# Expression of focal adhesion kinase and phosphorylated focal adhesion kinase in human gliomas is associated with unfavorable overall survival

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**Human glioma is a malignancy that has no effective systemic therapy. Focal adhesion kinase (FAK) is overexpressed in various invasive and metastatic tumor cells. To investigate its prognostic value in human gliomas, which currently is unknown, we examined the expression patterns of FAK and its activated form, phospho-FAK (FAK pY397), and analyzed the correlation between their expression and prognosis in patients with gliomas. Immunohistochemical staining was performed to detect FAK and phospho-FAK expression patterns in the biopsies from 96 patients with primary gliomas. Kaplan-Meier survival and Cox regression analyses were performed to evaluate the prognosis of patients. As a result, the immunohistochemical analysis revealed that FAK and phospho-FAK both were associated significantly with the Karnofsky performance scale (KPS) score and World Health Organization (WHO) grades of patients with gliomas. Especially, the positive expression rates of FAK and phospho-FAK were significantly higher in patients with a higher grade ($P$ = 0.01 and 0.02, respectively) and a lower KPS score ($P$ = 0.006 and 0.008, respectively). The patients with FAK positive expression correlated with a poor prognosis of human gliomas ($P$ = 0.006) as well as phospho-FAK ($P$ = 0.01). The survival rate of the patients with FAK+/phospho-FAK+ expression was the lowest ($P < 0.05$), and conjoined expressions of FAK/phospho-FAK were an independent prognostic indicator of human gliomas ($P < 0.05$). In conclusion, the results suggest that the elevated expression of FAK and phospho-FAK is an important feature of human glioma. A combined detection of FAK/phospho-FAK coexpression may benefit us in the prediction of the prognosis of human glioma. (Translational Research 2010;156:45–52)**

**Abbreviations:** ECM = extracelluar matrix; FAK = focal adhesion kinase; GBM = glioblastoma multiforme; GPNMB = glycoprotein nonmetastatic melanoma protein B; GST = glutathione S-transferase; KPS = Karnofsky performance scale; MAPK = mitogen-activated protein kinase; WHO = World Health Organizaiton

Glioma, the most common neoplasm in the human brain, remains a major medical challenge and one of the most malignant diseases. The World Health Organization (WHO) classification scheme divides gliomas into the following 4 grades in order of increasing malignancy[1]: Grades I and II are the least malignant phenotypes and grade III comprises the moderate malignant gliomas—such as anaplastic astrocytoma, anaplastic oligoastrocytoma, and anaplastic oligodendroglioma—whereas grade IV (glioblastoma

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## AT A GLANCE COMMENTARY

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### Background

Human glioma is a malignancy that has no effective systemic therapy. Focal adhesion kinase (FAK) is overexpressed in various invasive and metastatic tumor cells.

### Translational Significance

To investigate its prognostic value in human gliomas, which currently is unknown, we examined the expression patterns of FAK and its activated form, phospho-FAK (FAK pY397), and analyzed the correlation between their expression and prognosis in patients with gliomas.

multiforme, GBM) is the most malignant type of glioma. The patients with GBM have an average life expectancy of less than 1 year; even with recent advances in cancer diagnostic methodology and treatment, the prognosis of GBM has not improved.[2,3] The tumor invasion of normal brain represents a key contributor to treatment failure because an invasive tumor cannot be resected surgically and displays resistance to cytotoxic therapies. Several clinical reports show that the outcome of patients with gliomas in each grade is highly variable, and genetic differences among them may contribute to their different survival.

Integrins, as dynamic signaling molecules, play a role in the mediation of the cell adhesion to the extracelluar matrix (ECM).[4] Integrins allow normal, nontransformed cells to sense that they are adhered to ECM, thus providing a cell survival signal. Integrins also mediate cell migration as it occurs in normal processes such as angiogenesis, wound healing, immune system function, and development.[5] Aberrances in the expression and function of integrins contribute to many disease states including cancer. Focal adhesion kinase (FAK) is a signal transducer of integrins and becomes phosphorylated and activated during integrin-mediated cell adhesion.[6] It integrates signals from activated growth factor receptors and integrins to regulate cell motility, invasion, proliferation, apoptosis, and angiogenesis.[7] Growth factors and integrins lead to the autophosphorylation of FAK on tyrosine 397 (Tyr397), which creates an SH2 binding site for the Src family of tyrosine kinases. Src then is thought to lead to the phosphorylation of several other tyrosine residues, including Tyr861 and Tyr925.[8] FAK can promote tumor growth and originally was shown to be overexpressed at the mRNA or protein levels in invasive and metastatic tumors derived from several tissues including colon,[9] gastrointestinal tract,[10] prostate,[11] thyroid,[12] and ovary.[13] Subsequent immunohistochemical staining demonstrated that subpopulations of cells located within preinvasive (carcinoma in situ) areas within oral squamous cell carcinoma contain enhanced FAK immunoreactivity relative to neighboring cells.[14] In addition, the overexpression of FAK in glioma specimens also was detected. An increased regional expression of FAK is found at the invasive tumor edge, implicating FAK in tumor invasion.[15] Lipinski et al[16] found that the elevated FAK activity correlated with high proliferation and low migratory rates of 4 different human glioblastoma cell lines, which suggest that FAK function as an important signaling effecter in gliomas, and its regulation may be a determining factor in the temporal development of proliferative or migrational phenotypes. The role of FAK in tumor invasion is complex and may demonstrate contradictory results in different models of tumor invasion/motility; moreover, the existing studies only examined total FAK, which becomes activated on the autophosphorylation of tyrosine 397 (FAK pY397). Therefore, it is important to examine the distribution of this form of FAK as well.

In the current study, we analyzed the association of FAK and phospho-FAK, as a separate factor or in combination, with the clinicopathological features and survival of patients with gliomas to evaluate the clinical significance of these 2 markers in the pathological classification and the prognosis assessment of this disease.

## MATERIALS AND METHODS

**Patients and tissue samples.** Ninety-six glioma samples were obtained from 96 Chinese patients with gliomas of different grades. Patient characteristics, including the Karnofsky performance scale (KPS) score, were collected before the initial surgery. After surgical resection of their tumors, patients with a high-grade glioma received a course of external beam radiation therapy (standard doses: 40 Gy to the tumor with 3-cm margins, 20 Gy boost to the whole brain) and nitrosourea-based chemotherapy during the course of the disease. Surgically resected tissues were frozen immediately and stored at –80°C until processing. Tumors were classified histopathologically according to the WHO classification. Eligibility criteria included written informed consent and availability of frozen tumor tissue and of follow-up data. Clinical information was obtained by reviewing the medical records on radiographic images, by telephone or written correspondence, and by review of death certificate. A patient was considered to have recurrent disease if this issue was revealed either by magnetic resonance