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Original Article

Clinical and prognostic significance of prokineticin 1 in human gliomas

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Abstract: The objective of this study was to explore the expression and the clinical and prognostic significance of prokineticin 1 (PROK1) in human gliomas. The expression of PROK1 in 60 patients with glioma and in eight control cases (patients with traumatic brain injury) by immunohistochemistry (IHC). The associations between the differences in expression and pathology grades were analyzed statistically. The positive rates of PROK1 expression in normal brain and glioma tissue were 25.0% (2/8) and 93.3% (56/60), respectively. PROK1 expression in glioma tissue was higher than that in normal tissue (P<0.05). The positive rates of PROK1 expression in low-grade gliomas (LGGs, grades I and II) and high-grade gliomas (HGGs, grades III and IV) were 66.7% (8/12) and 100% (48/48), respectively, the positive rates in HGG were higher than those in LGG (P<0.01). PROK1 is an angiogenic growth factor that is related with metastatic ability of tumor, we also correlated PROK1 expression with NFAT expression. Expression of PROK1correlated significantly with expression of NFAT (r=0.524, P<0.01), but not with patient sex and age. Glioma patients with higher expressing PROK1 had a significantly shorter progression-free survival time, increasing levels of PROK1 expression significantly correlated with reduced survival times when all patients with glioma were considered (P<0.01). These results suggested that PROK1 positivity and protein expression levels are of significant clinical and prognostic value in human gliomas, which significantly correlates with the survival in gliomas, PROK1 may regulate the progression of glioma via the NFAT pathway.

Keywords: Prokineticin 1, NFAT, gliomas, prognosis

Introduction

Gliomas are the most common type of central nervous system tumors, and the majority of the histological findings of the gliomas are malignant [1]. Although the diagnosis and treatment of glioma has progressed, the overall prognosis of glioma patients remains poor, and the five-year survival rate is <25% [2]. The difficulties in treating malignant glioma are partly due to its malignant biological properties, such as its over-proliferation and high invasiveness [3]. Recent studies have shown that many molecular mechanisms, such as the EGFR [4], AKT [5], STAT3 [6], and beta-catenin [7] pathways are involved in glioma. However, the molecular pathology of glioma is not sufficient to investigate the development and invasive behavior of glioma. Therefore, it is quite necessary to identify the key gene targets of glioma, and to find a mechanism by which to reverse their malignant behavior.

Prokineticin 1 (PROK1) is an angiogenic growth factor that is expressed in endocrine cells, including the adrenal gland, ovary, and testis [8]. PROK1 which contains cysteines in its amino acid sequence promotes vascular endothelial growth under hypoxic conditions [8]. The primary function of PROK1 is to strengthen metastatic ability of colorectal cancer cells. Recent studies have shown that PROK1 is highly expressed in the primary lesions of some patients with colorectal cancer [9], prostate cancer [10], neuroblastoma [11], pancreatic duct cancer [12] and thyroid cancer [13], and is associated with cell-infiltrating ability of a colorectal cancer cells through an autocrine

mechanism [14] and malignant degree of tumors.

However, whether PROK1 is highly expressed in human gliomas is not known. The nuclear factor of activated T cells (NFAT) is a cell signaling molecule involved in complex adaptive systems particular to vertebrate biology related to the Rel family of cell signal proteins [15, 16], they may control transcriptional events governing functions as diverse as cell proliferation, survival and differentiation [17, 18], epithelial-to-mesenchymal transition (EMT) [19], and cell migration and invasion [19, 20]. NFAT is a potential regulator of tumor progression and metastasis. The aim of this study was to investigate PROK1 expression in human gliomas. To assess the role of PROK1, we detected NFAT and correlated PROK1 expression with glioma WHO classification, NFAT, and progression-free survival (PFS) time to further demonstrate the role of PROK1 in the proliferation and invasion of gliomas and its possible role as a prognostic biomarker and therapeutic target.

Materials and methods

Patients and samples

Tumor tissues were obtained at the first surgery in 60 previously untreated patients with glioma. The patient population comprised 31 males and 29 females, with a mean age of 54.2 years (27-75 years). All specimens were pathologically confirmed referring to the 2007 World Health Organization classification of tumors of the nervous system and grading criteria [21]. Twelve cases of low-grade glioma (LGG) were identified, including two cases of pilocytic astrocytoma, one cases of subependymoma, four cases of astrocytoma, five cases of diffuse astrocytoma. In addition, 48 cases of high-grade glioma (HGG) were identified, including 13 cases of anaplastic astrocytoma, two cases of anaplastic astrocytoma with local glioblastoma, one case of astrocytoma, five cases of glioblastoma multiforme, 27 cases of glioblastoma. Eight samples of normal brain tissue were obtained from brain injury decompression surgery as a control. Consent was received from the families of the patients to collect and preserve the specimens using cryopreservation at -80°C. The present study was approved by the Life Science Ethics committee of Capital Medical University (Beijing, China).

Immunohistochemical detection

Immunohistochemistry was performed to detect the expression of PROK1 and NFAT. The specimens were embedded, cut into serial 3-μm sections and placed on a slide after pretreatment that was undertaken by Beijing Bioss. In brief, pretreatment involved immersion of the slide in cleaning fluid of potassium dichromate sulfuric acid for 24 h and then rinsing under running water. After it was rinsed again at least three times with distilled water 95% ethanol was added and the slide was left to dry and immerse in poly-L-lysine (0.01%) for about 30 sec. Finally, the slide was drained and placed in the oven at 45°C for 1 h. Anti-PROK1 monoclonal antibodies (1:100; Beijing Biosynthesis Biotechnology Co., Ltd., Beijing, China) and Anti-NFAT monoclonal antibodies (1:50; Beijing Biosynthesis Biotechnology Co., Ltd., Beijing, China) were added to the sections for incubation overnight at 4°C, following by washing with phosphate-buffered saline including TWEEN (PBST) and incubation with biotin-labeled secondary antibody (Sigma-Aldrich, St. Louis, MO, USA) at room temperature for 20 min. Subsequent to further washing with PBST, horseradish peroxidase-labeled streptavidin working solution (Beijing Biosynthesis Biotechnology Co., Ltd.,) was added and the sections were incubated at room temperature for 15 min. 3,3'-diaminobenzidine chromogenic reagent was added for coloration, followed by rinsing, hematoxylin staining, conventional ethanol dehydration, xylene clearing, mounting with a neutral gum and observation under a microscope. Samples in which the cytoplasm was colored yellow or brown were classified as positive. An area with a strong immune response was selected in each slice, and five non-repetitive, high-power fields of view were observed (magnification, ×400). All controls provided satisfactory results. The immunohistochemical analysis was performed by one of the authors, who was blinded to the clinical data. Immunoreactivities were scored by the intensity of staining (0, no staining; 1, weak = light yellow; 2, moderate = yellow brown; 3, strong = brown) and the percentage of stained cells, (0, on staining; 1, 1-10%; 2, 11-35%; 3, 36-70%; 4, >70%). By multiplication of both values, a final score ranging between 0 and 12 was obtained [22]. PROK1 and NFAT overex-

Table 1. Expression of PROK1 in malignant gliomas of different WHO classes measured by immuohistochemical staining

WHO class	PROK1 expression/total (%)			Sex (M/F)	Age (Y)	NF-AT (%)
	-/+	++	+++			+++
I-II (n=12)	4-4/12 (66.7%)	3/12 (25%)	1/12 (8.3%)	4/8	43	16.7%
III (n=15)	2/15 (13.3%)	8/15 (53.3%)	5/15 (33.3%)	7/8	52.3	26.7%
IV (n=33)	2/33 (6.06%)	18/33 (54.5%)	13/33 (39.4%)	20/13	59	39.4%

Note: At least 1000 cells were counted for each section and 3 sections for each sample at ×400 magnification with a randomly selected microscope. Abbreviations: -, no expression; +, 1%-35%; ++, 35%-70%; +++, 70%.

Table 2. Association between PROK1 and the clinicopathological factors of glioma

Variable	PROK1 expression			χ^2	*P*-value
	n	Positive, n (%)	Negnative, n (%)		
Gender					
Male	31	29/31 (93.5%)	2/31 (6.45%)	4.340	0.631
Famale	29	27/29 (93.1%)	2/29 (6.90%)		
Age in years					
>45	47	47/47 (100%)	0/47 (0%)		
≤45	31	9/13 (69.2%)	4/13 (30.8%)	22.100	0.001
Pathological grade					
LGG	12	8/12 (66.7%)	4/12 (33.3%)		
HGG	48	48/48 (100%)	0/48 (0%)	29.105	<0.001

LGG, Low-grade glioma; HGG, High-grade glioma; PROK1, Prokineticin 1.

pression was defined as final score more than zero. For the purpose of statistical analysis, tumors with a final staining score of ≥3 were considered to be high expression.

Statistical analysis

All statistical analyses were performed using SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA), and $P<0.05$ was considered to indicate a statistically significant difference. Measurement data are expressed as the mean ± standard deviation. Differences between two symmetrical portions of the same group of patients at different times were analyzed using the paired Student's t-test. The correlation between PROK1 expression and NFAT, clinical pathological features was evaluated for statistical significance by χ^2 and Fisher's exact tests. Survival curves were calculated using the Kaplan-Meier method and compared by the log-rank test. The significance of various variables for survival was analyzed by the Cox proportional hazards model in the multivariate analysis. $P<0.05$ in all cases was considered statistically significant.

Results

PROK1 was up-regulated in glioma tissues

Immunohistochemical staining showed that the colored areas due to PROK1 expression were mainly located in the cytoplasm. PROK1 showed lower expression in the 8 normal brain tissue samples, and the positive expression rate was 25.0% (2/8). However, in the 60 glioma samples the PROK1 positive expression rate was 93.3% (56/60), which was higher than that in the normal brain tissue ($P<0.05$) (**Table 1**). We also observed that 4 (6.67%) exhibited no positivity for PROK1, 8 (13.3%) demonstrated weak positivity, 29 (48.3%) had moderated positivity, and in 19 (31.7%), strong positivity was observed (**Table 1**). According to evaluation method as described above, PROK1 expression of tumor cells was further reclassified into high or low expression. PROK1 was evaluated as high expression in 63.3% (38/60) of tumor samples.

High-expression of PROK1 is associated with grade of glioma

Table 2 showed the relationship between the high-expression of PROK1 protein and clinical characteristics. There was no significant correlation between the high-expression level of PROK1 protein and gender of patients with glioma ($P>0.05$). However, the difference in PROK1 expression between the different age groups was statistically significant ($\chi^2=22.100$, $P=0.001$). The positive expression rate of PROK1

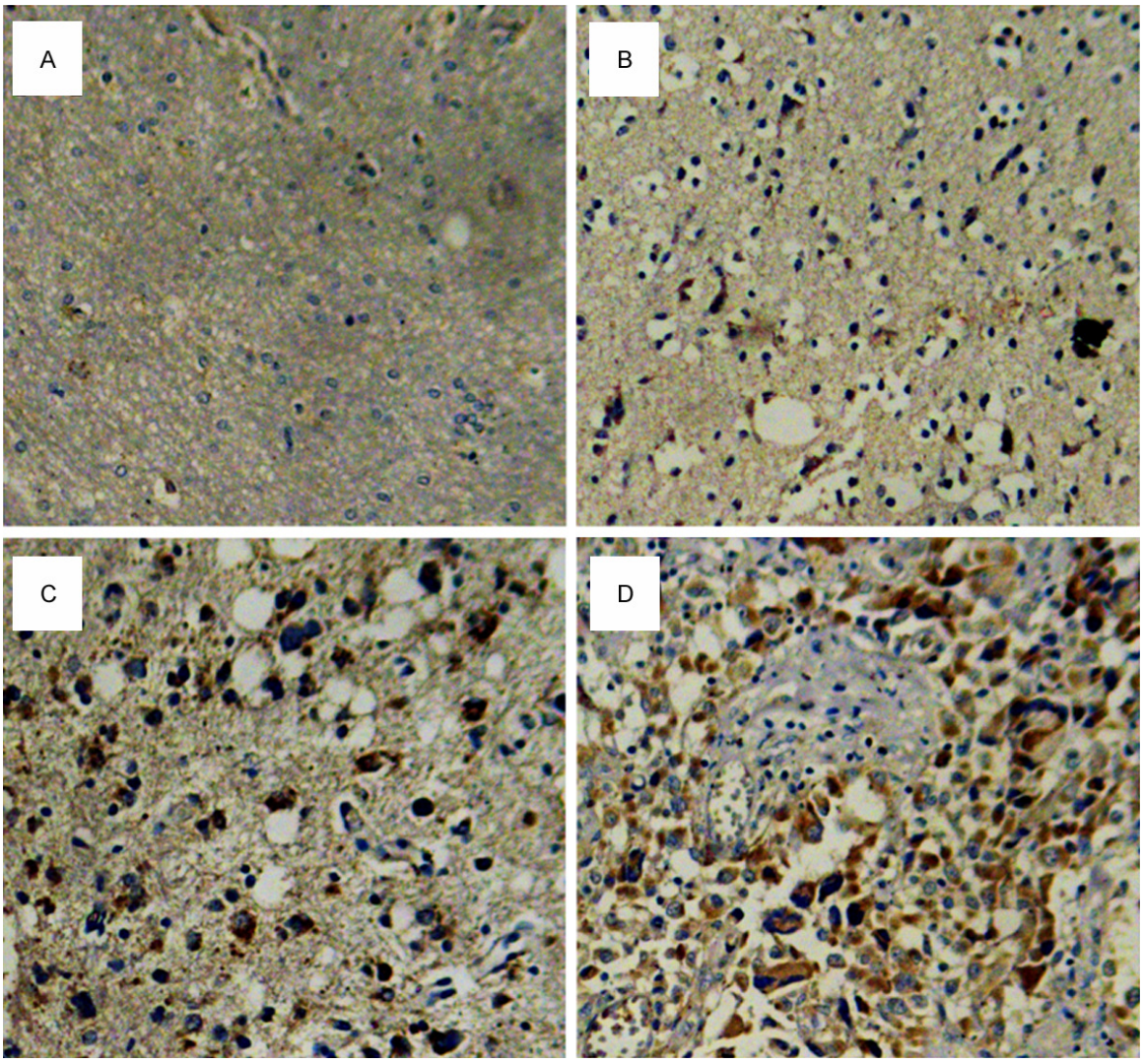

Figure 1. The expression of PROK1 in normal brain tissue and gliomas. Expression of PROK1 in the different grades of gliomas (streptavidin-peroxidase staining; magnification, ×400). A: Normal brain tissue; B: Diffuse astrocytoma (grade II); C: Anaplastic astrocytoma (grade III); D: Glioblastoma (grade IV).

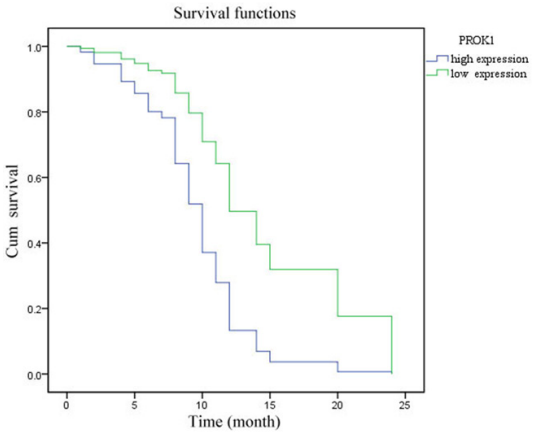

Figure 2. Kaplan-Meier curves showing PFS of patients with different WHO grade gliomas (n=60) according to PROK1 expression. Glioma patients with high expression of PROK1 were shown to have significantly shortened survival times compared with glioma patients with low expression of PROK1 (P=0.003). Of the 60 patients with glioma, immunohistochemical detection revealed that 38 were PROK1 high expression (bottom curve) and 22 were PROK1 low expression (top curve). PROK1 high expression (histochemical scores: ≥3); PROK1 low expression (histochemical scores: <3).

in the LGG and HGG groups was 66.7% (8/12) and 100% (48/48), respectively, and the difference between the two groups was statistically significant (χ^2=29.105, *P*<0.001). (**Figure 1**)

Prognostic value of PROK1 expression levels

As stated earlier, a positive correlation was identified between PROK1 expression levels

Table 3. Multivariate analysis of prognostic factors

Variable	B	SE	χ^2	*P*-value	OR	95% CI
Gender	0.058	0.289	0.041	0.840	1.060	0.602-1.868
Age	0.012	0.013	0.910	0.340	1.012	0.987-1.038
PROK1 expression	-1.095	0.424	6.687	0.01	0.334	0.146-0.767
Pathological	1.523	0.664	5.260	0.022	4.581	1.248-16.839

and the pathological grades of the gliomas. In the LGG and HGG groups, positive PROK1 expression was found in 8 and 48 cases, respectively. To further evaluate the prognostic value of PROK1 protein for glioma patients, we also analyzed the association between PROK1 protein expression and survival duration using Kaplan-Meier analysis with the log-rank test. The results revealed that high-level expression of PROK1 protein was correlated with short survival time of patients with glioma (Log Rank =8.784, *P*=0.003, **Figure 2**). The median survival time of patients with low PROK1 expression tumors was 14 months, compared with 8 months for patients with high PROK1 expression tumors. This suggests that PROK1 expression at the highest levels is associated with a more extreme malignant phenotype of glioma and may also be associated with increased treatment resistance.

Multivariate analysis of prognostic factors

In a model that included the presence or absence of PROK1 expression, pathological grade (LGG versus HGG), gender and age, pathological grade (P=0.022) and PROK1 expression (P=0.01) were significantly associated with reduced survival times, whereas age and gender were not (**Table 3**). The results showed that high-level expression of PROK1 protein is an independent prognostic factor for poor survival of patients with glioma.

Relation between PROK1 expression and NFAT

The results of immunohistochemical analysis: NFAT gene expression was significantly higher in HGG than in LGG (*P*<0.05), details are given in **Figure 3**. Spearman correlation analysis showed that there was a significant correlation between cytoplasm PROK1 expression and NFAT positive expression(r=0.524, *P*<0.01). (**Figure 3**)

Discussion

Glioma is the most common type of intracranial tumor and has the highest incidence and mortality. The adult incidence rate is <6/100,000 and the five-year survival rate for glioma is 20-30% [23, 24]. Glioma exhibits the biological features of infiltrative growth and unclear boundaries; therefore, total resection is difficult. Although comprehensive treatments, including radiotherapy and chemotherapy, are available, the tumor still recurs easily because of the infiltrative nature and abnormal proliferation [25]. Invasion and migration acquisition is the first step of cancer metastasis, and it is always combined with EMT [26], infiltration is an essential pathologic feature of gliomas. Emerging evidence has suggested that NFATs play important roles in the metastasis process. It was shown that NFATs are transcription factors for invasion and migration-associated genes, such as cyclooxygenase-2 (COX-2) and autotaxin, whose overexpression enhances cell invasion and metastasis [27, 28]. Matrix metalloproteinases (MMPs) are also induced by NFATs in myocytes, glomerular mesangial cells and astrocytes [29-31]. More powerful evidence in tumor angiogenesis also have shown that NFATs enhance transcription of some target genes involved in angiogenesis, such as COX-2, tissue factor (TF) and caspase-8 inhibitor cellular Fas-associated death domain-like interleukin 1β-converting enzyme inhibitory protein (c-FLIP) [32]. The results above suggest that the NFAT pathways are involved in tumor lymphangiogenesis, so NFATs can facilitate tumor angiogenesis and lymphangiogenesis. Consequently, NFATs can promote tumor progression and metastasis. Proliferation is a natural occurrence in the growth of neoplasms, which leads to tumor metastasis and is involved in high intracranial pressure, cerebral herniation, and hydrocephalus, eventually reducing the chance of patient survival [33]. NFAT is a ubiquitous transcription factor in vertebrates, whose dysregulation induces the expression of various target genes involved in cancer development. Some study showed that in pancreatic cancer NFAT2 can interacts with c-Myc, leading to cyclin D upregulation and anchorage-independent growth [34]. Additionally, sequential induction of NFAT2 and c-Myc facilitates TGF-β-promoted cell growth in various cancer cells [35]. It has been founded that Macrophages infiltrate into the tumor microenvironment and secrete epidermal growth factor (EGF) and col-

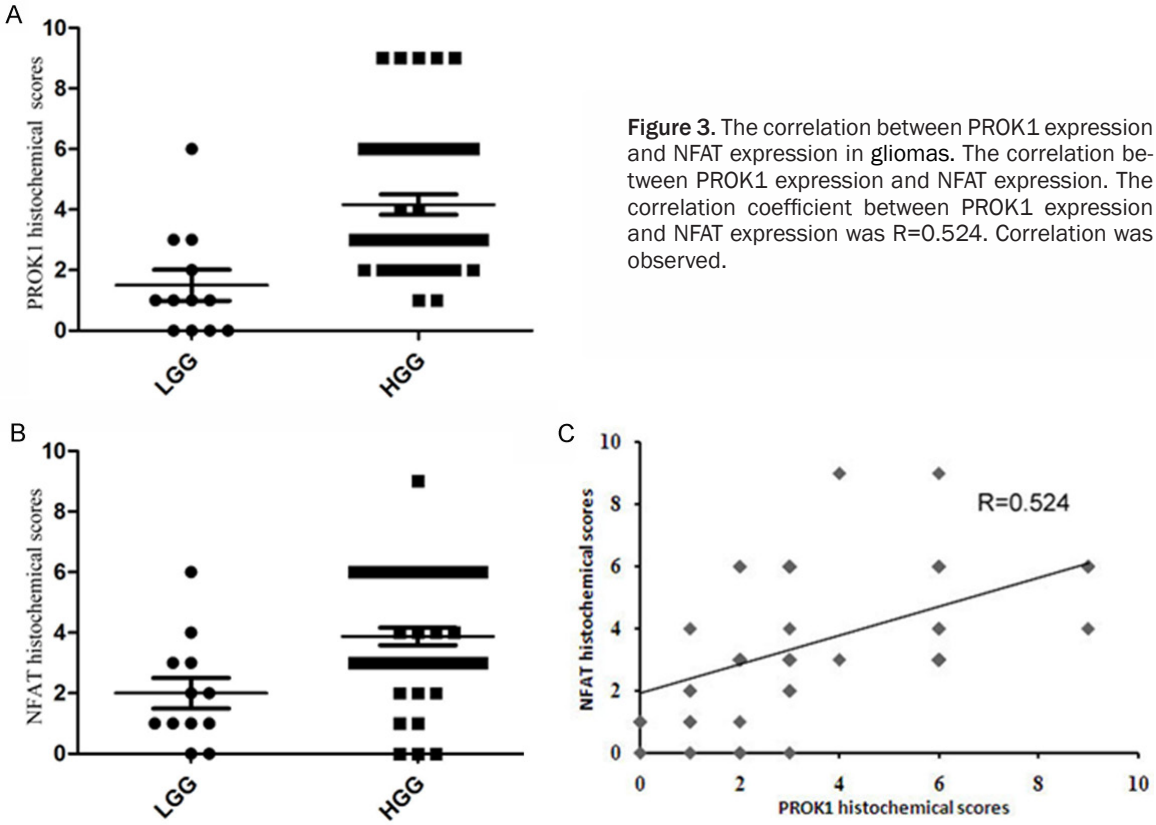

Figure 3. The correlation between PROK1 expression and NFAT expression in gliomas. The correlation between PROK1 expression and NFAT expression. The correlation coefficient between PROK1 expression and NFAT expression was R=0.524. Correlation was observed.

ony-stimulating factor 1 (CSF1) to facilitate tumor proliferation and migration [36]. Some study has proved that NFATs can be in involved in the regulation of the tumor microenvironment and progression by inducing CSF1 in both immune cells and tumor cells. Dysregulation of NFAT signaling confers malignant cells with pro-survival potential under stress conditions, the calcineurin/NFAT pathway can promote glioma cell survival during the apoptosis in rat glioma cells induced by CsA [37]. As discussed above, NFATs have been shown to function in tumorigenesis, proliferation, metastasis, drug resistance and the tumor microenvironment. Although comprehensive treatments, including radiotherapy and chemotherapy, are available, the curative effect of malignant gliomas requires improvement, the identification of novel effective targets related with invasive behavior of malignant gliomas is of essential importance for glioma's therapy. Prokineticin-1 (PROK1) is a multifunctional secreted protein which signals via the G-protein coupled receptor, PROKR1. The PROK/PROKR system has been associated with a considerable number of physiological and pathological functions. They are able to coordinate complex behaviors such as feeding, drinking, circadian rhythm, and hyperalgesia, but are also involved in neuron migration and survival, angiogenesis, hematopoiesis and inflammation, multiple signaling events can be activated by the system [38]. Therefore, the PROK/PROKR system has elicited great interest among cancer investigators because of its role as survival factors for certain tissue-specific cells, and for their ability to induce angiogenesis and coordinate a pro-inflammatory immune response. Prokineticins can participate actively in the tumorigenesis process (prostate, testicles, neuroblastoma, colon, and pancreas) where they participate as a growth factor for cancer cells, an angiogenic and a chemotactic factor for pro-inflammatory neutrophils [8-13]. Whether Prokineticins could be implicated in other cancers has not yet been investigated, such as brain cancer, where they serve as very important growth factors for nerve cells of the olfactory bulb [39]. Our study showed that PROK1 is highly expressed in gliomas but not in normal human brain, that PROK1 expression in GBM (WHO grade IV) and AA (WHO grade III and IV) was higher than in (WHO grade II and grade

I), the protein expression of PROK1 increased with an increase in glioma malignancy and was significantly associated with malignant gliomas. It can be found that overexpression of PROK1 closely correlated with high expression of NFAT, which was related to tumor progression and invasiveness. Some reports showed that in endometrial cells PROK1 can activate the calcineurin/NFAT pathway to induce IL-8 expression [40]. The correlation between PROK1 and NFAT is a particularly interesting finding in glioma that calls for further research to reveal the underlying mechanism of the interaction, PROK1 may regulate the progression of glioma via the NFAT pathway. Furthermore, PROK1 reflects poor prognosis of many malignant tumors [41]. In our study, a significant association of high PROK1 expression with glioma patients' shorter PFS time was seen, such that glioma patients with a high expression of PROK1 had a worse prognosis. Given that PROK1 enhances cell invasion and the degree of malignancy in a variety of tumor cells, we propose that PROK1 may be used as an indicator of glioma prognosis. However, there are limitations to our study. To eliminate interference factors, we examined follow-up for at least 3 years; therefore, patients receiving primary surgical treatment after December 2014 were not included.

In conclusion, our present study indicated that high PROK1 expression in malignant gliomas tended to increase in parallel with pathological grade. Moreover, our results showed that PROK1 correlated with a poor prognosis and with NFAT as well, which were correlated with tumor progression and invasion, respectively, in gliomas. We conclude that PROK1 has the potential to be a useful tool in the prognosis and treatment of gliomas.

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Disclosure of conflict of interest

None.

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References

[1] Kong BH, Park NR, Shim JK, Kim BK, Shin HJ, Lee JH, Huh YM, Lee SJ, Kim SH, Kim EH, Park EK, Chang JH, Kim DS, Kim SH, Hong YK, Kang SG and Lang FF. Isolation of glioma cancer stem cells in relation to histological grades in glioma specimens. Childs Nerv Syst 2013; 29: 217-229.

[2] Demuth T and Berens ME. Molecular mechanisms of glioma cell migration and invasion. J Neurooncol 2004; 70: 217-228.

[3] Warren KE. Diffuse intrinsic pontine glioma: poised for progress. Front Oncol 2012; 2: 205.

[4] Nagane M, Coufal F, Lin H, Bögler O, Cavenee WK and Huang HJ. A common mutant epidermal growth factor receptor confers enhanced tumorigenicity on human glioblastoma cells by increasing proliferation and reducing apoptosis. Cancer Res 1996; 56: 5079-5086.

[5] Nicoletti NF, Erig TC, Zanin RF, Pereira TC, Bogo MR, Campos MM and Morrone FB. Mechanisms involved in kinin-induced glioma cells proliferation: the role of ERK1/2 and PI3K/Akt pathways. J Neurooncol 2014; 120: 235-244.

[6] Priester M, Copanaki E, Vafaizadeh V, Hensel S, Bernreuther C, Glatzel M, Seifert V, Groner B, Kögel D and Weissenberger J. STAT3 silencing inhibits glioma single cell infiltration and tumor growth. Neuro Oncol 2013; 15: 840-852.

[7] Chen X, Hu W, Xie B, Gao H, Xu C and Chen J. Downregulation of SCAI enhances glioma cell invasion and stem cell like phenotype by activating Wnt/beta-catenin signaling. Biochem Biophys Res Commun 2014; 448: 206-211.

[8] LeCouter J, Kowalski J, Foster J, Hass P, Zhang Z, Dillard-Telm L, Frantz G, Rangell L, DeGuzman L, Keller GA, Peale F, Gurney A, Hillan KJ and Ferrara N. Identification of an angiogenic mitogen selective for endocrine gland endothelium. Nature 2001; 412: 877-884.

[9] Tabata S, Goi T, Nakazawa T, Kimura Y, Katayama K and Yamaguchi A. Endocrine gland-derived vascular endothelial growth factor strengthens cell invasion ability via prokineticin receptor 2 in colon cancer cell lines. Oncol rep 2013; 29: 459-463.

[10] Monnier J and Samson M. Prokineticins in angiogenesis and cancer. Cancer lett 2010; 296: 144-149.

[11] Ngan ES, Sit FY, Lee K, Miao X, Yuan Z, Wang W, Nicholls JM, Wong KK, Garcia-Barcelo M, Lui VC and Tam PK. Implications of endocrine gland-derived vascular endothelial growth factor/prokineticin-1 signaling in human neuroblastoma progression. Clin Cancer Res 2007; 13: 868-875.

[12] Pasquali D, Rossi V, Staibano S, De Rosa G, Chieffi P, Prezioso D, Mirone V, Mascolo M, Tra-

montano D, Bellastella A and Sinisi AA. The endocrine-gland-derived vascular endothelial growth factor (EG-VEGF)/prokineticin 1 and 2 and receptor expression in human prostate: up-regulation of EG-VEGF/prokineticin 1 with malignancy. Endocrinology 2006; 147: 4245-4251.

[13] Jiang X, Abiatari I, Kong B, Erkan M, De Oliveira T, Giese NA, Michalski CW, Friess H and Kleeff J. Pancreatic islet and stellate cells are the main sources of endocrine gland-derived vascular endothelial growth factor/prokineticin-1 in pancreatic cancer. Pancreatology 2009; 9: 165-172.

[14] Pasquali D, Santoro A, Bufo P, Conzo G, Deery WJ, Renzullo A, Accardo G, Sacco V, Bellastella A and Pannone G. Upregulation of endocrine gland-derived vascular endothelial growth factor in papillary thyroid cancers displaying infiltrative patterns, lymph node metastases, and BRAF mutation. Thyroid 2011; 21: 391-399.

[15] Crabtree GR and Olson E. NFAT signaling: choreographing the social lives of cells. Cell 2002; 109 Suppl: S67-79.

[16] Hogan PG, Chen L, Nardone J and Rao A. Transcriptional regulation by calcium, calcineurin, and NFAT. Genes Dev 2003; 17: 2205-2232.

[17] Wu H, Peisley A, Graef IA and Crabtree GR. NFAT signaling and the invention of vertebrates. Trends Cell Biol 2007; 17: 251-260.

[18] Horsley V, Aliprantis AO, Polak L, Glimcher LH and Fuchs E. NFATc1 balances quiescence and proliferation of skin stem cells. Cell 2008; 132: 299-310.

[19] Oikawa T, Nakamura A, Onishi N, Yamada T, Matsuo K and Saya H. Acquired expression of NFATc1 downregulates E-cadherin and promotes cancer cell invasion. Cancer Res 2013; 73: 5100-5109.

[20] Fougere M, Gaudineau B, Barbier J, Guaddachi F, Feugeas JP, Auboeuf D and Jauliac S. NFAT3 transcription factor inhibits breast cancer cell motility by targeting the lipocalin 2 gene. Oncogene 2010; 29: 2292-2301.

[21] Brat DJ, Scheithauer BW, Fuller GN and Tihan T. Newly codified glial neoplasms of the 2007 WHO classification of tumours of the central nervous system: angiocentric glioma, pilomyxoid astrocytoma and pituicytoma. Brain pathol 2007; 17: 319-324.

[22] Soumaoro LT, Uetake H, Higuchi T, Takagi Y, Enomoto M and Sugihara K. Cyclooxygenase-2 expression: a significant prognostic indicator for patients with colorectal cancer. Clin Cancer Res 2004; 10: 8465-8471.

[23] Goodenberger ML and Jenkins RB. Genetics of adult glioma. Cancer Genet 2012; 205: 613-621.

[24] Sahgal A, Ironside SA, Perry J, Mainprize T, Keith JL, Laperriere N, Tsao M and Paszat L. Factors influencing overall survival specific to adult low-grade astrocytoma: a population-based study. Clin Oncol 2013; 25: 394-399.

[25] Xia H, Qi Y, Ng SS, Chen X, Li D, Chen S, Ge R, Jiang S, Li G, Chen Y, He ML, Kung HF, Lai L and Lin MC. MicroRNA-146b inhibits glioma cell migration and invasion by targeting MMPs. Brain Res 2009; 1269: 158-165.

[26] Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2002; 2: 442-454.

[27] Yiu GK and Toker A. NFAT induces breast cancer cell invasion by promoting the induction of cyclooxygenase-2. J Biol Chem 2006; 281: 12210-12217.

[28] Chen M and O'Connor KL. Integrin alpha6beta4 promotes expression of autotaxin/ENPP2 autocrinemotility factor in breast carcinoma cells. Oncogene 2005; 24: 5125-5130.

[29] Mott JD and Werb Z. Regulation of matrix biology by matrix metalloproteinases. Curr Opin Cell Biol 2004; 16: 558-564.

[30] Saygili E, Rana OR, Meyer C, Gemein C, Andrzejewski MG, Ludwig A, Weber C, Schotten U, Krüttgen A, Weis J, Schwinger RH, Mischke K, Rassaf T, Kelm M and Schauerte P. The angiotensin-calcineurin-NFAT pathway mediates stretch-induced upregulation of matrix metalloproteinases-2/-9 in atrial myocytes. Basic Res Cardiol 2009; 104: 435-448.

[31] Neria F, del Carmen Serrano-Perez M, Velasco P, Urso K, Tranque P, Cano E. NFATc3 promotes Ca(2+)-dependent MMP3 expression in astroglial cells. Glia 2013; 61: 1052-1066.

[32] Armesilla AL, Lorenzo E, Gómez del Arco P, Martínez-Martínez S, Alfranca A and Redondo JM. Vascular endothelial growth factor activates nuclear factor of activated T cells in human endothelial cells: a role for tissue factor gene expression. Mol Cell Biol 1999; 19: 2032-2043.

[33] Pang B, Fan H, Zhang IY, Liu B, Feng B, Meng L, Zhang R, Sadeghi S, Guo H and Pang Q. HMGA1 expression in human gliomas and its correlation with tumor proliferation, invasion and angiogenesis. J Neurooncol 2012; 106: 543-549.

[34] Buchholz M, Schatz A, Wagner M, Michl P, Linhart T, Adler G, Gress TM and Ellenrieder V. Overexpression of c-myc in pancreatic cancer caused by ectopic activation of NFATc1 and the Ca2+/calcineurin signaling pathway. EMBO J 2006; 25: 3714-3724.

[35] Singh G, Singh SK, König A, Reutlinger K, Nye MD, Adhikary T, Eilers M, Gress TM, Fernandez-Zapico ME and Ellenrieder V. Sequential activation of NFAT and c-Myc transcription fac-

tors mediates the TGF-beta switch from a suppressor to a promoter of cancer cell proliferation. J Biol Chem 2010; 285: 27241-27250.

[36] Wyckoff J, Wang W, Lin EY, Wang Y, Pixley F, Stanley ER, Graf T, Pollard JW, Segall J and Condeelis J. A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. Cancer Res 2004; 64: 7022-7029.

[37] Mosieniak G, Figiel I and Kaminska B. Cyclosporin A, an immunosuppressive drug, induces programmed cell death in rat C6 glioma cells by a mechanism that involves the AP-1 transcription factor. J Neurochem 1997; 68: 1142-1149.

[38] Ngan ES and Tam PK. Prokineticin-signaling pathway. Int J Biochem Cell Biol 2008; 40: 1679-1684.

[39] Negri L, Lattanzi R, Giannini E and Melchiorri P. Bv8/prokineticin proteins and their receptors. Life Sci 2007; 81: 1103-1116.

[40] Maldonado-Pérez D, Brown P, Morgan K, Millar RP, Thompson EA, Jabbour HN. Prokineticin 1 modulates IL-8 expression via the calcineurin/NFAT signaling pathway. Biochim Biophys Acta 2009; 1793: 1315-1324.

[41] Goi T, Nakazawa T, Hirono Y and Yamaguchi A. Prokineticin 1 expression in gastrointestinal tumors. Anticancer Res 2013; 33: 5311-5315.