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AKT2 expression in histopathologic grading and recurrence of meningiomas

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Abstract

Background: AKT2 (protein kinase B), an important protein in PI3K signaling pathway, is overexpressed in a variety of malignant tumors. However, in patients with meningiomas, the potential correlation between AKT2 and clinical outcome remains unknown.

Methods: The expression of AKT2 and Ki-67 in meningioma tissues were evaluated immunohistochemically in 94 patients with meningiomas. The correlation of AKT2 immunoreactivity with clinicopathological features and the prognostic value of AKT2 in patients were also analyzed.

Results: In this study, we examined the expression of AKT2 in meningiomas and unveiled its possible relationship with the clinical outcome. Immunohistochemical analysis revealed high AKT2 expression in 46 patients (46/94, 48.9%) and low AKT2 expression in the remaining 48 patients (48/94, 51.1%). There was a positive correlation between AKT2 and Ki-67 immunoreactivity ($r = 0.35$, $P = 0.01$). Clinicopathological evaluation suggested that AKT2 expression was associated with pathological grade and recurrence ($P < 0.05$). Univariate and Cox analysis indicated a significant correlation between high levels of AKT2 immunoreactivity and high rates of tumor recurrence ($P < 0.05$).

Conclusions: We conclude that AKT2 may play an important role in the development of meningioma. High AKT2 labeling index indicates higher grade of meningioma, and therefore AKT2 may be a useful molecular marker for predicting the prognosis of meningioma.

Keywords: AKT2; Meningioma; Ki-67; Tumor grade; Recurrence

Introduction

Meningiomas are divided into 3 grades according to the 2007 World Health Organization (WHO) classification of tumors of the central nervous system (CNS).[1] Although 90% of meningiomas are classified as benign and can be curable after surgery, a minority of them shows aggressive clinical features, and is classified as atypical (WHO grade II) and malignant (WHO grade III).[2] Even with aggressive intervention like surgery, radiotherapy and other therapeutic modalities, effective treatment for malignant meningiomas is still unavailable.[3] In addition, the molecular mechanisms underlying the initiation, maintenance and progression of high grade meningiomas still remain largely unclarified.[4] Hence, identification and characterization of the regulatory molecules involved in the meningioma development may offer important targets for treatment strategies.

Protein kinase B or AKT is a family of three serine/threonine kinases, including AKT1, 2 and 3.[5] Recently, compared with their corresponding normal tissues, aberrant high expression of AKT2 has been demonstrated in a variety of malignancies, including pancreatic, prostate, ovarian, thyroid, and breast cancers.[6–9] AKT2, an AKT isoform, plays a critical role in tumoral growth, angiogenesis, metastasis, and the inhibition of apoptosis, and has also been held responsible for given chemoresistance.[8–11] We have previously showed that AKT2 overexpression is also common in gliomas, suggesting the therapeutic effect of AKT2 inhibitors

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in such situations.[12] However, the expression pattern and biological function of AKT2 in meningiomas are unknown.

The goal of this study was to examine AKT2 expression in human meningiomas, and its correlations with Ki-67 expression, pathological grade and postoperative recurrence. By analyzing the relationship between AKT2 expression and the progression of meningioma, we might be able to find effective targets for gene therapy of this malignancy.

Materials and methods

Tissue samples

The study protocol and acquisition of tissue specimens were approved by the Specialty Committee on Ethics of Biomedicine Research, Second Military Medical University, China. The acquisition and use of human tissue in this study complied with the National Regulations of Clinical Sampling in China. 249 tissue specimens were obtained from archived tissue samples from patients with meningiomas who underwent surgical treatment at Changzheng Hospital from January 1998 to December 2010. Meningioma was diagnosed according to the 2007 WHO Classification of Tumors of the Central Nervous System and by two experienced pathologists independently. The follow-up was carried out in all patients, with survival time being censored in December 2011. The follow-up was conducted every 6 months with telephone interview. The last follow-up was July 2012. The tumor size, location, extent of surgical resection, and the recurrence-free survival time were recorded. The extent of resection was defined in each case according to the Simpson grading system (grade I, complete tumor and dura resection; grade II, complete tumor resection and coagulation of the dura; grade III, complete tumor resection without coagulation of the dura; grade IV, incomplete tumor resection; and grade V, tumor biopsy). The selection criteria were as follows: 1) the subject had a diagnosis of meningioma and no history of other tumors; 2) the subject had complete clinical data, such as age, gender, clinical manifestations, mean tumor diameter (MTD, defined as the geometric mean of the 3 diameters on MRI scan), extent of resection and adjuvant therapy; 3) the subject underwent evaluation by enhanced head MRI scans for tumor relapse or progression after surgery at least once every six months. Patients receiving radiotherapy prior to the surgery were excluded. According to the selection criteria, 94 meningioma samples were left (Fig. 1).

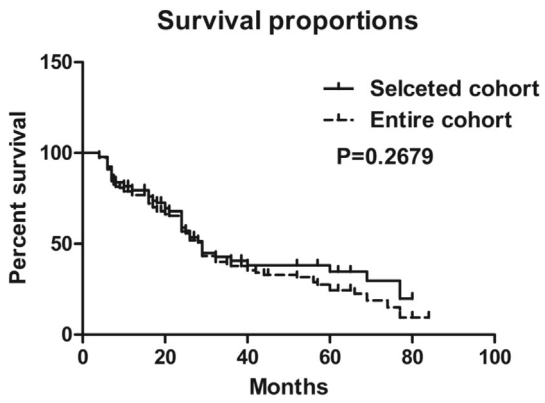

Figure 1. KM survival curve for the entire cohort compared to the selected cohort, we found there is no significant difference between the entire cohort (249 patients) and the selected cohort (94 patients), $P = 0.2679$.

Immunohistochemistry and expression analysis

Formalin-fixed, paraffin-embedded, 3-μm tissue sections were deparaffined in xylol and rehydrated in a graded ethanol series. A microwave antigen retrieval procedure was conducted for 20 min in 1 mM EDTA buffer (pH 8.0). Thereafter, endogenous peroxidase activity was eliminated after processing the specimens with 3% methanolic hydrogen peroxide solution for 30 min. The slides were incubated in nonimmune serum for 30 min. The primary AKT2 monoclonal antibody (Abnova, H000000208-M04) at 1:500 dilution ratio was then added and the sections were incubated overnight at 4 °C. After washing in Tris Buffered Saline Tween (TBST), the sections were incubated with biotin-conjugated secondary antibody for 20 min at room temperature, then with peroxidase-conjugated biotin–streptavidin complex (Dako, Glostrup, Denmark) for 20 min, and were finally visualized with 3, 3′-diaminobenzidin and counterstained with hematoxylin. To ensure the specificity of the immunostaining, some sections were incubated with nonimmune serum instead of the primary antibody before incubation in the secondary antibody and subsequent staining.

Immunostaining for AKT2 was classified according to the percentage of positive cells and to the staining intensity. Scores for the percentage of positive cells were assigned as follows: score of 0 if $\leq$10% of cells positive, score of 1 if 11%–25% of cells positive, score of 2 if 26%–50% of cells positive, score of 3 if 51%–75% of cells positive, and score of 4 if >75% of cells positive. Scores for staining intensity were assigned as follows: 0 for no staining, 1 for light brown, 2 for brown, and 3 for dark brown. Overall scores were obtained by multiplying the percent positive score by the intensity score. Overall scores $\leq$3 were defined as negative or low score, and overall scores >3 were defined as positive or high score. Two independent pathologists examined 5 random fields (1 field = 0.159 mm^2 at ×100 magnification) from each sample, and scored each sample without knowledge of patient outcome (double-blinded).

Statistics

The Kaplan–Meier survival analysis was used to compare the overall survival times in meningioma patients. The Kruskal–Wallis test was used to analyze the AKT2 expression and clinicopathological characteristics. The correlation between AKT2 and Ki-67 immunoreactivity was examined by Spearman's correlation coefficient. Univariate