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FOXO4 expression is associated with the occurrence and outcome of seizures: An RNA-sequencing analysis of low-grade gliomas

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ABSTRACT

Purpose: Epileptic seizures account for most of the initial symptoms in patients with low-grade gliomas (LGGs). Nevertheless, the molecular mechanisms of tumor-associated seizures remain unclear. This study investigated the genetic changes associated with the occurrence and outcome of seizures in patients with LGGs.

Methods: The clinical characteristics and gene profile data of 86 patients with LGGs were collected from the Chinese Glioma Genome Atlas database. Gene expression was analyzed based on whole-genome RNA sequencing. The genes with significantly different expressions between patients with and without seizures were identified. Additionally, the Engel Epilepsy Surgery Outcome Scale was applied to evaluate the seizure outcomes at 6 months after tumor resection.

Results: In patients with LGGs, the expression of Forkhead Box O4 (FOXO4) was significantly different between the seizure and non-seizure groups, and high FOXO4 expression was found to be associated with a low risk of seizure occurrences ($p = 0.026$). This result was validated by using the clinical information and RNA sequence data from The Cancer Genome Atlas database ($p = 0.005$). FOXO4 was additionally identified as a predictor of seizure outcomes in patients with LGGs at 6 months after tumor resection ($p = 0.018$).

Conclusions: The results of our genomic analysis suggest that low FOXO4 expression is a significant risk factor for epileptic seizures in patients with LGGs and is associated with the seizure outcome. FOXO4 may be a potential therapeutic target for tumor-associated epilepsy.

1. Introduction

Brain tumors can cause intractable seizures. Gliomas, which are the most common type of brain tumor, account for the majority of tumor-associated seizures [1–3]. Patients with low-grade gliomas (LGGs) often experience secondary epileptic seizures as their chief complaint [4–6]. In contrast, patients with glioblastomas, the most malignant type of glioma, are unlikely to experience seizures as the tumor progresses [7,8]. Thus, identifying the mechanisms underlying the generation of LGG-related seizures is critical for determining the prognoses of and appropriate treatment strategies for patients with LGGs.

The genetic background of brain tumors has been linked to the tumor's ability to invade the normal brain tissue and peritumoral microenvironment [9,10]. Moreover, since epileptic seizures are generated as a result of chronic damage to neurons, the genetic characteristics of the tumor may be related to the epileptogenesis in patients with LGGs [11]. Indeed, evidence suggests that genes, which are associated with glutamate transporter expression, synaptic transmission, and cell apoptosis, are involved in the generation of LGG-related seizures [12–14]. However, the molecular profiles that contribute to glioma-induced seizures are currently unclear [13,15].

Identifying seizure-associated genes in gliomas requires the gene expression of patients with and without seizures to be compared using a whole-genome analysis and large number of tumor samples. Therefore, in the current study, we employed a whole-transcriptome sequencing analysis based on the data from the Chinese Glioma Genome Atlas (CGGA, http://www.cgga.org.cn, a pure Chinese population dataset). Correlations between the expression of specific genes and LGG-related epilepsy were investigated. Furthermore, the results of this study were validated with RNA sequence data from an independent database, The

Abbreviations: FOXO4, Forkhead box O4; LGG, low-grade glioma; CGGA, Chinese Glioma Genome Atlas; TCGA, The Cancer Genome Atlas.

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Cancer Genome Atlas (TCGA, cancergenome.nih.gov, a mixed population with mostly Caucasian individuals). The prognostic roles of the identified genes were also investigated.

2. Methods

2.1. Data collection

A testing group consisting of 86 patients with LGGs (World Health Organization grade II) was enrolled in this study. The clinical information of patients and the RNA sequencing data obtained from CGGA database. All samples from the patients in the testing group were collected during the initial brain surgery. A validation group of patients was collected from the TCGA database. The current study was approved by the ethics committee of Beijing Tiantan Hospital, and all participants provided informed consent allowing their information to be collected in the database and analyzed.

2.2. Assessment of seizure occurrences and outcomes

Tumor-associated epileptic seizures were identified based on the clinical presentation of the patients. LGG-associated epilepsy was defined as a history of one or more seizure presentation in patients with LGGs [2]. Seizure types, including partial and generalized seizures, were classified according to the seizure terminology defined by the International League Against Epilepsy [16]. Patients were placed in either the seizure or non-seizure group based on their presentations.

The seizure outcome was also assessed during patient follow-up at 6 months after surgery. In this study, the Engel Epilepsy Surgery Outcome Scale was used to describe the seizure outcomes, which were classified as follows: Class I, free of disabling seizures; Class II, rare disabling seizures; Class III, worthwhile improvement; or Class IV, no worthwhile improvement or a worsened condition [17].

2.3. RNA sequencing and data analyses

CGGA RNA sequencing was performed as previously described [18]. Briefly, frozen tumor samples were disrupted and homogenized with a QIAshredder and pestle (Qiagen, Germany). Libraries were sequenced on the Illumina HiSeq 2000 platform using the 101-bp pair-end sequencing strategy. Short sequence reads were aligned to the human reference genome (Hg19Refseq) using the Burrows-Wheeler Aligner (version 0.6.2-r126). The reads per kilobase transcriptome per million method was used to assess the gene expression [19]. Normalization allowed the direction of gene expression to be compared between individual cases. In addition, TCGA RNA sequencing data and the corresponding genetic profiles [20] were collected from the online TCGA database and used as the validation group in this study.

2.4. Statistical analysis

The mean expression of all genes was calculated and compared between the seizure and non-seizure groups using t-statistics. The clinical characteristics associated with the occurrence of seizures were identified using chi-squared tests. A univariate analysis was performed to identify the significant ($p \leq 0.05$) risk factors for LGG-associated seizures. The prognostic role of the gene expression (high vs. low) was investigated with log-rank tests and demonstrated in Kaplan-Meier curves.

3. Results

3.1. Clinical characteristics

A group of 86 patients with LGGs were reviewed, including 35 (40.7%) with astrocytomas, 17 (19.8%) with oligodendrogliomas, and 34 (39.5%) with oligoastrocytomas. The mean age of the patients at diagnosis was 39 years (range: 21–62 years). Preoperative epileptic seizures occurred in 57 (66.3%) patients, while 29 patients were free of tumor-related seizures. Generalized seizures secondary to the brain tumor were observed in 38 (66.7%) of the patients with seizures. Partial seizures were observed in 6 (10.5%) cases with complex partial seizures and 13 (22.8%) cases with simple partial seizures. There were 82 (95.3%) patients who underwent radiotherapy, while only 6 (7.0%) cases underwent additional chemotherapy.

3.2. Gene analysis

Gene data based on RNA sequencing were collected from the CGGA database. The gene expression patterns of the tumor samples were compared between patients with tumor-associated seizures and those without seizures. The results revealed that 370 genes were differentially expressed between the two groups ($p \leq 0.05$). In addition, several genes from the TCGA database were differentially expressed between the seizure and non-seizure groups (Supplementary material 2). One of the identified genes, Forkhead Box O4 (FOXO4), is reportedly associated with tumor development [21,22]. Therefore, we focused our investigation on

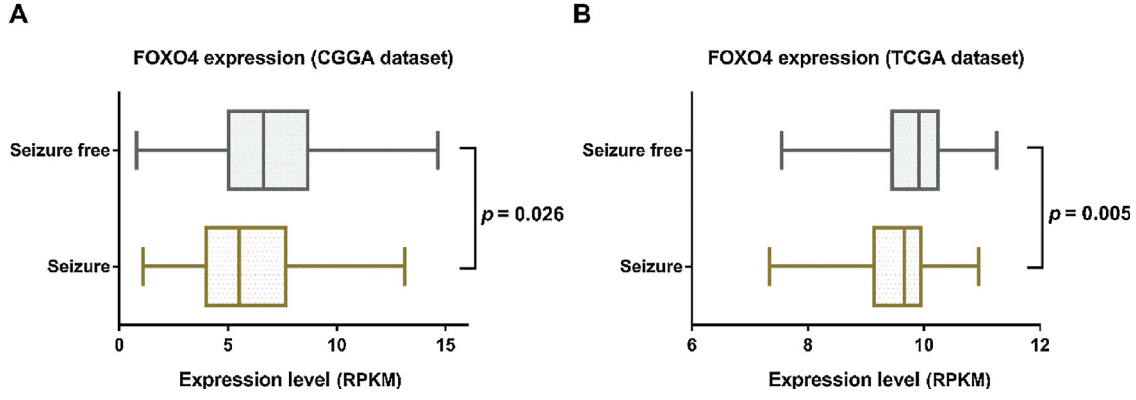

Fig. 1. Comparisons of the expression of FOXO4 in LGG patients with or without seizures. (A) Comparison in the CGGA dataset ($p = 0.026$, t-test). (B) Comparison in the TCGA dataset ($p = 0.005$, t-test). Lower expression of FOXO4 was associated with an increased seizure risk in both the CGGA glioma and TCGA glioma datasets. RPKM: reads per kilobase per million.