Neuroscience Letters 654 (2017) 1–5

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Overexpression of *STMN1* is associated with the prognosis of meningioma patients

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HIGHLIGHTS

- There is a part of the pathological type of the meningioma has a malignant tendency and patients have poor prognosis.
- *STMN1* plays an important role in the maintenance, metastasis, invasion, and differentiation of malignant tumor cells.
- High expression of *STMN1*, along with high Ki-67 scores and WHO grades, act as independent prognostic factors for survival times.
- *STMN1* represents a potential biomarker for predicting meningioma progression.

ARTICLE INFO

Article history:
Received 23 April 2017
Received in revised form 13 June 2017
Accepted 14 June 2017
Available online 16 June 2017

Keywords:
Meningioma
Stathmin
Biomarker
Prognosis

ABSTRACT

There is a small part of the pathological type of the meningioma has a malignant tendency and patients have the poor prognosis. Looking for effective biomarkers to predict the degree of malignancy of the tumors, will help us to better manage the patient and guide the treatment. The present study aims at investigating the prognostic value of the expression of *Stathmin* in a series of meningiomas of different grade. We integrated eight published microarray datasets of meningiomas to screen grade biomarkers in meningiomas patients using the WebArrayDB platform. We focused on *Stathmin*, Using formalin-fixed paraffin-embedded (FFPE) tumor samples, we corroborated the relationship between *Stathmin* and patient outcomes using qRT-PCR for gene expression. We also found expression of *Stathmin* that atypical/anaplastic meningiomas have higher expression than benign meningiomas ($p < 0.01$). No correlation between *Stathmin* expression and age, gender and tumor extent of resection was found ($p > 0.05$). Moreover, increased *Stathmin* expression was correlated to higher meningioma grade and shorter disease-free survival (DFS) of meningioma patients with Simpson I resection. *Stathmin* might be promising targets to improve the cure rates in meningiomas.

1. Introduction

Meningiomas are thought to arise from arachnoidal cap cells of intracranial neoplasms in adulthood, and they account for 35.8% of all primary central nervous system (CNS) tumors in the United States [1]. They are currently classified into several histotypes and three grades of malignancy according to the criteria of the World Health Organization (WHO) classification scheme for tumors in the central nervous system (CNS). Pathological grade has been associated with significantly greater rates of morbidity and mortality, even with the provision of multimodality treatments [2]. Although atypical (WHO II) and anaplastic (WHO III) meningiomas only account for 10% of all meningiomas [3], they are more likely than WHO I meningiomas to display malignant behavior, characterized by invasion, growth, recurrence, and poor prognosis, after surgical resection [4].

Characterization of the genetic or epigenetic processes associated with malignant transformation may provide insights that inform the development of novel diagnostic and therapeutic tools to address this aggressive tumor subtype [5]. Previous studies have identified several biomarkers that correlate with the progression of meningiomas. Insulin-like growth factor binding protein 2 *(IGFPB2)* amplification, tumor protein 73 *(TP 73)* methylation, lysine-specific demethylase 5C *(KDM5C)* mutation, and vascular

Abbreviations: CNS, central nervous system; DFS, disease-free survival; FFPE, formalin-fixed paraffin-embedded; H&E, hematoxylin and eosin; qRT-PCR, quantitative real-time polymerase chain reaction.

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http://dx.doi.org/10.1016/j.neulet.2017.06.020
0304-3940/

endothelial growth factor *(VEGF)* amplification, among other proteins, were observed more frequently in samples from aggressive rather than non-aggressive tumors [6–8]. Although meningioma research at the molecular level has progressed greatly, effective assessments of biomarkers are still lacking for clinical practice. Microarray techniques could aid in analyzing the genomic landscape to identify prognostic biomarkers for the survival of patients with meningiomas. However, analyses of microarray datasets require large numbers of samples to reduce bias. Unfortunately, the number of samples from malignant tumors has been very limited in past studies. Therefore, it will be important to generate data from larger cohorts for robust detection of differences by WHO grade. In our study, we analyzed microarray data integrated from multiple array sources using the WebArrayDB platform [9] to compensate for inadequate sample sizes. Among the genes upregulated in WHO II and WHO III groups, we focused on the significantly upregulated gene stathmin 1 (*STMN1*).

STMN1 also plays an important role in the maintenance, metastasis, invasion, and differentiation of malignant tumor cells [10,11], which can affect the curative effect of some chemotherapy drugs on microtubules [12]. The correlation between *STMN1* and tumor histological grade has already been documented in other CNS neoplasias [13–15].

In the present study, for the first time, we investigated the mRNA expression levels of *STMN1* in meningiomas of different pathological grades and determined the correlation between *STMN1* levels and survival time in Simpson I resection patients. *STMN1* represents a potential biomarker for predicting meningioma progression.

2. Materials and methods

2.1. Patient samples

Samples from 73 cases of meningioma were collected from Department of Neurosurgery, 1 st Affiliated Hospital of Jilin University, from 2005 to 2010. Meningioma tumors were obtained from 42 females and 31 males (at surgery: mean age, 49.9 ± 14.7 years; range, 18–78 years). No patient had a pretreatment history before surgery; however, the Simpson grade of the extent of survival resection was available in all cases [16], as were follow-up data on recurrences and disease-free survival (DFS). Recurrence was defined as the detection of a recurrent tumor by radiological investigations of patients with a previous complete surgical excision (Simpson grade I). All patients were histologically reviewed according to the WHO 2007 classification scheme for CNS tumors [2]. The meningiomas samples comprised 41 benign (WHO grade I), 23 atypical (WHO grade II), and 9 anaplastic (WHO grade III) samples. This study was approved by the Ethics Committee of 1st Affiliated Hospital of Jilin University (IRB 00008484).

2.2. Total RNA extraction and quality assessment

Total RNA was extracted from FFPE using a QuickExtract FFPE RNA Extraction Kit protocol (Epicentre). Hematoxylin and eosin (H&E) sections from FFPE specimens were reviewed by a pathologist to select the most informative blocks. Four 10 mm-thick sections per FFPE block were cut, followed by one H&E control slide. The tumor area selected for analysis was marked on the control slide to ensure, as far as possible, that greater than 80% of neoplastic cells were within that area of a section. RNA deparaffinization, extraction, and purification were performed using the QuickExtract FFPE RNA Extraction Kit protocol (Epicentre). The concentration of isolated RNA was measured with a Qubit RNA BR assay (Invitrogen, Carlsbad, CA, USA) using a Qubit 2.0 fluorometer. Purity absorbance ratios ($A_{260}/_{280}$) were measured using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies).

2.3. Real-time quantitative reverse transcription-PCR (qRT-PCR) analysis

The relative expression levels of *STMN1* were analyzed by real-time qRT-PCR using the SYBR Green approach. cDNA synthesis was performed using a SuperScript II Reverse Transcriptase Kit (Invitrogen) with 300 ng total RNA according to the manufacturer's protocol. All gene expression assays were run on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using the standard settings. All assays were prepared with 1 × SYBR Green PCR Supermix (BioPioneer, San Diego, CA, USA). mRNA expression levels were normalized to that of the housekeeping gene for β-actin. Relative fold changes were calculated using the Pfaffl method [17] for each gene after correction for the β-actin level. The primers were synthesized by Takara as follows: *STMN1* sense: 5′- TGTCGCTTG TCTTCTATTCACCAT -3′; *STMN1* antisense: 5′- CTTTTGACCGAG GGCTGAGA -3′; *β*-actin sense: 5′-CCACGAAACTACCTTCAACTCCA-3′; *β*-actin antisense 5′-GTGATCTCCTTCTGCATCCTGTC-3′. The absence of primer dimers was demonstrated by agarose gel electrophoresis. Each sample was run in triplicate.

2.4. Microarray dataset analysis

We performed an integrated microarray analysis to select genes associated with meningioma progression. Eight independent external microarray datasets containing all 231 meningioma samples were analyzed, including 158 benign (WHO grade I), 60 atypical (WHO grade II), and 13 anaplastic (WHO grade III) samples. The microarray datasets were analyzed using the WebArrayDB cross-platform analysis suite to screen candidate genes (Table 1) [9]. Data were analyzed with an ANCOVA model to correlate gene expression with meningioma WHO grade in the WebArrayDB platform. Genes were sorted in ascending order according to the *p* values associated with WHO tumor grade.

2.5. Statistical analysis

Variables are presented as means ± standard deviations. For comparisons of different groups, the ANOVA test was used. Cox proportional hazards model for multivariate survival analysis was used to assessing predictors of survival. DFS was assessed by the Kaplan-Meier method, and survival curves were calculated. A *p* value less than 0.05 was considered statistically significant. The statistical analysis was performed using SPSS software v.17.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Clinical characteristics and WHO grades

A total of 73 meningioma patients who were treated within the defined study period were included in the study. The mean age at diagnosis was 49.9 years (range 18–78 years), and most patients had undergone Simpson I resections (49/73, 67.1%). In patients who had Simpson I resections, those in the atypical/anaplastic group had higher recurrence rates than those in the benign meningioma group (85.7% vs. 25.0%, $p < 0.01$). Moreover, the atypical/anaplastic group had higher Ki-67 scores (cut-off score, 4%) than those in the group of patients with benign meningiomas (87.5% vs. 12.2%, $p < 0.01$) (Table 2).