Focal Adhesion Kinase Expression in Human Neuroblastoma: Immunohistochemical and Real-time PCR Analyses

Elizabeth A. Beierle,1 Nicole A. Massoli,2 Joseph Hartwich,1 Elena V. Kurenova,1 Vita M. Golubovskaya,1 William G. Cance,1 Patrick McGrady,3 and Wendy B. London3

Abstract Purpose: The focal adhesion kinase (FAK) is a nonreceptor protein tyrosine kinase important in signaling between cells and their extracellular matrix. Studies have shown that FAK expression is up-regulated in several human tumors and is related to tumor progression. We recently found an increase in p125FAK expression in human neuroblastoma cells lines and wished to determine its expression in human neuroblastoma specimens and evaluate for a possible correlation between p125FAK expression and known prognostic factors for neuroblastoma. We hypothesized that p125FAK expression would be up-regulated in advanced human neuroblastomas.

Experimental Design: Using immunohistochemical techniques with monoclonal antibody 4.47 specific for p125FAK expression, we analyzed 70 formalin-fixed, paraffin-embedded human neuroblastoma specimens for p125FAK staining. In addition, real-time PCR was used to determine the abundance of FAK mRNA in 17 matched human neuroblastoma mRNA specimens.

Results: FAK staining was present in 51 of the 70 tumor specimens (73%). Immunohistochemical staining of p125FAK in the ganglion-type tumor cells correlated with advanced International Neuroblastoma Staging System tumor stages and FAK mRNA abundance. In addition, p125FAK staining was significantly increased in stage IV tumors with amplification of the N-MYC oncogene.

Conclusions: These novel findings provide evidence that FAK is expressed by advanced-stage neuroblastoma and provide a rationale for targeting FAK in the treatment of this tumor.

Focal adhesion kinase (FAK) is a 125-kDa, nonreceptor protein tyrosine kinase that was originally isolated from embryonic chick fibroblasts transformed by v-src (1). FAK is localized to focal adhesions or contact points between cells and their extracellular matrix and has been shown to have important functions in integrin signaling, cellular motility, and cellular apoptosis. Integrins bind to FAK through their β subunits, leading to the phosphorylation of FAK (2), thereby creating a high-affinity binding site for the Src family kinases (3–5). The FAK-Src complex activates protein kinases that eventually result in the activation of nuclear transcription factors leading to cell differentiation and growth. In addition, phosphorylation of FAK results in the binding and activation of other signaling molecules (6) resulting in decreased apoptosis and increased cellular survival.

FAK has been shown to be overexpressed in several human tumors (7), with numerous reports of significantly increased FAK protein expression in primary and metastatic breast cancer (8–11) and in colon (8, 12, 13), thyroid (14, 15), ovarian (16), and esophageal cancers (17), head and neck tumors (18), and melanoma (19). The expression of FAK mRNA has also been shown to correlate with tumor aggressiveness, with an increased abundance of FAK mRNA being seen during the progression of epithelial tumors to metastatic phenotypes (20) and also in the hepatic metastasis of human colorectal carcinomas (12). Data correlating increased fak gene copy number with increased protein expression are not consistent, however. Agochiya et al. showed that human cancer cell lines derived from invasive epithelial tumors have an increased dosage of the fak gene, which may contribute to the elevated protein expression commonly seen in these tumors (21). In contrast, a recent study with head and neck squamous cell carcinomas failed to correlate the increased FAK protein expression seen in these malignancies with an increase in fak gene copy number (22). Neuroblastoma is the most common extracranial solid tumor of childhood. Despite aggressive medical and surgical interventions, there has been little change in the overall survival (OS) in the last decade for children presenting with advanced-stage disease (23). New biomarkers and potential therapeutic targets will be needed to provide novel strategies for the detection and treatment of this tumor. We have recently reported an increase in both FAK mRNA and protein expression in human neuroblastoma cell lines that have amplification of the N-MYC oncogene (24). To date, there have been no reports detailing the expression of FAK in human neuroblastoma specimens. We
hypothesized that FAK expression may be increased in human neuroblastoma tumor specimens. In addition, we wished to determine whether FAK expression in human neuroblastoma specimens would correlate with known neuroblastoma prognostic factors such as International Neuroblastoma Staging System (INSS) stage, amplification of the N-MYC oncogene, and patient age at diagnosis or with patient outcome. We used immunohistochemistry and real-time PCR analyses to investigate FAK expression in human neuroblastoma specimens. Here, we report that human neuroblastoma specimens do stain for p125FAK. In addition, we show that FAK mRNA abundance correlates with FAK staining in the ganglion-type cells. Finally, we found increased FAK staining in N-MYC amplified advanced INSS stage neuroblastoma tumor specimens.

Materials and Methods

Tissue specimens. Before the initiation of this study, we obtained approval from the University of Florida Institutional Review Board (140-2003). The Children’s Oncology Group tumor bank was petitioned for formalin-fixed, paraffin-embedded neuroblastoma tumor specimens. Tumor specimens from 70 neuroblastoma patients were provided in 5 µm sections on positive slides. In addition, mRNA was provided from 17 of the 70 patients. The specimens were completely masked for all patient data including patient demographics, tumor stage, and N-MYC amplification status, and patient outcomes.

Immunohistochemistry. Immunohistochemical analyses were done as described previously (7, 8) with the following modifications: Slides were deparaffinized and rehydrated through xylene and decreasing concentrations of ethanol including quenching of endogenous peroxidase activity (3% hydrogen peroxide in methanol). Slides were transferred to 1 x TBS. During heat-induced antigen retrieval, sections were heated with a steamer while submerged in Citra buffer (pH 6.0; Biogenex) for 30 min. Sections were subsequently rinsed in 1 x TBS. A universal protein blocker, Sniper (Biocare Medical) was applied for 15 min at room temperature followed by rinsing in 1 x TBS. Slides were incubated overnight at 4°C with anti-FAK 4.47 mouse monoclonal antibody (1:200; Upstate Biotechnology) or MsIgG1 (clone MOPC21/321; Biosource International). The slides were rinsed in 1 x TBS followed by application of a conjugated secondary polymer, Mach 2 Mouse HRP Polymer (Biocare Medical), for 30 min at room temperature. The chromogenic reaction was done with Cardasian 3,3-diaminobenzidine (Biocare Medical) for 6.75 min at room temperature. Slides were counterstained with hematoxylin (Vector Laboratories) for 1 min followed by bluing with 1 x TBS for 1 min. Slides were then mounted and coverslipped with cytoseal XYL (Richard-Allen Scientific). Positive and negative (MsIgG1) controls were included with each run. The specificity of our analyses was tested by blocking the immunoreactivity in the tissue specimens by incubating the antibody with recombinant FAK protein. In addition, the specificity of FAK 4.47 antibody for detection of p125FAK by immunohistochemistry has been shown by several authors in previous publications (8, 12, 17).

Immunohistochemistry scoring. A single board-certified pathologist (N.A.M.), blinded to the specimens, reviewed each tissue section for FAK expression and assigned a stain score. Scoring was based on a system that has been used previously (7, 8, 12). The slides were examined and the staining was evaluated by measuring the intensity of stain (0, none; 1, weak; 2, moderate; 3, strong, 4, extremely strong), the percentage of positive cells (0-100) and the tumor cell type staining positive (ganglion-type cells or small, round blue cells). A stain score was calculated based on the percent positive cells and the amount of staining. For example, if the specimen showed moderate staining (2) in 50% of the cells and strong staining (3) in 20% of the cells, the calculated stain score would be (2 x 50) + (3 x 20) = 160.

Measurement of FAK mRNA using real-time quantitative PCR. For TaqMan quantitative PCR, the following protocol was employed. TaqMan PCR primers and probes for FAK were obtained from Applied Biosystems. Probes were labeled with a reporter dye, 6-carboxyfluorescein phosphoramidate at the 5' end and with 6-carboxytetramethyl-rhodamine as a quencher dye at the 3' end. TaqMan PCR was done with 10 ng cDNA in 50 µL reaction volume containing TaqMan Universal PCR Master Mix and TaqMan gene expression assay (Applied Biosystems). Amplification was done using an ABI PRISM 7700 sequence detection system (Applied Biosystems). Cycling conditions were 50°C for 2 min, 95°C for 10 min followed by 40-cycle amplification at 95°C for 15 s and 60°C for 1 min. The ABI PRISM 7700 Cycler software calculates a threshold cycle number (CT) at which each PCR amplification reaches a significant threshold level. The threshold cycle number is proportional to the number of FAK RNA copies present in the sample reaction mix.

Cyclophilin A was used as an internal standard for all assays and FAK sRNA was used as a positive control and as an absolute standard in all assays. As described previously (12), full-length FAK cDNA was subcloned in to the pcDNA3 vector bearing the T7 promoter. This construct was used in vitro to transcribe FAK sRNA using the MEGAscript kit (Ambion). Amplification of serial dilutions of the FAK sRNA was used to construct standard curves and determine the FAK message levels. Using this absolute standard, FAK message levels were measured accurately over the 6-log range from 475 to 475 million template copies (1-1x107 fg). Any DNA contamination was resolved by treating all total RNA isolates with DNase (RNase-free; Ambion). To minimize the experimental variability, all of the neuroblastoma specimens were analyzed for FAK mRNA abundance using the same master reaction mix. Each sample was tested in triplicate and the mean femtogram expression level calculated and converted to copy number using the formula (6.02 × 1012 copies/mol) × (measured FAK g) / (molecular weight of FAK message). The molecular weight of the linearized FAK sRNA calculated from its nucleotide sequence was M, 1266501.

Statistical methods. A Pearson correlation test was used to determine association between FAK mRNA abundance and immunohistochemical staining. To determine possible associations between neuroblastoma risk factors and FAK immunohistochemical staining and FAK mRNA abundance, an exact two-sided Wilcoxon rank-sum test was used. A Fisher’s exact test was used to evaluate the stage IV cohort separately based on staining and N-MYC amplification. Statistical significance was determined at the P < 0.05 level.

In addition, Kaplan-Meier survival curves were generated (25), and the log-rank test was used to determine if the level of p125FAK staining was predictive of outcome. Within subgroups, FAK expression levels were dichotomized on the median value for the given cohort, and FAK mRNA abundance, an exact two-sided Wilcoxon rank-sum test was used. For the overall cohort (n = 70), the expression level was dichotomized on the value that maximized the difference in outcome for low versus high expression, that is, the cutoff point with the lowest log-rank test value for both EFS and OS. To make adjustment for multiple testing, P values less than 0.01 were considered statistically significant. All of the statistical analyses were done with either SAS statistical software (version 9; SAS Institute) or SPSS for Windows (version 14: SPSS).

Results

Immunohistochemical analysis of p125FAK expression in human neuroblastomas. We did immunohistochemistry for FAK

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The expression on 70 human neuroblastoma samples. The median age for the staining cohort was 2.4 years (range, 0.03-10.9). Eleven of the patients were <1 year old and 59 were ≥1 year old. The cohort was evenly divided between males and females with 36 and 34, respectively. A majority of the specimens were from patients with INSS stage IV tumors (46), leaving 24 of the specimens from INSS stage I, II, III, or IVS disease. Immunohistochemistry results are presented in Table 1. FAK staining was detected in 51 of the 70 neuroblastoma specimens (73%). Standard histologic examination of human neuroblastoma specimens shows the presence of two cell types: small, round blue cells, usually more immature cells, and larger, more mature ganglion-type cells. The specimens in this study consisted of tumors with both of these types of commonly encountered cells. The small, round blue cell types were present in all 70 of the specimens (Fig. 1A) and FAK staining was seen in these cells in 45 (64%) of the specimens. Figure 1B is a photomicrograph showing positive staining for p125FAK by immunohistochemistry. In this example, over 90% of the cells in the displayed field show positive staining for p125FAK (Fig. 1B). Seventeen of the 70 specimens (24%) also had areas that displayed ganglion-type cells, and there was positive FAK staining in these cells in 16 of those 17 tumors (94%; Fig. 1C). The mean stain score for the entire staining cohort (n = 70) was 83.4 and the median stain score was 50. The staining cohort sample was further stratified by known neuroblastoma biological risk factors and is presented in Table 2. FAK staining based on the neuroblastoma cell type was tested for associations with demographic and biological factors known to influence the survival in neuroblastoma, mainly tumor stage (INSS) at diagnosis, amplification of the N-MYC oncogene, and patient age at diagnosis. Using an exact two-sided Wilcoxon rank-sum test, no correlation was noted between FAK staining and patient age at diagnosis in either cell type (Table 2). There was a tendency for FAK staining in the small, round blue cell type to correlate with amplification of the N-MYC oncogene and a strong correlation between FAK staining in the ganglion-type cells with advanced INSS stage (stage IV) tumors (P = 0.0091; Table 2). Further evaluation of advanced-stage tumors (stage IV) as a single cohort in relation to known biological prognostic factors revealed that FAK staining in the small, round blue cells

<table>
<thead>
<tr>
<th>Table 1. FAK protein staining and mRNA abundance in human neuroblastoma tissue samples (Cont’d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
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<tr>
<td>---------</td>
</tr>
<tr>
<td>64</td>
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<td>68</td>
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<tr>
<td>69</td>
</tr>
<tr>
<td>70</td>
</tr>
</tbody>
</table>

*Blank = cell type not present.  
†0 = cell type present with no staining.

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was associated with amplification of the N-MYC oncogene ($P = 0.016$; Table 3). These results clearly show that $p125^{FAK}$ staining is increased in advanced-stage neuroblastomas and is associated with amplification of the N-MYC oncogene in stage IV tumors.

**Real-time PCR analysis of FAK mRNA abundance in human neuroblastoma.** Conflicting reports in the literature regarding the correlation of FAK protein with FAK mRNA prompted us to extend our investigations in neuroblastoma to the mRNA level using real-time PCR analysis. We were provided with mRNA from 17 neuroblastoma patients matched with those from whom we had received tissues for immunohistochemistry. The median age for the PCR cohort was 2.7 years (range, 0.03-7.4), with 2 of the patients being $<1$ year old and 15 of the specimens coming from patients that were $>1$ year old. Again, sex distribution was equal with 9 males and 8 females. Also, most of the specimens were from advanced INSS stage IV tumors (11), but most were from tumors that did not have amplification of the N-MYC oncogene (12). A standard curve was generated using sRNA for FAK for the purpose of calculating abundance of FAK mRNA in the specimens. All 17 specimens expressed mRNA for FAK (Table 1), with a median FAK mRNA copy number of 31,552 (range, 9,350-214,704 copies). The PCR cohort sample was stratified by neuroblastoma risk factors and is presented in Table 2. The mRNA data were then analyzed for correlation with demographic and biological factors known to influence the survival in children with neuroblastoma, that is, INSS tumor stage at diagnosis, amplification of the N-MYC oncogene, and patient age $>1$ year at diagnosis. No statistically significant correlations between FAK mRNA and these known risk factors were seen (Table 2). FAK mRNA abundance was then compared with $p125^{FAK}$ staining as detected by immunohistochemistry. There was a statistically significant positive correlation between abundance of FAK mRNA (median copy number, 31,552) and $p125^{FAK}$ staining (median stain score, 87.5) in the ganglion-type cells ($P = 0.05$). Therefore, these data clearly show that FAK mRNA is present in human neuroblastoma specimens and that the abundance of mRNA does correlate with FAK immunohistochemical staining in the ganglion type cells.

**Analysis of $p125^{FAK}$ staining and FAK mRNA with neuroblastoma outcomes.** In the final analysis of these data, Kaplan-Meier survival curves were generated, and the log-rank test was used to determine if mRNA and staining level was predictive of outcome (Fig. 2). Levels of FAK staining were dichotomized on the median value for the given cohort, and EFS and OS rates for low versus high expression were compared. In the cohorts with $\leq 17$ patients (mRNA data and ganglion-type cell immunohistochemical staining data), the survival analyses were underpowered due to the low number of events and deaths that occurred, and no significant correlation could be detected between FAK mRNA abundance and ganglion-type cell staining with either EFS or OS. The patient cohort with the data for small, blue cell FAK staining ($n = 70$) was large enough for survival analyses overall (Kaplan-Meier; Fig. 2). Those patients with staining greater than the median tended to do more poorly in the first year, but no statistically significant correlation was detected between FAK mRNA abundance and ganglion-type cell staining with either EFS or OS. The patient cohort with the data for small, blue cell FAK staining was also large enough for survival analyses within the following subsets: INSS stages I, II, III, or IVS; stage IV; N-MYC nonamplified; N-MYC amplified; age $<1$ year at diagnosis; and age $\geq 1$ year at diagnosis.
diagnosis, by the median FAK staining of that particular subset. No statistically significant relationships with outcome, either EFS or OS, were found (Table 4).

**Discussion**

These results provide definitive evidence that FAK is elevated in human neuroblastoma tumors as detected by both mRNA and immunohistochemical analyses and that the abundance of FAK mRNA correlated with the FAK immunohistochemical staining in the ganglion-type cells. In addition, the amount of FAK staining in the ganglion-type cells correlated with advanced-stage disease at presentation. Finally, p125FAK staining in stage IV tumor specimens was associated with amplification of the N-MYC oncogene, the primary adverse prognostic determinant for this tumor. To our knowledge, this current report is novel in its investigation of FAK in human neuroblastoma specimens.

The mechanisms by which FAK contributes to neuroblastoma aggressiveness and metastasis are currently not known, but it is interesting to discuss the possible reasons for the high levels of FAK expression in this tumor. FAK is known to be a survival signal for anchorage-dependent growth (27–29). It is possible that FAK expression is up-regulated by the neuroblastoma tumors to maintain survival as the tumor proceeds from anchorage-dependent to anchorage-independent growth and metastasis. FAK has also been shown in other human tumors to have antiapoptotic properties through the up-regulation of the phosphatidylinositol 3-kinase-Akt pathway with subsequent increases in the antiapoptotic Bcl-2 protein and decreases in the proapoptotic protein Bax (6, 30–32). In neuroblastoma, increased expression of Bcl-2 and decreased expression of Bax have been shown to correlate with poorer prognosis (33, 34) and decreased sensitivity to chemotherapeutic drugs (35). Therefore, it is conceivable that FAK is serving as an upstream survival signal in neuroblastoma.

Amplification of the N-MYC oncogene is the primary adverse prognostic indicator in neuroblastoma. In the current study, we analyzed FAK staining in the stage IV tumors independently, and this analysis clearly showed that in advanced-stage IV tumors, increased FAK staining correlated with amplification of the N-MYC oncogene. These in vivo findings support our recent findings in vitro of increased FAK expression by N-MYC-expressing neuroblastoma cell lines (24).

It is interesting to note that the ganglion-type neuroblastoma cells had significant FAK staining. There are several explanations

**Table 2. Association between neuroblastoma biological risk factors and FAK staining and FAK mRNA abundance**

<table>
<thead>
<tr>
<th>Biological risk factor</th>
<th>Ganglion-type cells FAK (n = 17)</th>
<th>Small, round blue cells FAK (n = 70)</th>
<th>PCR cohort (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INSS stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I, II, III, or IVS</td>
<td>10</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>IV</td>
<td>7</td>
<td>46</td>
<td>11</td>
</tr>
<tr>
<td><em>P = 0.0091</em></td>
<td></td>
<td><em>P = 0.7985</em></td>
<td><em>P = 0.6165</em></td>
</tr>
<tr>
<td>N-MYC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonamplified</td>
<td>11</td>
<td>34</td>
<td>12</td>
</tr>
<tr>
<td>Amplified</td>
<td>6</td>
<td>36</td>
<td>5</td>
</tr>
<tr>
<td><em>P = 0.5968</em></td>
<td></td>
<td><em>P = 0.0696</em></td>
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<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>2</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>≥1</td>
<td>15</td>
<td>59</td>
<td>15</td>
</tr>
<tr>
<td><em>P = 0.5956</em></td>
<td></td>
<td><em>P = 0.7945</em></td>
<td><em>P = 0.2092</em></td>
</tr>
</tbody>
</table>

*Fig. 2. Kaplan-Meier survival curves for small, blue cell FAK staining and EFS and OS. A and B, Kaplan-Meier survival curves examining the correlation between FAK staining in the small, round blue type cells and EFS and OS. Using n = 70 with a median stain score of 25, there was no significant correlation with either EFS (*P = 0.7434*) or OS (*P = 0.9722*).*
for this finding. Ganglion-type cells are commonly associated with maturing or differentiating neuroblastomas. However, in our study population, 60% of the ganglion-type cells were found in either advanced INSS stage or N-MYC oncogene amplified tumor specimens. Therefore, the stronger FAK staining in this cell type was seen in more advanced tumors and may be due to sampling bias of our specimens. However, we have encountered findings similar to these in the past, when we noted FAK to be overexpressed in premalignant lesions including breast ductal carcinoma in situ (36) and in premalignant villous adenomas of the colon (7). As reported previously (7, 36), we did not find a linear relationship between FAK staining and patient outcomes but clearly showed that increased FAK immunohistochemical staining was statistically associated with more advanced tumors.

We conclude from these studies that FAK is associated not only with malignant tumor survival but also with tumor progression from the premalignant to the malignant and metastatic states.

Several previously mentioned reports describe an association between patient survival and FAK expression. We were unable to correlate FAK mRNA expression with neuroblastoma prognostic markers or EFS or disease-free survival. Other authors have found similar results with other tumors (9, 22, 37). A recent report evaluating node-negative breast tumors found no statistically significant association of FAK overexpression and EFS or disease-free survival (37). These findings in our study are likely related to the sample size (n = 17), providing too little power to detect modest associations and only strong associations would be detected with the entire cohort (n = 70).

Immunohistochemistry using the monoclonal FAK-specific 4.47 antibody showed positive staining for FAK in 73% of the tumors. There was a correlation between the p125FAK staining in the ganglion-type tumor cells and advanced-stage disease and a correlation between N-MYC amplification and p125FAK expression in stage IV tumors but no statistically significant correlation with other neuroblastoma prognostic factors or patient survival.

Again, other reports have shown a lack of a linear correlation between FAK immunostaining and patient outcome (22, 37). There are several possible explanations for these findings. As reported previously, the FAK epitope is affected by formalin fixation and paraffin embedding, and tumor handling may affect results (8, 12), but in our study, antigen retrieval was used to overcome this difficulty. There is also significant heterogeneity in neuroblastoma tumors and the specific section of the tumor examined may alter the findings. The tumors consist of several types of cells ranging from small, round blue cells with little cytoplasm to large purely ganglion cells with an abundance of cytoplasm. Because FAK is chiefly a cytoplasmic epitope, it would inherently be more difficult to detect FAK staining in these cells with less cytoplasm, making statistical comparisons with small sample sizes difficult. In addition, it is possible that it is not FAK alone that is important for patient prognosis, but the presence of multiple survival signaling pathways in addition to FAK, such as Src, epidermal growth factor receptor, or vascular endothelial growth factor receptor that, when acting in concert, will contribute to patient prognosis. In fact, we have shown previously that simultaneous inhibition of FAK and Src (38) enhances apoptosis in human colon cancer cells and dual inhibition of FAK and epidermal growth factor receptor (39) or FAK and vascular endothelial growth factor receptor 3 (40) induces apoptosis in breast cancer cell lines.

In summary, these results are the first definitive biological evidence that human neuroblastomas express p125FAK. There are previous studies suggesting that FAK is a potential target for therapy in human tumors. Attenuation of FAK in vitro with either a dominant-negative FAK protein (41–43) or FAK antisense oligonucleotides (44, 45) results in cellular rounding, detachment, and significant apoptosis in human tumor cells. Further, down-regulation of FAK in nontransformed fibroblasts (44), normal mammary cells (43), or other normal cell types (46) that express FAK does not result in increased apoptosis in these normal cells. Currently, there are at least two FAK inhibitors that are being investigated as cancer treatments (47–50). One of these inhibitors is a dual inhibitor of both FAK and insulin-like growth factor (48, 49). We have done initial investigations with a FAK inhibitor (TAE226) in neuroblastoma cell lines and have found it to be effective in inhibiting tumor cell growth in vitro. The associations between FAK immunostaining and advanced-stage disease and N-MYC amplification found in this study of human neuroblastoma specimens suggest that FAK is a potential target for therapeutic interventions in neuroblastomas.

Acknowledgments

We thank the Children’s Oncology Group Neuroblastoma Tumor Bank for providing the specimens and Dr. Martha Campbell-Thompson and Tracy Clarke and the other members of the University of Florida Molecular Pathology and Immunology Core Laboratory for contributions to the immunohistochemistry studies.

Table 4. Survival analysis based on FAK staining and mRNA abundance

<table>
<thead>
<tr>
<th>Expression cohort</th>
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<th>Median expression</th>
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<th>OS P</th>
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<td>FAK mRNA (copies)</td>
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<td>31,552</td>
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References


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