0.6

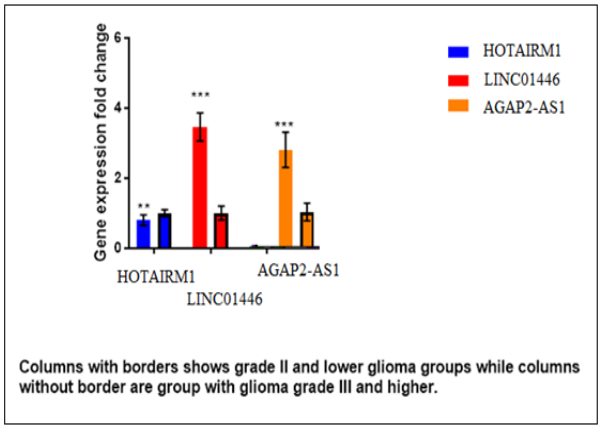

Figure 1. The Relative Expression of Selected Genes in High- and Low-grade Gliomas

Discussion

Recent microarray studies have revealed significant changes in the expression patterns of many lncRNAs in tissues of different subtypes of glioma and normal brain tissue. Using the lncRNA classification pipeline, Zhang et al. identified 1970 lncRNAs across different types and grades of human gliomas [14]. Wang et al. reported that maternally expressed gene 3 (MEG3) expression was markedly decreased in astrocytoma tissues compared to adjacent normal tissues. Recently, several studies have indicated that aberrant expression of lncRNAs may affect glioma initiation and progression [15]. Therefore, lncRNAs may act as biomarkers for glioma diagnosis, prognosis and target therapy [16]. AGAP2-AS1, an antisense lncRNA, is located at human chromosome 12q14.1 and 1567 nucleotides in length [17]. Originally, AGAP2-AS1 was identified as a novel lncRNA in lung cancer, and has found to be overexpressed in non-small–cell lung cancer tissues [18]. Then, Fan et al also showed that the AGAP2-AS1 expression was elevated in non-small–cell lung cancer tissues, and served as a useful biomarker for discriminating non-small–cell lung cancer tissues from normal lung tissues [18]. Ebrahimi et al, showed that the expression of ADAMTS9- AS2 and HOXA11-AS genes significantly increased with increasing tumor grade in Glioma patients. Also the expression of CASC2 gene significantly decreased with increasing tumor grade. They concluded that ADAMTS9-AS2 and HOXA11-AS and CASC2 are promising lncRNA markers in prognosis of glioma [19].

In conclusion, Our results showed that the expression of AGAP2-AS1 and LINC01446 genes significantly increased with increasing tumor grade (with fold-change ratio of 2.1 and 3.8 respectively). Also the expression of HOTAIRM1 gene increased with increasing tumor grade but this increase was not statistically significant (P value=0.6). We concluded that AGAP2-AS1 and LINC01446 are promising lncRNA markers in prognosis of glioma.

Ethics approval and consent to participate

All participants signed the informed consent form. The project was approved by the Local Research Ethics Committee (Shahid Beheshti medical university,Tehran, Iran).

Competing Interests

The authors declare that they have no competing interest.

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