Overexpression of Focal Adhesion Kinase in Primary Colorectal Carcinomas and Colorectal Liver Metastases: Immunohistochemistry and Real-Time PCR Analyses

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ABSTRACT

Purpose: Focal adhesion kinase (FAK), a protein tyrosine kinase that functions in signaling events between cells and their extracellular matrix, is up-regulated in colorectal cancer, we analyzed FAK mRNA and protein levels in primary colorectal tumors and colorectal liver metastases.

Experimental Design: p125FAK expression in formalin-fixed paraffin-embedded (FFPE) tissue was studied using immunohistochemical assays on 24 matched primary colorectal carcinomas and colorectal liver metastases as well as 18 different colorectal liver metastases using monoclonal anti-FAK 4.47. FAK mRNA expression was quantitated by real-time PCR on 39 matched normal colorectal mucosa and primary colorectal carcinomas as well as on 17 separate liver metastases.

Results: Elevated levels of p125FAK expression were demonstrated in both primary colorectal tumors and colorectal liver metastases compared with normal colorectal mucosa. Immunohistochemistry experiments demonstrated equivalent FAK expression in matched samples of colorectal primary tumors and liver metastases. Using real-time PCR in 39 matched samples, FAK mRNA copy number was significantly higher in primary colorectal tumors compared with normal colorectal mucosa. FAK expression was analyzed by both real-time PCR and immunohistochemistry in a separate set of colorectal liver metastases. Immunohistochemistry demonstrated high levels of FAK expression in 89% of samples. Furthermore, FAK mRNA copies in these unmatched liver metastases were significantly higher than the primary tumor FAK mRNA copies.

Conclusion: These experiments have shown that both primary colorectal cancers and colorectal liver metastases express high levels of FAK mRNA and p125FAK protein. Furthermore, the majority of colorectal liver metastases demonstrated robust FAK expression equivalent to or greater than that in the primary colorectal tumor.

INTRODUCTION

Originally isolated from v-src-transformed chicken embryo fibroblasts (1), FAK3 is a protein tyrosine kinase involved in signaling events between cells and their extracellular matrix. The M125,000 FAK protein functions in cellular motility (2), integrin-signaling pathways (3–6), and apoptosis (7–9). In human tumors, FAK has been shown to function as a survival signal for tumor cells to resist the apoptotic stimuli of invasion and metastasis. Previously, our group has examined a variety of tumor cell lines and shown that loss of adhesion and subsequent apoptotic cell death resulted when FAK expression was attenuated by antisense oligonucleotides (7) or dominant-negative gene constructs that disrupt FAK signaling (10).

The human FAK gene was first isolated from a primary human sarcoma (11). Initial studies of FAK expression in human tumors linked elevated levels of FAK mRNA to the progression of epithelial and mesenchymal tumors to invasive and metastatic phenotypes (12). Studies at the protein level consistently demonstrated that FAK overexpression as a neoplastic tissue developed the capacity for invasion and metastasis (13, 14). In addition, FAK has been shown to be overexpressed in a variety of tumors, including breast (13, 15), thyroid (13), ovarian (16), head and neck (17), and colon (13, 15, 18).

Although several studies have demonstrated that primary and metastatic colorectal tumors overexpress FAK (12, 13, 18), a recent report by Ayaki and colleagues reported reduced levels of FAK expression in colorectal liver metastases (19). To clarify these disparate findings, we examined FAK expression at both the mRNA and protein level in matched samples of colorectal primary tumors with corresponding liver metastases in addition to a separate group of liver metastases from known colorectal...
cancer of the colon and rectum. Using real-time PCR as well as immunohistochemical analyses, we have demonstrated elevated levels of FAK expression in colorectal liver metastases at both mRNA and protein levels.

**MATERIALS AND METHODS**

**Tissue Specimens**

FFPE as well as snap-frozen colon and liver tissue were obtained from the University of North Carolina Department of Pathology and University of North Carolina-Linberger Comprehensive Cancer Center Tissue Procurement and Analysis Facility. FFPE-matched tissue samples of primary colorectal carcinoma and liver metastasis from the same patient were obtained from 24 patients. FFPE and snap-frozen tissue samples of liver metastasis from a known primary colorectal carcinoma were obtained from 18 and 17 patients, respectively. Snap-frozen matched tissue samples of normal colorectal mucosa and primary colorectal carcinoma were obtained from 39 patients. Serial 5-μm sections were cut from each paraffin block, placed on positive coated slides, and stored at 4°C.

**Immunohistochemistry**

Immunohistochemical analyses were performed as described previously (20, 21) with the following modifications: slides were heated at 60°C for 1 h before deparaffinization, rehydration, and quenching of endogenous peroxidase activity (3% hydrogen peroxide in methanol). The hydration process was completed by rinsing for 5 min in DAKO Tris buffered saline buffer (DAKO Corporation, Carpinteria, CA) containing 0.05% Tween 20 (DAKO Corporation). During heat-induced epitope recovery (22, 23), sections were steamed-heated in a standard steamer (Black and Decker) while submerged in antigen-retrieval Citra buffer (BioGenex, San Ramon, CA) for 30 min. Sections were blocked in normal horse serum (Vectastatin Elite kit; Vector Laboratories, Burlingame, CA) for 15 min and then incubated with anti-FAK 4.47 monoclonal antibody (1:250; Upstate Biotechnology, Lake Placid, NY) overnight at 4°C. The antibody reactivity in paraffin-embedded samples, we used a GST-FAK fusion protein (GST-FAK-NT), containing the NH2-terminal portion of FAK that generated the monoclonal antibody 4.47 (15). GST-FAK-NT (1 μg/μl) and GST (1 μg/μl) control were incubated with the primary antibody, anti-FAK 4.47, for 1 h. To remove antibody reactivity, the GST antibody mixture was centrifuged three times at 6000 rpm with glutathione Sepharose 4B (Amersham Pharmacia Biotech, Buckinghamshire, England, United Kingdom) before application of the primary antibody to the tissue sections.

**Immunohistochemistry Scoring**

A single board-certified pathologist (C.A.L.) scored each tissue section for FAK expression based on a scoring system that measured intensity (0, none; 1, borderline; 2, weak; 3, moderate; 4, strong), percentage positive cells (0–100), cellular localization (cytoplasm, nucleus, membrane, or combination thereof), and overall distribution (homogeneous or heterogeneous).

**Western Blot Analysis**

Lysates of colorectal liver metastases were prepared as described previously (13), and 50 μg of cell lysate were analyzed for FAK and β-actin expression by Western blotting using anti-FAK 4.47 monoclonal antibody (1:1000 dilution) and anti-β-actin AC-15 monoclonal antibody (1:2000 dilution; Sigma, St. Louis, MO). To demonstrate specificity of anti-FAK 4.47, we used recombinant FAK protein GST-FAK-NT to block immunoreactivity. Anti-FAK 4.47 monoclonal antibody (1:1000 dilution) was incubated with GST (1 μg/μl) and GST-FAK-NT (1 μg/μl) for 30 min on ice. The membrane was then probed with the antibody/fusion protein mixtures for 1 h at room temperature with shaking. After washing the membrane with washing buffer, the membrane was probed with goat antismouse IgG-horseradish peroxidase (1:5000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA). Protein was visualized by enhanced Chemiluminescence detection system (Amersham, Arlington Heights, IL).

**Measurement of FAK mRNA Using Real-Time Quantitative PCR**

**Synthesis of a FAK-specific Probe.** A FAK-specific and nonextendable oligonucleotide probe was designed from a cDNA sequence obtained from GenBank using the GCG analysis programs (University of Wisconsin). FAK-specific 5′ and 3′ oligonucleotides and an intervening fluorescent dye-labeled probe were fashioned using Primer Express (ABI/Perkin-Elmer). The probe was synthesized, labeled with 3′ TAMRA (6-carboxy-tetramethylrhodamine; Ref. 24) and 5′ FAM 6-carboxyfluorescein, and purified by high-performance liquid chromatography (Integrated DNA Technologies, Coralville, IA).

**Real-Time PCR Conditions.** The probe was hybridized to the target cDNA between the 5′ and 3′ oligonucleotides and, as amplification proceeded, the 5′-fluorophor was cleaved off the probe by 5′ nuclease activity of the polymerase. Free in solution, the 5′-fluorophor was no longer quenched by TAMRA, resulting in increased fluorescence at 518 nm. Ribogreen ( Molecular Probes, Eugene, OR) fluorescence was used to accurately quantitate starting levels of 10 ng/μl total-RNA extracted from the tumor samples. Fluorescence intensity produced during the PCR amplifications in each of 96 wells was monitored every 8.5 s using the 96-well thermal cycler ABI PRISM 7700 (Perkin-Elmer-ABI, Foster City, CA). A real-time amplification plot was generated for each well, for which the number of amplification cycles was plotted on the X axis and...
the log of change in fluorescence over baseline ($\Delta R_n = \text{fluorescence-baseline fluorescence}$) on the Y axis. The instrument’s software calculated a threshold cycle number (Ct) at which each PCR amplification reached a significant threshold level. This threshold cycle was directly proportional to the number of specific FAK template copies present in the sample.

FAK sRNA was used as positive control and absolute standard in all assays. In brief, full-length FAK cDNA was subcloned into the pcDNA3 vector bearing the T7 promoter. This linearized construct was used in vitro to transcribe FAK sRNA using the MEGAscript kit (Ambion, Austin, TX). Amplification of 2-fold serial dilutions of the FAK sRNA was used to construct standard curves and determine the dynamic range of the assay. Using this absolute standard, FAK message levels were measured accurately over the five-log range from 200 copies to 90 million template copies (0.1 femtograms to $2 \times 10^5$ femtograms).

**RNA Isolation and Quantitation of FAK mRNA Expression.** Total RNA was isolated using the guanidinium isothiocyanate-based system (RNaseasy; Qiagen, Valencia, CA). Snap-frozen tumor specimens were power homogenized (Power Gen 125; Fisher Scientific, Pittsburgh, PA) before chloroform extraction. Any DNA contamination was resolved by treating all total-RNA isolates with DNase (RNase-free; Ambion). Each mRNA unknown was tested for DNA contamination by including reactions lacking the reverse transcriptase as a control.

All of the colorectal carcinomas and liver metastases were analyzed for FAK expression levels in individual experiments using the same master reaction mix to minimize experimental variability. Each tissue sample was tested in triplicate and the mean femtogram expression level calculated and converted to copy number using the formula $(6.02 \times 10^{23} \text{copies/mol}) \times (\text{measured FAK grams})/(\text{molecular weight of FAK message})$. The molecular weight of the linearized FAK sRNA calculated from its nucleotide sequence was $M_r$ 1266501.

**Statistical Methods**

We used the nonparametric Wilcoxon signed-rank test on calculated difference scores (colorectal primary tumor FAK mRNA copies minus normal colorectal mucosa FAK mRNA copies).
copies, and log10 colorectal primary tumor FAK mRNA copies minus log10 normal colorectal mucosa FAK mRNA copies. We used the Wilcoxon two-group test (using normal scores) for group comparisons between unmatched primary colorectal tumors and liver metastases. P-values adjusting for multiple comparisons were calculated using the Bonferroni method. We used Fisher’s exact test for data categorized into two-by-two frequency count tables. All of the analyses were performed with SAS statistical software (version 8.2; SAS Institute, Cary, NC).

RESULTS

Immunohistochemical Analysis of p125FAK Expression in Colorectal Carcinoma

Validation of p125FAK Immunohistochemistry. Initially, we performed experiments to validate specific immunostaining for the M, 125,000 FAK protein. Using the FAK-specific monoclonal antibody 4.47 that has been shown to recognize FAK on both Western blots as well as FFPE sections (15), we performed immunohistochemistry and Western blot experiments. Normal colorectal mucosa expressed minimal levels of p125FAK (Fig. 1A), whereas FAK was up-regulated in both the matched primary invasive colorectal carcinoma (Fig. 1B) and the liver metastasis (Fig. 1C). The specificity of the FAK staining was demonstrated by a complete abrogation of immunostaining in the adjacent section of liver metastasis, when the antibody was preincubated with GST-FAK-NT fusion protein (Fig. 1D). The specificity of the antibody reactions was further demonstrated biochemically in Western blot experiments using this liver metastasis sample. The M, 125,000 FAK band was detected in the liver metastasis (Fig. 2, Lane 1) but was completely blocked by incubation of the monoclonal antibody 4.47 with GST-FAK-NT (Fig. 2, Lane 2). These results confirm, at both the immunohistochemical and the Western blot levels, that the staining pattern on immunohistochemistry was specific for p125FAK.

Colorectal Primary Cancer and Liver Metastases.

Next, we performed immunohistochemical analyses for FAK expression on 24 matched colorectal carcinoma and liver metastases from individual patients (Table 1). Robust levels of FAK expression were detected in both the colorectal primaries and their matched liver metastases, with all of the samples in this group expressing an intensity of 3 or 4, with at least 30% of the tumor cells demonstrating FAK immunoreactivity. FAK expression in the available liver metastasis was equivalent or greater than the available matched colorectal primaries in 14 (78%) of 18 samples scored by intensity and in 15 (83%) of 18 samples scored for percentage of tumor cells positive for FAK. Furthermore, tumors that expressed the highest levels of p125FAK (intensity of 4 and >90% positive cells) were more commonly seen in the liver metastasis group than the matched colorectal primary (74 versus 52% by Fisher’s exact test; Table 1). Although there was a trend for FAK expression to be greater in the liver metastases, no statistically significant difference was detected between the matched colorectal primary tumors and liver metastases.

Table 1  FAK expression by immunohistochemistry in matched tissue samples of primary colorectal carcinoma and liver metastasis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Colorectal primary</th>
<th>Liver metastasis</th>
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<tbody>
<tr>
<td></td>
<td>Intensitya % positive</td>
<td>Intensity % positive</td>
</tr>
<tr>
<td>1</td>
<td>NDb</td>
<td>ND</td>
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<tr>
<td>2</td>
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<td>95</td>
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<tr>
<td>24</td>
<td>3</td>
<td>90</td>
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</table>

a 0, none; 1, borderline; 2, weak; 3, moderate; 4, strong.
b ND, no data.
Real-Time PCR Analysis of FAK Expression in Colorectal Carcinoma

Validation of FAK mRNA Expression in Matched Normal Colorectal Mucosa and Primary Colorectal Cancer. After showing high levels of p125FAK expression in the liver metastases samples, we wished to extend our observations to the mRNA level, using real-time PCR analyses. Initially, we validated this technique by examining the expression of FAK mRNA in 39 matched normal colorectal mucosa and colorectal primary tumor samples (Table 2). In these matched samples, FAK mRNA copies are significantly higher in the primary colorectal tumors compared with the normal colorectal mucosa (adjusted \( P = 0.001 \), paired Wilcoxon signed-rank test). Although the liver metastases are not matched to the primary colorectal tumors, FAK mRNA copies in these liver metastases is significantly higher compared with the primary tumor (adjusted \( P = 0.002 \), Wilcoxon two-group test).

![Fig. 3](https://example.com/f3.png)

Fig. 3  Real-time PCR analysis of FAK expression in 39 matched samples of normal colorectal mucosa and primary colorectal cancer as well as a different set of colorectal liver metastases (\( n = 17 \)). In the matched samples, FAK mRNA copies are significantly higher in the primary colorectal tumors compared with the normal colorectal mucosa (adjusted \( P = 0.001 \), paired Wilcoxon signed-rank test). Although the liver metastases are not matched to the primary colorectal tumors, FAK mRNA copies in these liver metastases is significantly higher compared with the primary tumor (adjusted \( P = 0.002 \), Wilcoxon two-group test).

### Table 2  Real-time PCR FAK expression in matched samples of normal colorectal mucosa and primary colorectal cancer and in a separate set of colorectal liver metastasis

<table>
<thead>
<tr>
<th>Sample</th>
<th>Minimum mRNA copies</th>
<th>Maximum mRNA copies</th>
<th>Median mRNA copies</th>
<th>Mean mRNA copies 95% CI</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Normal and Primary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal colorectal mucosa</td>
<td>39 204</td>
<td>22,591</td>
<td>6,809</td>
<td>7,643</td>
<td>6,002–9,283</td>
</tr>
<tr>
<td>Primary colorectal cancer</td>
<td>39 252</td>
<td>32,790</td>
<td>10,699</td>
<td>12,828</td>
<td>10,504–15,151</td>
</tr>
<tr>
<td>Difference of primary – normal</td>
<td>39 −14,969</td>
<td>32,453</td>
<td>4,474</td>
<td>5,185</td>
<td>2,388–7,982</td>
</tr>
<tr>
<td>B. Metastasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorectal liver metastasis( ^a )</td>
<td>17 6,491</td>
<td>85,584</td>
<td>22,690</td>
<td>28,688</td>
<td>17,627–39,749</td>
</tr>
</tbody>
</table>

\( ^a \) CI, confidence interval.

\( ^b \) Colorectal liver metastasis (\( n = 17 \)) separate data set from the matched normal and primary colorectal samples.

Real-Time PCR Analysis of FAK Expression in Colorectal Carcinoma

Validation of FAK mRNA Expression in Matched Normal Colorectal Mucosa and Primary Colorectal Cancer. After showing high levels of p125FAK expression in the liver metastases samples, we wished to extend our observations to the mRNA level, using real-time PCR analyses. Initially, we validated this technique by examining the expression of FAK mRNA in 39 matched normal colorectal mucosa and colorectal primary tumor samples (Table 2A). In these matched samples, FAK expression was significantly higher in the primary colorectal carcinoma (median mRNA copy number of 10,699), compared with matched normal colorectal mucosa (median copy number of 6,809; Table 2A). We calculated two sets of difference scores in these data (colorectal primary tumor FAK mRNA copies minus normal colorectal mucosa FAK mRNA copies, and log10 colorectal primary tumor FAK mRNA copies minus log10 normal colorectal mucosa FAK mRNA copies). The number of FAK mRNA copies in the primary colorectal tumors was significantly higher compared with normal colorectal mucosa (adjusted \( P = 0.001 \), Wilcoxon signed-rank test on absolute difference scores; Fig. 3). Likewise, examining these data on the logarithmic scale also showed a significant increase in FAK mRNA copies in the primary tumors compared with normal colorectal mucosa (adjusted \( P = 0.002 \), Wilcoxon signed-rank test on log10 difference scores).

Dual Analysis of FAK mRNA and Protein Expression in Liver Metastases. In a final series of experiments, we analyzed a different group of liver metastases for FAK expression at both the mRNA level by real time PCR and the expression of p125FAK by immunohistochemistry (Tables 2B and 3). These liver metastases demonstrated high levels of FAK expression (>50% of cells positive) in 16 (89%) of 18 samples by immunohistochemistry. FAK mRNA expression was up-regulated in the liver metastases, with a median mRNA copy number of 22,690 (Table 2B). Although these samples were not matched to the normal and primary samples, marked up-regulation of FAK mRNA expression was demonstrated. In fact, the unmatched liver metastases FAK mRNA copies were significantly
higher than the primary colorectal tumor FAK mRNA copies (adjusted \( P = 0.002 \), Wilcoxon two-group test; Fig. 3).

In this series of liver metastases, there was variability between the FAK mRNA expression and the \( p125^{\text{FAK}} \) expression (Table 3). Some tumors had high mRNA copies with lower immunohistochemical scores (patients 25 and 31). In contrast, other tumors expressed higher levels of \( p125^{\text{FAK}} \) by immunohistochemistry, with lower mRNA copies (patients 32 and 35). Representative examples of the immunohistochemical and real-time PCR results are shown in Fig. 4. Although there was variability between the message and protein levels, these results demonstrate that both FAK mRNA and \( p125^{\text{FAK}} \) protein are expressed at high amounts in colorectal liver metastases samples.

**DISCUSSION**

These results provide evidence at both the mRNA and the protein levels that the expression of \( p125^{\text{FAK}} \) is elevated in colorectal carcinomas and colorectal liver metastases. In this series of experiments, the level of FAK expression in colorectal liver metastases was equal to or greater than the level of expression in primary colorectal tumors. These results support previous investigation from our group and others who have studied FAK expression in colon carcinoma. The first evidence of overexpression of FAK mRNA in colorectal carcinomas and liver metastases was reported by Weiner et al., (12), who found that normal colorectal mucosa expressed negligible levels of FAK but that 85% of invasive primary carcinomas and 100% of liver metastases overexpressed FAK. Subsequently, Owens et al. (13) extended these results to the protein level by demonstrating elevated FAK expression in 17 of 17 invasive and metastatic colonic lesions by Western blot, using a polyclonal rabbit anti-FAK antibody. In addition, another group of investigators examined a large series of colorectal cancer liver metastases by Western blotting using a different monoclonal antibody (Transduction Laboratories, Lexington, KY) and showed FAK overexpression in 29 of 30 liver metastases (18). Finally, our group developed a FAK-specific monoclonal antibody that recognized \( p125^{\text{FAK}} \) on Western blots, immunoprecipitates, and FFPE tissue sections (15). Using this antibody, we have previously shown FAK expression to be borderline-to-weak in normal colonic epithelium but moderate-to-strong in 13 of 15 invasive colonic cancers by immunohistochemistry. This current report has extended our previous findings by using both real-time PCR analyses and immunohistochemical analyses to demonstrate elevated levels of FAK expression in primary and metastatic colorectal cancers.

In contrast to these results, Ayaki et al. (19) recently reported lower levels of FAK expression in colorectal liver metastases than in the primary colorectal tumors. These investigators used a different monoclonal anti-FAK antibody for Western blot experiments and a polyclonal anti-FAK antibody for immunohistochemical studies and found that both FAK and paxillin expression were generally lower in liver metastases than in primary colorectal specimens. Nonetheless, FAK immunohistochemical expression was positive in 8 (88%) of 9 liver metastases, compared with 2 (20%) of 10 normal mucosal specimens. It is not clear whether these investigators used antigen-retrieval techniques in their immunohistochemical experiments (22, 23, 25). We have found that the FAK epitope is masked by the formalin fixation and paraffin embedding processes, and epitope retrieval was necessary for optimum immunohistochemical analyses (15). Although this technique has the possibility of introducing false-positive staining (26), we demonstrated the specificity of our analyses by abrogating immunoreactivity in the tissue specimens by incubating the monoclonal antibody with recombinant FAK protein.

Within our series of immunohistochemical experiments, we observed variability between FAK expression in the matched sample of liver metastases as compared with the unmatched liver metastases. The biological nature of genetic variability may explain this observation; however, we have also observed that the FAK antigen deteriorates as the duration of formalin fixation and paraffin embedding increases (data not shown). In fact, the unmatched liver metastases were older samples (at least 4 years of storage), and we hypothesize that the variability that we observed is secondary to some deterioration of the FAK antigen. Similarly, the variability that we observed between the unmatched liver metastases \( p125^{\text{FAK}} \) and their corresponding FAK mRNA expression was likely secondary to this FAK deterioration in the immunohistochemistry samples.

The up-regulation of FAK in primary and metastatic colorectal tumors is consistent with the role of FAK in suppressing apoptosis in tumor cells (10, 27). Evidence for reduced apoptosis in metastatic colorectal tumors has been presented by Hashimoto et al., (28) who examined intranuclear DNA strand breaks localized with \textit{in situ} nick translation to measure the frequency of apoptosis in human colorectal cancer specimens. They found that the labeling indices of carcinomas that did metastasize to lymph nodes or the liver were lower than that of carcinomas that did not metastasize. Thus, liver metastases were more likely to have less apoptotic cells (28). These findings support the conceptual framework whereby tumor cells that become invasive...
Fig. 4 Immunohistochemical and real-time PCR analysis of FAK expression in colorectal liver metastasis. A, representative examples of immunohistochemical staining that demonstrated different levels of FAK protein expression in four colorectal liver metastases samples. B, real-time PCR analysis for FAK mRNA in the same four colorectal liver metastases as in A. A1 and B, patient 31 illustrates the least amount of FAK protein expression in this set (n = 18) of colorectal liver metastases with FAK mRNA copy number slightly below the mean, 21,454. A2 and B, patient 39 represents the lowest mRNA FAK expression, 6,491, with high p125FAK expression (>50% of cells positive). A3 and B, patient 38 has high FAK protein expression with the highest FAK mRNA copy number, 85,584. A4 and B, patient 36 illustrates FAK up-regulation at both the message and protein with the majority of colorectal liver metastasis cells staining strongly and 29,967 copies of FAK mRNA.
and metastatic are subjected to intense apoptotic pressure. This would select for cells that are resistant to apoptosis so that they could successfully grow and become sites of distant metastasis. Thus, our finding that FAK expression is up-regulated in colorectal liver metastases fits into this framework of enhanced survival signaling through FAK to promote continued growth of the metastatic colorectal tumor cells (7).

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REFERENCES


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