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Review

Focal adhesion kinase and cancer

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Summary. Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that resides at the sites of integrin clustering, known as focal adhesions. The FAK protein has a molecular mass of 125kDa and is encoded by the FAK gene located on human chromosome 8q24. Structurally, FAK consists of an amino-terminal regulatory FERM domain, a central catalytic kinase domain, two proline-rich motifs, and a carboxy-terminal focal adhesion targeting domain. FAK has been shown to be an important mediator of cell growth, cell proliferation, cell survival and cell migration, all of which are often dysfunctional in cancer cells. Our lab was the first to isolate FAK from primary human tissue and link it to the process of tumorigenesis. We analyzed FAK mRNA expression in normal, invasive and metastatic human tissues and demonstrated through Northern blot analysis that normal tissues had very low levels of FAK mRNA while primary and metastatic tumors significantly overexpressed FAK. We also demonstrated and confirmed FAK overexpression in colorectal carcinoma and liver metastases with real-time PCR. In this review we summarized immuno-histochemical data of FAK expression and role in different cancer types tumors and discussed FAK inhibition therapy approaches.

Key words: Focal adhesion kinase, Cancer, Tumorigenesis

Breast cancer

We found that p125FAK was significantly elevated in 17 (100%) of 17 invasive and metastatic colonic lesions and in 22 (88%) of 25 invasive and metastatic breast tumors (Fig. 1), suggesting that FAK can be a marker of invasive tumors (Owens et al., 1995) We have shown that dual inhibition of FAK and EGFR (which are both overexpressed in breast tumors) signaling pathways cooperatively enhanced apoptosis in breast cancers (Golubovskaya et al., 2002). We have also shown that overexpression of an activated form of the Src tyrosine kinase in breast cancer cells (BT474 and MCF-7) suppressed the loss of adhesion and apoptosis induced by dominant-negative, adenoviral FAK-CD, indicating cooperative FAK and Src signaling in breast tumorigenesis (Park et al., 2004; Madan et al., 2006; Mitra and Schlaepfer, 2006). Overexpressed HER2 has been shown to be involved in tumor malignancy and metastatic ability of breast cancer through a FAK and Src signaling pathways (Schmitz et al., 2005) . We analysed FAK expression in DCIS breast tumors and demonstrated up-regulation of focal adhesion kinase (FAK) expression in ductal carcinoma in situ (DCIS) is an early event in breast tumorigenesis (Lightfoot et al., 2004). Subsequently, we analyzed FAK expression in 629 formalin-fixed, paraffin-embedded tissue sections (Lark et al., 2005). High FAK expression was associated with poor prognostic indicators, including high mitotic index, nuclear grade 3, architectural grade 3, estrogen and progesterone receptor negative, and overexpression of HER-2/neu (Lark et al., 2005). Recently, it has been demonstrated in transgenic mouse Cre/loxP model in vivo that disruption of FAK blocked mammary tumor progression in a transgenic breast cancer model (Lahlou et al., 2007). The association of FAK and Her-2 in breast tumors indicated its important cooperative signaling to promote breast tumorigenesis (Lark et al., 2005). Recently, we showed in breast cancer tumors that mutations in p53 resulted in an increase in FAK mRNA and protein expression (Golubovskaya et al., 2008a). We analyzed 600 breast cancer tumors and found high positive correlation between FAK overexpression and p53 mutations, demonstrating that p53 regulates FAK expression during breast tumorigenesis (Cance and Golubovskaya, 2008).

Recently FAK has been proposed a potential target in cancer therapy (van Nimwegen and van de Water

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2007). We also showed that the novel FAK kinase inhibitor TAE226 (Novartis) induced apoptosis in breast cancer cells (Golubovskaya et al., 2008b). Another group used the Src kinase inhibitor SKI-606 (bosutinib) to block breast carcinogenesis and found that it blocked FAK phosporylation and suppressed human breast cancer cell migration and invasion (Vultur et al., 2008). Another inhibitor PF-271 (Pfizer) blocked FAK phosphorylation and breast cancer and other cancers types (pancreatic, prostate) tumorigenesis, suggesting it as a potential therapeutic inhibitor (Roberts et al., 2008).

Neuroblastoma

Treatment of neuroblastoma cells with okadaic acid (OA), a serine phosphatase inhibitor, increasing serine/threonine phosphorylation and inhibiting tyrosine phosphorylation, induced focal adhesion loss, actin cytoskeleton disorganization, and cellular detachment, which corresponded to a loss of FAK Tyr397 (Kim et al., 2003). The authors suggested that inhibitors, causing FAK dephosphorylation may be potentially therapeutic drugs in neuroblastoma cells. We have demonstrated that N-Myc can regulate FAK expression in neuroblastoma through binding to the FAK promoter (Beirle et al., 2007). Recently, FAK expression was analyzed on 70 formalin-fixed, paraffin-embedded human neuroblastoma specimens (Beierle et al., 2008a). The authors demonstrated that FAK was expressed in 73% of neuroblastoma tumor samples (Beierle et al., 2008a). FAK staining was significantly increased in stage IV tumors with the amplification of the N-MYC oncogene, providing basis for targeting FAK in neuroblastoma treatment (Beierle et al., 2008a).

Most recently the novel FAK molecule inhibitor TAE226 has been used on neuroblastoma cells, and the drug decreased cell viability and increased apoptosis suggesting that FAK is a potential therapy target of

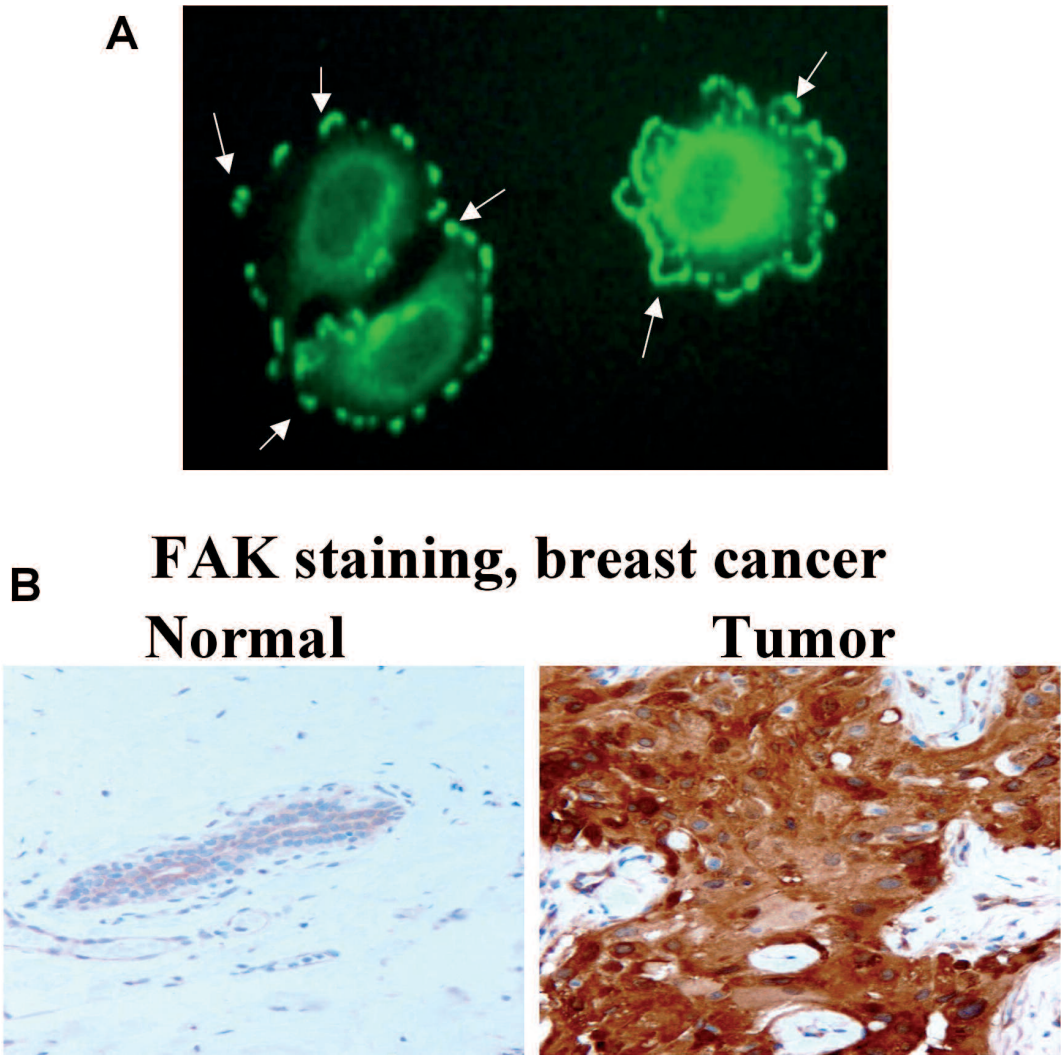

Fig. 1. FAK staining in cancer cells. **A.** FAK staining in human breast cancer cells. Immunistaining with FAK 4.47 N-terminal antibody in breast cancer cells. White arrows show FAK in focal adhesions. **B.** Overexpression of FAK in breast cancer tumors. Immunohistochemistry staining was performed on breast tumor (right) and matched normal (left) tissue samples.

neurobalstoma (Beierle et al. 2008b).

Pancreatic cancer

Inhibition of FAK with FAKsiRNA potentiated gemcitabine-induced cytotoxicity in pancreatic cells (Duxbury et al., 2003). FAK siRNA treatment down-regulated AKT activity (Duxbury et al., 2003). In addition, down-regulation of FAK with siRNA caused cell increased anoikis in pancreatic adenocarcinoma PANC-1, BxPC3 and MiaPaCa-2 cell lines (Duxbury et al., 2004). FAKsiRNA also inhibited metastasis in a nude mouse model (Duxbury et al., 2004). Furthermore, down-regulating FAK with siRNA decreased cell motility and invasiveness in pancreatic cells (Huang et al., 2005). Analysis of FAK expression in 50 patients with pancreatic invasive ductal carcinoma by immunohistochemistry showed its detection in 24 samples (48%) (Furuyama et al., 2006). There was a statistically significant correlation between FAK expression and tumor size (P=0.004), although FAK expression did not significantly correlate with other factors such as tumor histological grade, lymph node metastasis, distant metastasis, histological stage, and overall survival. The authors conclude that focal adhesion kinase expression may not be a prognostic marker for pancreatic cancer patients (Furuyama et al., 2006). The more extended study of pancreatic cancer needed to be analyzed for FAK expression, as it correlated with tumor size. Recently, FAK was shown to promote survival in pancreatic adenocarcinoma by interacting with IGF-IR and activating pathways that lead to cell proliferation and survival (Liu et al., 2008).

Ovarian cancer

We have shown that FAK expression was increased in ovarian cancers (26 cancer samples from patients with Stage I-IV ovarian carcinoma and two ovarian carcinoma cell lines) compared to normal samples (Judson et al., 1999). The authors suggested that FAK may be a potential target for therapeutic disruption of ovarian carcinoma progression (Judson et al., 1999). Recently FAK overexpression in ovarian cancer and absence in normal ovarian epithelial cells was also demonstrated by several other groups (Gabriel et al., 2004; Grisaru-Granovsky et al., 2005; Sood et al., 2004). It has also been suggested that FAK activated the PI3-Akt signaling pathway and induced expression of KFL8 (Kruppel-like factor 8), a transcription factor important in cell cycle regulation, oncogenic transformation, and epithelial to mesenchymal transition in human ovarian epithelial and cancer cells (Wang et al., 2008).

Ovarian cancer cells mobility has been inhibited recently by inhibiting FAK phosphorylation and focal adhesion assembly with the potential anti-cancer agent TGZ (Troglitazone) (Yang et al., 2007). TAE226 (Novartis) has also been used in combination with docetaxel to significantly inhibit ovarian cancer cell growth (Halder et al., 2007). The authors suggested that TAE226 can be an effective therapeutic approach in ovarian carcinoma (Halder et al., 2007).

Cervical cancer

FAK has been shown to be activated in human cervical cancer (McCormack et al., 1997). FAK overexpression has been suggested to be a marker for malignant transformation of cervical cancer (Oktay et al., 2003). The authors found minimal FAK expression in benign cervical epithelium and in low-grade squamous dysplasia (CIN I and CIN I-II) of the cervix, while most of the invasive SCCs of the cervix (13 of 16 cases) were positive for FAK (Oktay et al., 2003) In another studies, FAK phosphorylation was linked to the cervical cancer invasion (Moon et al., 2003), and a poor prognosis (Gabriel et al., 2004).

Osteosarcoma

Expression analysis of 16 osteosarcoma tumors, 5 osteosarcoma cell lines and 6 normal tissues demonstrated FAK overexpression in high grade osteosarcomas (Schroder et al., 2002). FAK expression analysis performed in our group in 13 high grade sarcomas showed high levels in all tumor samples compared to benign, noninvasive mesenchymal specimens (Owens et al.,1995). This analysis shows that FAK can be a potential target in these tumors, although more detailed study with correlation analysis of other tumor markers will be critical.

Kidney cancer

Comparative analysis of FAK expression in metastatic and non-metastatic renal carcinoma cells revealed that FAK and paxillin mRNA expression were up-regulated 2.0-2.5 fold in the metastatic Caki-1 cells over normal renal cortex epithelial cells (RCEC), suggesting its potential role as a marker of metastasis (Jenq et al., 1996).

Expression of FAK and HGF (hepatocyte growth factor) synergistically increased transformation of Madin-Darby canine kidney epithelial cells (Chan et al. 2002).

Lung cancer

In lung surgical specimens, phosphorylated FAK has been shown to be the major component among 100-130 kDa phosphorylated proteins correlating with poor patient prognosis (Nishimura et al., 1996). In addition, increased phosphorylation of FAK has been demonstrated in lung cancer samples and its absence in normal tissues (Imaizumi et al., 1997). The increased phosphorylation of FAK was closely correlated with the nodal involvement of cancer and disease-free survival

time (Imaizumi et al., 1997). Expression of laminin 5, regulating cell adhesion and anoikis, and increased phosphorylation of FAK in lung adenocarcinoma cells suggested the importance of the laminin-integrin-FAK pathway in tumorigenesis (Kodama et al., 2005). Stimulation of small cell lung cancer cells with HGF (hepatocyte growth factor) activated c-Met and increased phosphorylation of Y397 FAK, suggesting this pathway as a therapeutic target in lung tumorigenesis (Maulik et al., 2002). FAK signaling has been demonstrated to be important in the early stages of mammary adenocarcinoma lung metastasis (van Nimwegen et al., 2005). In an experimental model of mammary metastasis, lung metastasis formation was prevented when the dominant-negative FAK inhibitor, FRNK, was expressed one day before tumor cell injection, whereas at 11 days after injection expression of FRNK did not affect lung metastasis formation (van Nimwegen et al., 2005). Immunohistochemical staining of FAK in 60 formalin-fixed and paraffin-embedded non-small cell lung cancerNSCLC) tumors demonstrated increased FAK levels compared with non-neoplastic tissues (Carelli et al., 2006). Moreover, Western blotting and real-time RT-PCR showed a statistically significant correlation between FAK up-regulation and higher disease stages (I+II versus III+IV, p=0.019 and 0.028, respectively), indicating FAK involvement in tumorigenesis (Carelli et al., 2006).

Prostate cancer

Increased FAK protein and mRNA levels and phosphorylation was observed in metastatic prostate cancer cells that correlated with the progression and metastasis of tumors (Tremblay et al., 1996). FAK signaling has been suggested to play a critical role in invasiveness and motility of prostate cancers (Zheng et al., 1999, 2006).

Brain tumors

Anaplastic astrocytoma brain tumors expressed higher levels of FAK and autophosphorylation compared to non-neoplastic adult normal brain tissues (Hecker et al., 2002). Overexpression of FAK promoted Ras activity through the formation of FAK/p120RasGAP complex in malignant astrocytoma cells cultured in aggregate suspension or as monolayers adherent to vitronectin (Hecker et al., 2004). Overexpression of FAK in serum-starved glioblastoma cells plated on recombinant (rec)-osteopontin resulted in enhancement of basal migration and in a more significant increase of PDGF-BB-stimulated migration. Both expression of mutant FAK(397F) and FAK down-regulation with small interfering siRNA inhibited basal and PDGF-stimulated migration (Natarajan et al., 2006). Recently, a novel kinase inhibitor of FAK (TAE226) has been shown to increase apoptosis in brain tumors (Shi et al., 2007).

Melanoma cancer

Increased FAK constitutive phosphorylation was observed in human metastatic melanoma cells (Scott and Liang, 1995). Focal adhesion increased expression correlated with motility of human melanoma cells (Akasaka et al., 1995). FAK expression appeared to be important for tumor cell adhesion in melanoma (Maung et al., 1999). Constitutive activation of FAK was regulated by the cytoskeleton in melanoma cells important for aggressive tumor growth (Kahana et al., 2002). We treated melanoma cell lines with antisense FAK oligonucleotides, inhibiting FAK expression, and demonstrated increased cell apoptosis and sensitivity to the chemotherapy drug 5-fluorouracil (Smith et al., 2005). Treatment of melanoma cells with dominant-negative FAK-related FRNK increased the aggressive phenotype of the cells, demonstrated by decreased invasion and motility and inhibiting Erk1/2 mediated signaling pathway (Hess and Hendrix, 2006). Recently FAK has been linked with the enhanced malignant properties of ganglioside GD-3-expressing melanoma cells (Hamamura et al., 2008).

The compound geraniin, a form of tannin separated from geranium, has been shown to induce apoptosis in human melanoma cells by promoting caspace-3 mediated cleavage of FAK (Lee et al., 2008). A synthetic agent, Manbeta(1-4)[Fucalpha(1-3)]Glcbeta1-Cer, (glycosphingolipid 7), which was identified in the millipede Parafontaria laminata armigera, had an inhibitory effect on proliferation of the melanoma cells (Sonoda et al., 2008). Glycosphingolipid 7 suppressed the activation of the FAK-Akt and Erk1/2 pathways, which caused decrease in the expression of cyclin D1 and CDK4 (Sonoda et al., 2008). The authors suggest that glycosphingolipid 7 might be a candidate for an inhibitor of cell proliferation in melanomas (Sonoda et al., 2008).

Thyroid cancer

We analyzed p125FAK expression in 30 human thyroid tissue samples that included paired normal and malignant specimens (Owens et al., 1996). The highest levels of p125FAK were seen in follicular carcinomas and tumors associated with distant metastatic foci (Owens et al., 1996). FAK was not expressed in normal tissue and nodular hyperplasia but was expressed in all of the follicular, papillary, medullary, and anaplastic thyroid carcinomas (Kim et al., 2004). This result indicates that the up-regulation of FAK may play a role in the development of thyroid carcinogenesis.

Oral cancer

FAK was found to be overexpressed in oral cancers (Kornberg, 1998). Overexpression of FAK in low-invading cells resulted in a 4.5-fold increase in the rate

of invasion compared with control cell lines, suggesting that enhanced expression of FAK in oral carcinoma cells may lead to a selective growth advantage and increased invasive potential of the primary oral tumor (Schneider et al., 2002). In another study, authors suggested that activation of FAK by phosphorylation of c-Met could mediate the HGF/SF-induced motility of human oral squamous cell carcinoma cells, and that Rho protein could regulate the tyrosine phosphorylation of FAK through translocation from the nucleus to the membrane (Kitajo et al., 2003). Increased FAK phosphorylation and expression was observed in oral carcinoma of the larynx (Aronsohn et al., 2003).

Head and neck cancer

In a recent study, hepatocyte growth factor/scatter factor HFG/SF inducedphosphorylation of FAK and Erk in cultured squamous cell carcinoma of the head and neck, indicating its role in tumorigenesis (Fleigel et al., 2002). Inhibition of Focal Adhesion Kinase with a dominant-negative C-terminal FAK, Ad-FRNK caused increased apoptosis and decrease of cell motility in epithelial cells from head and neck carcinomas (SCCHN cells) (Kornberg, 2005). FAK expression analysis by immunohistochemistry in 211 head and neck squamous cell carcinoma (HNSCC)tissue samples, including 147 primary tumors, 56 lymph node metastases, 3 benign hyperplasias, and 5 dysplasias demonstrated elevated FAK expression in 62% of tumor samples (Canel et al., 2006). Positive immunostaining was also detected in benign hyperplasias and preinvasive dysplastic lesions, indicating that FAK overexpression is an early event during tumorigenesis. FAK DNA copy number ratios were higher in 39% of the tumors compared with normal matched samples. However, not all cases of FAK overexpression FAK contained gene amplification indicating additional mechanisms of FAK expression regulation (Canel et al., 2006). Inhibiting of FAK by adenoviral Ad-FRNK and introduction of exogenous p53 with Ad-p53 cause increased apoptosis of the carcinoma cells and improved cytotoxic effect of chemotherapy drugs (Kornberg, 2006). Photodynamic therapy, effective in treatment of head and neck invasive cancers, decreased cell motility and FAK-phosphorylation and downstream survival signaling (Yang et al., 2007). Recently, FAK was shown to be important in cellular invasion of head and neck cancer (Canel et al., 2008). Disruption of FAK caused decreased cell attachment, motility and invasion while FAK overexpression increased cell invasion.

Hepatocellular carcinoma

FAK expression analysis in 60 hepatocellular carcinoma demonstrated overexpression of FAK mRNA and protein in tumor samples compared to matched non-tumor liver samples (Fujii et al., 2004). The fact that FAK overexpression correlated significantly with the tumor size (P=0.034) and serum AFP level (P=0.030) led to the suggestion that FAK can be a prognostic therapeutic factor in these tumors (Fujii et al., 2004). Down-regulation of FAK with the dominant-negative FRNK caused metastatic adhesion of carcinoma cells within liver sinusoids (von Sengbusch et al., 2005). The hepatitis B virus (HBV) involved in hepatic cell transformation activated FAK, suggesting importance of FAK signaling in HBV-associated hepatocellular carcinogenesis (Bouchard et al., 2006).

Colon cancer

We have shown that FAK was overexpressed in invasive and metastatic colon tumors on both protein and mRNA levels (Weiner et al., 1993; Lark et al., 2003). FAK protein overexpression was seen in 32 out of 80 cases of colon adenocarcinoma (Theocharis et al., 2003). An increase of tyrosine phosphorylation of FAK (Y-397) was detected in colorectal cancer cells (Yu et al., 2004). Phospho-FAK correlated with invasiveness and lymph node metastasis in colon cancers (Yu et al., 2006). We have demonstrated that simultaneous inhibition of FAK and Src pathways caused increased apoptosis in colon cancer cell lines, suggesting that multiple signaling is involved in tumorigenesis (Golubovskaya et al., 2003). Recently, FAK has been suggested to mediate pressure-induced colon cancer cell adhesion (Thamilselvan et al., 2007).

Acute myeloid leukemia

FAK expression was analyzed in 60 cases of acute myeloid leukemia (AML) and both the FAK protein and mRNA were detected in about 42% of the cases. Aberrant expression of FAK was significantly correlated with high blast cell mount, early death and shorter survival rate. FAK expression in enhanced blast cell migration and celularity, and equated to a poor prognosis (Recher et al., 2004). Understanding the role of FAK in this cancer may be critical in the development of an effective treatment.

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