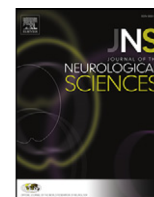
Journal of the Neurological Sciences 368 (2016) 314–317

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

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



Predictive and prognostic value of TLR9 and NFKBIA gene expression as potential biomarkers for human glioma diagnosis

Ata Miyar [a], Iman Habibi [b], Ali Ebrahimi [c], Delaram Mansourpour [d], Aram Mokarizadeh [e], Alireza Rajabi [f], Rozhin Farshgar [g,*], Mehdi Eshaghzadeh [b], Mohamad Zamani-Ahmadmahmudi [h], Seyed Mohamad Hossein Tabatabaei Nodushan [b]

[a] Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[b] Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[c] Students' Research Committee, Baqiyatallah University of Medical Sciences, Tehran, Iran
[d] Brain and Spinal Cord Injury Research Center, Neuroscience Institute, Tehran University of Medical Sciences, Tehran, Iran
[e] Cellular & Molecular Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran
[f] Graduated from the Faculty of Veterinary Medicine, Islamic Azad University, Karaj Branch, Karaj, Iran
[g] Veterinarian in Private Sector
[h] Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

ARTICLE INFO

Article history:
Received 30 June 2016
Received in revised form 16 July 2016
Accepted 19 July 2016
Available online 20 July 2016

Keywords:
TLR9 and NFKBIA
Glioma
Survival
IHC
PCR
Patient
Biomarker

ABSTRACT

Background: Malignant gliomas are the most common form of primary intracranial tumors with the highest mortality rates. Various gene alterations are considered as prognostic markers in glioma. But, the relevant molecular mechanisms in this setting are not well-understood.
Objective: The aim of this study was to assess the association and prognostic value of TLR9 and NFKBIA with clinical significance and also their impact on patient survival in human glioma.
Methods: Expression of TLR9 and NFKBIA mRNA in the tissues was determined by immunohistochemistry and qRT-PCR methods. Kaplan-Meier curves and Cox proportional hazards regression model were used to assess the association of TLR9 and NFKBIA with clinical outcomes of patients.
Results: Quantitative real-time PCR analysis showed that TLR9 mRNAs is markedly expressed in glioma tissues than in non-neoplastic tissues (mean $\pm$ SD: 3.26 $\pm$ 0.40 vs. 0.71 $\pm$ 0.36, $P < 0.001$). There was also a significant difference between TLR9 mRNAs and high grade glioma ($P < 0.001$).NFKBIA mRNAs was significantly identified in non-neoplastic tissues compared with glioma specimens (mean $\pm$ SD: 2.76 $\pm$ 0.30 vs. 0.94 $\pm$ 0.35, $P < 0.001$). Lower levels of NFKBIA mRNA were significantly related to advanced grade of gliomas ($P < 0.001$). Furthermore, Immunoreactivity for high expression of TLR9 was detected in 65% of cases (26/40) that was associated with high grade glioma ($P = 0.001$). No statistically significant correlation was found between TLR9 and other clinical parameters ($P > 0.05$). Immunoreactivity for high expression of NFKBIA was observed in 32.5% (13/40) of cases and NFKBIA expression was decreased in patients with high grad glioma ($P = 0.014$). There was no significant correlation between NFKBIA protein expression and age, sex, and relapse. The Kaplan-Meier analysis indicated that patients with high expression of TLR9 and low expression of NFKBIA are significantly related to poorer OS ($P < 0.001$). In addition, the multivariate Cox regression model revealed that TLR9 and NFKBIA protein expressions (low/high) and tumor grade were potentially an independent predictor of survival in patients (hazard ratio, 2.132, 2.411, 2.13 [95% confidence interval, 1.825–3.782, 1.61–3.231, 1.542–3.92]; $P = 0.012$,$P = 0.018$, $P = 0.001$).
Conclusion: These data indicate that TLR9 and NFKBIA protein expressions act as independent predictor of survival for the diagnosis of glioma and a prognostic biomarker for those with a tumor at an advanced pathological grade.

© 2016 Published by Elsevier B.V.

1. Introduction

Gliomas are known to be the most common primary tumors in adults with highly poor prognosis and a very low 5-years survival because of either metastasis or recurrent [1–4]. Current efforts have been performed to prevent the progression of disease because of improvement of the patient's survival. Furthermore, understanding of the molecular pathological mechanism of glioma is important to establish effective therapeutic targets. Certainly, biomarkers that are involved in the regulation of gliomas may develop better opportunities.

* Corresponding author.
E-mail address: rozhinfarshgar3000@gmail.com (R. Farshgar).

http://dx.doi.org/10.1016/j.jns.2016.07.046
0022-510X/© 2016 Published by Elsevier B.V.

Synthetic CpG oligonucleotides (CpG DNA) have been currently indicated to be a treatment option in cancer immunotherapy. As a matter of fact, CpG was applied as vaccine adjuvants. It has been shown that CpG mimic microbial DNA and has fundamental role in activating the immune system via the binding of toll-like receptor 9 (TLR9), [5].

TLR9 is known as a member of TLR family that can be expressed by many types of the immune system cells such as the subsets of B cells and myeloid cells in human, as well as it is a negative regulator of wound healing. This protein leads to the production of inflammatory mediators such as IL-12 and type-I interferon [6]. TLR9 expression has been detected in gastric, prostate, lung, renal and breast cancer cells [7–13]. TLR9 expression was not showed to be of prognostic significance in GBMs [14].

The nuclear factor-κ-light chain enhancer of activated B cells (NF-κB) family is involved in various biological processes, such as innate immunity, inflammation, proliferation and apoptosis [15]. Furthermore, aberrant expression of NF-κB is reported to be involved in cancer initiation and progression [16]. NF-κB is a transcription factor that current data considered a tumor suppressor gene. The aberrant activation of NF-κB was found glioblastomas [17,18]. Moreover, it has been indicated that NF-kB Inhibitor alpha gene (NFKBIA) act as a tumor suppressor in glioblastomas. NFKBIA deletion or decreased expression has been reported to be associated with poorer survival [19]. This study was aimed to determine TLR9 expression and NFKBIA in patients with glioma using quantitative RT-PCR and immunochemistry; also we investigate their impact on patient survival.

2. Materials and methods

2.1. Ethical approval

The protocol for the present study was approved through the Ethics Committee of the private clinics dependent on hospitals and written consents were obtained from all those patients included in this study.

2.2. Patients and tissue specimens

40 glioma tumor specimens from surgically treated patients without preoperative radiation or chemotherapy were collected at Tehran hospitals between 2009 and 2013. Informed consent was collected from each case. Non-neoplastic brain tissues were collected during temporal lobectomy from epilepsy surgery, and all collected tissues were snap-frozen in liquid nitrogen. Tumor tissues were confirmed histologically, based on hematoxylin and eosin staining (H&E), and diagnosed by three pathologists. The overall survival (OS) of each patient was recorded from surgery until the follow-up deadline or date of death. Clinicopathologic features for the included cases in the current study were indicated in Table 1. The present study was approved by the Ethical Committee of hospitals and was according to the criteria of the Helsinki convention.

2.3. Quantitative real-time PCR

Total RNA was extracted from the tissues using miRNeasy kit (Qiagen), according to the manufacturer's instructions. 1 μg RNA was applied and were reverse-transcribed to synthesize the complementary DNA (cDNA) using Transcriptor First Strand cDNA Synthesis Kit (Roche). The genes were amplified using SYBR Green PCR master mix (Applied Biosystems). GAPDH was used as gene control. Real-time RT-PCR was conducted using the following primers:

Human TLR9 forward: 5′-TGTAATAACAGTTGCCGTCCAT-3′, and reverse: 5′-CAGCCTTTCCTTGTCCT. FKBIA forward, 5′-CTC CGA GAC TTT CGA GGA AAT AC-3′ and reverse, 5′-GCC ATT GAA GTT GGT AGC CTT CA-3′. GAPDH forward, 5′-CTTCATT GACCTCAACTAC-3' and reverse, 5′-GCCATCCACAGTCTTCTG-3′. The relative expression level was calculated using the 2 − ΔΔCT method.

Table 1
Clinicopathological parameter of patients based on the expression of TLR9 and NFKBIA.

Clinicopathological parameters	Number: 40 cases	Expression of TLR9		Expression of NFKBIA		P value of TLR9	P value of NFKBIA
		Low: 14	High: 26	Low: 27	High: 13		
Age							
≤45	17	6	11	13	4	P > 0.05	P > 0.05
>45	23	8	15	14	9		
Gender							
Male	24	8	16	18	6	P > 0.05	P > 0.05
Female	16	6	10	9	7		
Tumor grade							
Low (I/II)	15	10	5	3	12	P = 0.001	P = 0.014
III + IV	25	4	21	24	1		
Relapse							
No	18	5	13	11	7	P > 0.05	P > 0.05
Yes	22	9	13	16	6		

2.4. Immunohistochemistry method

4-μm formalin-fixed paraffin sections were deparaffinized with xylene, rehydrated, endogenous peroxidase activity was blocked at room temperature for 30 min by using 0.5% H2O2 in methanol fallowed by heating each section in 0.01 M sodium citrate buffer (pH 6.0) for 20 min at 95 °C. Blocking nonspecific binding was performed with 20% normal horse serum at room temperature for 30 min. Sections were then incubated with a 1:100 dilution of primary anti- NFKBIA antibody (Sigma-Aldrich), a 1:100 dilution of anti- TLR9 (1:100; Sigma-Aldrich) at room temperature overnight.

The sections were incubated with horse reddish peroxidase (HRP)-labeled anti-rabbit secondary antibody according to the manufacturers protocol, and visualized with diaminobenzidine (DAB), counterstained with hematoxylin.

The markers immunoreactivity was categorized into a low- or high-expression levels based on the percentage of stained tumor cells if staining intensity was 0–50% or >50%, respectively.

2.5. Statistical analysis

The SPSS 21.0 was used for statistical analysis. The correlation between NFKBIA and TLR9 expression and clinicopathological parameters were analyzed by the fisher's exact test and Student's *t*-test. Kaplan–Meier curves and the log-rank test were done based on survival data. Multivariate analysis was performed by Cox proportional hazards regression models to identify independent prognostic factors for OS. Differences were considered statistically was accepted at $P < 0.05$.

3. Results

Quantitative real-time PCR analysis revealed that TLR9 mRNAs is markedly expressed in glioma tissues than in non-neoplastic tissues (mean ± SD: 3.26 ± 0.40 vs. 0.71 ± 0.36, $P < 0.001$, Fig. 1). There was also a significant difference between TLR9 mRNAs and high grade glioma ($P < 0.001$).

NFKBIA mRNAs was significantly identified in non-neoplastic tissues compared with glioma specimens (mean ± SD: 2.76 ± 0.30 vs. 0.94 ± 0.35, $P < 0.001$, Fig. 1). Lower levels of NFKBIA mRNA were significantly related to advanced grade of gliomas ($P < 0.001$).

Download English Version:

https://daneshyari.com/en/article/1912987

Download Persian Version:

https://daneshyari.com/article/1912987

Daneshyari.com