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ORIGINAL ARTICLE

Long noncoding RNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1): A molecular predictor of poor survival in glioblastoma multiforme in Egyptian patients

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KEYWORDS

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LncRNA;
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Abstract *Background:* Long noncoding RNAs (lncRNAs) are a recently discovered class of transcribed RNA molecules with a length of more than 200 nucleotides. Recent studies have shown that lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) could play an important role in carcinogenesis and cancer progression in several types of malignancies.

Objective: As little is known about the role and clinical significance of lncRNA MALAT1 in glioblastoma multiform (GBM) patients in Egyptian population, this study aimed to investigate the expressions of lncRNA-MALAT1 in human GBM samples and to correlate these expressions with the available clinicopathological features including patient survival data.

Subjects and methods: The relative expression of *MALAT1* was determined in 37 human glioblastoma formalin-fixed paraffin embedded (FFPE) tissue samples and 10 FFPE non-neoplastic brain tissues using quantitative reverse transcription polymerase chain reaction (qRT-PCR) technology.

Results: The current results revealed that lncRNA MALAT1 expression was down-regulated in all tumor specimens compared to normal tissues. A receiver operating characteristic (ROC) curve analysis showed high diagnostic performance; area under curve (AUC) = 0.925 ± 0.038 ($P < 0.001$), 95% CI = 0.850–1.00, with 94.6% sensitivity, and 72.7% specificity. Lower *MALAT1* expression was associated with poor prognosis; higher frequency of recurrence ($P < 0.044$), lower overall survival ($P < 0.005$), and shorter disease-free survival ($P < 0.004$).

Abbreviations: DFS, disease-free survival; FFPE, formalin-fixed paraffin embedded; GBM, glioblastoma multiform; lncRNAs, long non-coding RNA; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; NEAT2, nuclear-enriched abundant transcript 2; OS, overall survival; qRT-PCR, quantitative reverse transcription polymerase chain reaction; ROC, receiver operating characteristic; TBP, tata binding protein

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Conclusion: Taken together, we could postulate that MALAT1 might have a tumor-suppressive function in GBM in Egyptian population and this specific type of lncRNAs may be included in the lists of both potential prognostic biomarkers and the future therapeutic targets for glioblastomas.
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1. Introduction

Glioma is by far the most common primary brain tumor associated with poor outcome and survival [1]. It has been classified into well-differentiated low grade astrocytomas (grade I–II), anaplastic astrocytomas (grade III), and glioblastoma multiforme (GBM; grade IV) according to World Health Organization (WHO) [2]. Despite standard treatment that typically includes neurosurgery, chemotherapy and, radiation, recent clinical trials have reported a median survival of only 14–16 months with a 26–33% 2-year survival rate [3]. It has been suggested that the WHO criteria to predict the patient clinical outcomes may not be sufficient alone to estimate patient prognosis [4]. More recently, improved understanding of glioma molecular genetics has led to identifying new potential biomarkers for early diagnosis, prognosis prediction, and novel therapeutic targets [5].

Long noncoding RNAs (lncRNAs) are a recently discovered class of transcribed RNA molecules with a length of more than 200 nucleotides. Similar to protein-coding genes, they have promoter structure, and are transcribed by RNA polymerase II, polyadenylated, and subjected to splicing. However, unlike mRNA, they do not encode proteins [6]. LncRNAs are thought to be important regulators of gene expression at transcription, translation and epigenetic levels [7,8]. An accumulating number of evidences suggested that lncRNAs may have critical roles in a wide range of biological processes [9]. Screening lncRNAs expression profile in glioma has revealed a significant contribution to pathogenesis [10], development and progression [11–13] by regulating cell growth and metastasis; indicating that lncRNAs play significant roles in glioma tumorigenesis [14].

The functional lncRNA-MALAT1 (metastasis-associated lung adenocarcinoma transcript 1); encoded by *MALAT1* gene, which is located at chromosome 11q13.1. [15], and also known as nuclear-enriched abundant transcript 2 (NEAT2), was one of the first lncRNAs found to have a pathogenic role [16] and it has been linked to various cancers besides lung adenocarcinoma [17]. Its expression profile has been found to be dysregulated and correlated with clinical parameters and prognosis in several types of human cancer, such as hepatocellular carcinoma [18], osteosarcoma [19], lung cancer [20], bladder cancer [21], and glioblastoma multiforme [22].

As there are no previous studies, up to the researchers' knowledge, on the expression of this type of lncRNAs in GBM patients among the Arab population, this study for the first time will aim to determine the expression levels of *MALAT1* in a sample of GBM patients and correlate these expressions with the available clinicopathological features including patient survival data in a sample of Egyptian population.

2. Subjects and methods

2.1. Patients and tissue samples

The present study included 37 formalin-fixed paraffin embedded (FFPE) glioblastoma samples, fulfilling the WHO criteria of GBM and 10 FFPE non-neoplastic brain tissue specimens. GBM patient samples (9 females and 28 males, aged 35 to 60 years old) have been assessed retrospectively from the archive of the Pathology Departments, Mansoura University Hospitals and Suez Canal University Hospitals, Egypt, from 2010 to 2013. Detailed patients' data were retrieved from their medical follow up records. All patients had GBM (i.e. grade IV), undergone surgical removal and post-operative irradiation, and followed for more than 3 years. The work has been carried out in accordance with the code of ethics of the world medical association (Declaration of Helsinki) for experiments on humans. All patients gave written informed consent, except for deceased individuals or patients who provided archived tissue samples and can't be traced.

2.2. Total RNA extraction

Following deparaffinization in xylene and washing with alcohol, the total RNA was extracted from tumor and control FFPE tissue sections (4–5 μm) collected in sterile eppendorf tubes. Qiagen RNeasy FFPE Kit (Cat. No. 73504, Qiagen, Hilden, Germany) has been used following the protocol supplied by the manufacturer. Extracted total RNA concentration and purity at the absorbance ratio 260/280 nm were determined by NanoDrop ND-1000 spectrophotometer (NanoDrop Tech., Inc. Wilmington, DE, USA). In addition, RNA degradation and contamination were assessed by 1.5% agarose gel electrophoresis.

2.3. Reverse transcription

High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, P/N 4368814) was used for reverse transcription (RT) reaction. For each 20 μl RT reaction, 10 μl (10 ng) RNA sample was combined with 10 μl of 2× RT reaction mix containing 2 μl of 10× RT Buffer, 0.8 μl of 25× dNTP Mix (100 mM), 2 μl of 10× RT random primers, 1 μl of MultiScribe™ Reverse Transcriptase, 1 μl of RNase inhibitor, and 3.2 μl of nuclease-free water. RT was carried out in a T-Professional Basic, Biometra PCR System (Biometra, Goettingen, Germeny) at 25 °C for 10 min, followed by 37 °C for 120 min, and finally 85 °C for 5 min, then held at 4 °C. Appropriate negative controls were included in each experiment.

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