

Review

Focal adhesion kinase-regulated signaling events in human cancer

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Abstract

Focal adhesion kinase (FAK) is a non-receptor protein tyrosine kinase that is highly expressed or activated in many human cancers. Under specific scenarios, FAK can regulate cell proliferation, cell survival, cell migration and invasion, and has been implicated in the control of tumorigenesis and metastasis. FAK has both catalytic and scaffolding activity, and triggers downstream signals by activation of a number of pathways, including the Ras/mitogen-activated protein kinase pathway, the phosphatidylinositol 3'-kinase/Akt pathway, and Rho family GTPases. Recent evidence also suggests novel signaling interactions between FAK and p53. These signaling events were defined primarily from studies on cells in culture, and elucidating which of these signaling pathways are pathologically relevant downstream of FAK in human cancer remains an important goal in determining the molecular mechanisms of tumorigenesis and metastasis. This review discusses select evidence of these signaling pathways with an emphasis on studies linking these to animal models of cancer and human disease. The role of FAK in the process of epithelial-to-mesenchymal transition and in cancer stem cells and recent therapeutic advances targeting FAK are also discussed.

Keywords: cancer; Cas family; epithelial-to-mesenchymal transition; focal adhesion kinase; mitogen-activated protein kinase; p53; PI3K; Rho; Src; stem cells.

Introduction

Focal adhesion kinase (FAK) is a 125-kDa non-receptor protein tyrosine kinase, which plays a critical role in multiple

cellular processes, including cell spreading, adhesion, migration, survival, and proliferation (1). It is expressed ubiquitously, and is essential for development. The N-terminal FERM domain of FAK participates in intermolecular and intramolecular interactions, the latter serving to inhibit FAK catalytic activity (2). The catalytic domain of FAK, which includes the ATP and substrate binding sites, is in the middle of the protein and a focal adhesion targeting (FAT) domain is located at the C-terminus. The FAT domain functions in subcellular localization resulting in co-localization of FAK with the integrins, which are receptors for extracellular matrix proteins and major regulators of FAK activity. Integrin-dependent cell adhesion results in FAK activation and autophosphorylation at Y397 (1). The phosphorylation of Y397 creates a binding site for the SH2 domain of Src, which in turn can phosphorylate additional residues in FAK. Phosphorylation of these tyrosines regulates catalytic activity and the assembly of additional signaling complexes. Phosphorylation of Y397 is critical for most biological activities controlled by FAK, and a mouse model where the FAK locus is replaced by a mutant lacking Y397 exhibits embryonic lethality (3).

The *Ptk2* gene, which encodes FAK, is located on chromosome 8 at 8q24 in humans. There are two interesting features of the *Ptk2* gene. First, there is an internal promoter that drives expression of a second protein, known FAK-related non-kinase (FRNK), which shares the carboxyl-terminal region of FAK extending from residues 668 to 1052 (4). FRNK has been utilized as a dominant-negative inhibitor of FAK as it displaces FAK from focal adhesions and promotes dephosphorylation of FAK. Second, the *Ptk2* locus also harbors microRNA 151 (miR151), which is located in intron 22 of the *Ptk2* transcript (5). The significance of miR151 is discussed below. FAK has clearly been implicated in the development of cancer. Many studies describe overexpression of FAK or elevated activation of FAK in a variety of cancers (6). In some cases, e.g., ovarian cancer, FAK overexpression alone or in combination with other markers is prognostic for the disease (7), whereas in other cases FAK expression is not prognostic. Recent results from the literature describing FAK expression in clinical samples and its prognostic value are summarized in Table 1. Older and more descriptive studies have been compiled elsewhere (8, 9). In many experimental animal models of cancer, FAK has been implicated as an important player in both tumorigenesis and metastasis. As miR151 is contained within the *Ptk2* locus, its overexpression might also be anticipated in cancer. This has been documented in hepatocellular

Table 1 Relation of FAK expression/amplification in tumors and prognosis.

Disease	Sample size	Approach	Result	Conclusion	Reference
FAK expression/amplification and poor prognosis AML	36	Flow cytometry for FAK expression	FAK expression in cancer samples (median=65% +ve cells)	FAK expression correlates with poor survival (U, $p=0.044$), poorer survival if co-expressed with CXCR4 or VLA4	(98)
AML	60	Western blot for FAK	FAK expression detected in some samples (42%)	FAK expression correlates with poor survival (KM, $p=0.049$; M, $p=0.04$)	(99)
Breast cancer	435	FISH (amplification) and IHC for FAK on TMA	High FAK expression in some tumors (27.5%) Amplification in some tumors (17.7%)	Amplification but not expression level correlates with poor survival (KM, $p<0.001$; M, $p<0.001$)	(100)
Breast cancer – triple negative	1200 breast cancers, 184 triple negative	IHC for many markers (including FAK) on TMA	Variable FAK expression in tumors	FAK expression is prognostically significant in breast cancer (U, $p<0.001$) and in triple-negative breast cancer (U, $p<0.01$)	(101–103)
Colorectal cancer	104	IHC on FAK, paxillin and Src	Variable FAK expression in tumors	Combined high FAK and Src expression correlates with shorter time to recurrence (M, $p=0.005$) but not with survival	(43)
Esophageal squamous cell carcinoma	91	IHC for FAK and other markers	FAK overexpression in tumors (59.3%)	High FAK expression correlates with poor survival (KM, $p<0.0001$)	(104, 105)
Gastric cancer	444	FISH (amplification) and IHC for FAK on TMA	High FAK in tumors Amplification (high in 9%, lower in 22%)	High FAK expression correlates with invasion/metastasis (K, $p<0.001$) and poor survival (KM, $p<0.001$)	(106)
Gastric cancer	59	IHC for FAK and pFAK (pY397)	High FAK expression (69.5%) High pFAK (35.6%)	Amplification correlates with invasion/metastasis (K, $p<0.03$) and poor survival (KM, $p<0.001$) pFAK but not FAK correlates with recurrence and lower survival (χ^2 , KM, $p<0.02$)	(107)
Gastric adenocarcinoma – intestinal	30	IHC for FAK	FAK expression in tumors (78%)	High FAK expression correlates with poor survival (KM, $p=0.092$)	(108)
Glioma	96	IHC for FAK and pFAK (pY397)	FAK expression in tumors (89.6%) pFAK in tumors (50%)	FAK and pFAK correlate with tumor grade (χ^2 , $p=0.01$ – 0.02) and poor survival (KM, $p<0.01$). Prognosis for survival is worse if FAK and pFAK are detectable	(109)
Hepatocellular carcinoma	50	Q-RT-PCR (RNA) and IHC for FAK	FAK protein expression in tumors (50%), RNA higher in tumors than normal tissue	FAK protein (KM, $p<0.001$) and RNA (U, $p=0.009$) expression correlates with poor survival	(110)
Hepatocellular carcinoma	89	IHC for FAK	FAK expression in tumors (43%)	FAK expression correlates with stage and invasion (χ^2 , $p<0.05$)	(111)
Hepatocellular carcinoma	60	Q-PCR (RNA)	Higher FAK mRNA expression in tumors	Evidence for hepatitis B virus infection correlates with FAK (χ^2 , $p=0.005$) High FAK mRNA correlates with poor survival (KM, $p=0.041$)	(112)

Table 1 (continued)

Disease	Sample size	Approach	Result	Conclusion	Reference
Hepatocellular carcinoma	64	IHC for FAK	FAK expression in tumors (28.1%)	FAK expression correlates with poor survival (KM, $p=0.035$; M, $p=0.0071$)	(113)
Laryngeal premalignancy	82	Q-PCR (amplification) and IHC	High FAK in lesions	High FAK expression (not amplification) predicts cancer risk (U, $p=0.002$)	(114)
Lung adenocarcinoma	249	IHC for FAK	FAK expression in lung adenocarcinoma (88%) and bronchioloalveolar carcinoma (57%)	High FAK expression in lung carcinoma correlates with poor survival (KM, $p=0.0056$)	(115)
Ovarian cancer	79	IHC for FAK	High FAK expression in some tumors (68%)	High FAK expression correlates with poor survival (KM, $p=0.008$)	(7)
Squamous cell carcinoma – supraglottic larynx	95	IHC for FAK and E-cadherin	High FAK expression in some tumors (56%)	High FAK combined with low E-cadherin correlates with poor survival (KM, $p=0.005$)	(116)
Thyroid cancer	108	IHC for FAK and pFAK (pY861)	FAK (39.8%) and pFAK detected in tumors	FAK and pFAK expression can distinguish malignant from benign lesions (χ^2 , $p=0.00001$)	(42)
Tongue cancer	80	IHC for FAK	High FAK expression in tumors (69%), stronger staining at invasive front, lymph node metastasis are FAK +ve	FAK expression correlates with lymph node metastasis (χ^2 , $p<0.01$)	(117)
No correlation between FAK expression and prognosis					
Breast cancer – node negative	162	IHC for FAK	FAK expression in tumors (92%), some with high expression (18.5%)	FAK expression does not correlate with patient survival	(47)
Breast ductal invasive carcinoma	73	IHC for FAK	FAK expression in tumors (88%), some with high expression (21%)	FAK correlates with tumor size but no other clinicopathological parameters	(118)
Colon adenocarcinoma	80	IHC for FAK	FAK expression in tumors, but variable	FAK expression does not correlate with survival	(119)
Endometrial adenocarcinoma	43	IHC for FAK and pFAK (pY861)	High FAK in tumors	High FAK does not correlate with survival	(120)
Endometrial carcinoma	137	IHC for FAK	Moderate to strong FAK expression in tumors (89%)	FAK expression correlated with tumor grade (χ^2 , $p=0.02$) but not with survival	(121)
Hepatocellular carcinoma	49	IHC for FAK and Pyk2	High FAK expression in tumors (57%)	High Pyk2 correlates with poor survival (KM, $p=0.02$) but FAK expression does not (KM, $p=0.09$)	(122)
Neuroblastoma	70	IHC for FAK	FAK expression in tumors (73%)	High FAK expression correlates with stage and N-myc amplification but not with survival	(123)
Pancreatic cancer – invasive ductal adenocarcinoma	50	IHC for FAK	FAK expression in tumors (48%)	FAK expression correlates with tumor size but not with survival	(124)
SCLC	85	IHC on TMA	High FAK in tumors	FAK expression is not a prognostic biomarker	(125)
FAK expression/amplification and good prognosis					
Cervical cancer	162	IHC for FAK	Moderate to strong FAK expression in tumors (66%)	Moderate and high FAK expression correlates with increased survival (KM, $p=0.002$)	(126)
Gastric adenocarcinoma – diffuse type	36	IHC for FAK	FAK expression in tumors (89%)	High FAK expression correlates with increased survival (KM, $p=0.014$)	(108)

Table 1 (continued)

Disease	Sample size	Approach	Result	Conclusion	Reference
Ovarian cancer	51	Reverse-phase protein array/Western pleural/ peritoneal perfusions IHC for FAK	High FAK in malignant samples	In malignant samples, high FAK is prognostic for increased survival (U, $p=0.012$)	(127)
Extrahepatic bile duct carcinoma	76	IHC for FAK	High FAK in normal tissue (100%), variable expression in tumors	High FAK expression correlates with better survival (KM, $p=0.01$; U, $p=0.21$)	(128)
Intrahepatic cholangiocarcinoma	56	IHC for FAK	High FAK expression in some tumors (29%)	High FAK expression correlates with better survival (KM, $p<0.01$)	(129)
Correlation not assessed					
Breast cancer	55	Western blot and IHC for FAK	FAK expression is higher in tumors than normal tissue		(130)
Breast cancer	119	IHC for FAK	Moderate to high FAK expression in tumors (58%), including ductal carcinoma <i>in situ</i> (66%, n=51)		(131)
Breast – circulating tumor cells	45	Immunofluorescence for pFAK	pFAK detected in CK19 +ve circulating tumor cells (n=28 patients), not in CK19-ve peripheral blood mononuclear cells (n=17 patients)		(132)
Hepatocellular carcinoma	56	IHC for Src and FAK	FAK expression in tumors (58.9%)		(133)
Paget's disease – extramammary	33	IHC for pFAK (pY397) and pERK	pFAK detected in all tumors, not in normal skin		(134)
Prostate	35	IHC for Fyn, FAK and paxillin on TMA	FAK expression is higher in tumors		(135)
SCLC	42	FISH (amplification) and IHC on TMA	FAK expression (90%) and amplification (17%) seen in tumors		(136)

AML, acute myelogenous leukemia; SCLC, small cell lung carcinoma; IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridization; TMA, tissue microarray; Q-RT-PCR, quantitative reverse transcription-polymerase chain reaction; K, Kendall rank correlation test; KM, Kaplan-Meier log rank analysis; U, univariate analysis of survival; M, multivariate analysis of prognostic factors; χ^2 , correlation established using χ^2 -test.

carcinoma, and expression of miR151 has been associated with increased metastasis in a liver cancer model (5).

A long list of FAK binding partners has been identified and a large number of signaling pathways can be regulated downstream of FAK under specific conditions. The most significant signaling pathways in the context of tumor formation and progression of disease remain to be firmly established. This review will focus on the regulation of intracellular signaling pathways by FAK, specifically in the context of cancer proliferation, invasion, and metastasis.

Mitogen-activated protein kinases

It is well known that the Ras signaling pathway culminating in the activation of ERK1/ERK2 plays an important role in carcinogenesis, controlling cell proliferation, and controlling cell migration (10, 11). Activated FAK can directly and indirectly recruit adaptor proteins and Ras regulatory proteins into complexes to contribute to the regulation of these important signaling pathways. Many biochemical studies have demonstrated that FAK can activate multiple mitogen-activated protein kinase (MAPK) signaling pathways, including the ERK1/ERK2, JNK, p38 (MAPK), and ERK5 pathways. These are very likely important signaling pathways contributing to tumorigenesis and metastasis downstream of FAK (Figure 1).

A number of studies demonstrate a correlation between FAK signaling to MAPKs and biological response *in vitro*.

Anchorage-independent growth and cell motility of melanoma cells in response to syntenin overexpression depends on FAK-mediated p38 MAPK activation (12). FAK regulates ERK activation in melanoma cells to promote cell adhesion, invasion, and extracellular matrix-dependent proliferation (13). FAK also controls ERK5 activation, which leads to increased adhesion and motility of MDA-MB-231 breast cancer cells (14). Integrin-mediated ERK activation regulates adhesion and motility in colon cancer cells and this effect is FAK dependent (14). Fibronectin stimulates the migration and invasion of the A549 lung cancer cell line through activation of FAK and multiple downstream signaling pathways, including the ERK pathway (15). Glioma cell invasion is stimulated through activation of ERK1/2 and JNK1 and is regulated by FAK (16). These and other studies demonstrate a role for FAK in controlling the activation of MAPKs and a role for these signaling pathways in controlling biological responses that are altered in cancer.

Several tumor models support a role for the Ras/ERK pathway downstream of FAK *in vivo*. The murine 4T1 cell line forms mammary gland tumors in an orthotopic model, and injection of FAK short hairpin RNAs into the tumors modestly impairs tumor growth. These tumors also exhibit reduced ERK activation following injection of FAK short hairpin RNAs (17). A dominant-negative approach blocking FAK function also impairs tumorigenesis in 4T1 cells (18). This block can be bypassed by constitutive activation of the ERK pathway. V-*Src*-transformed mouse embryo

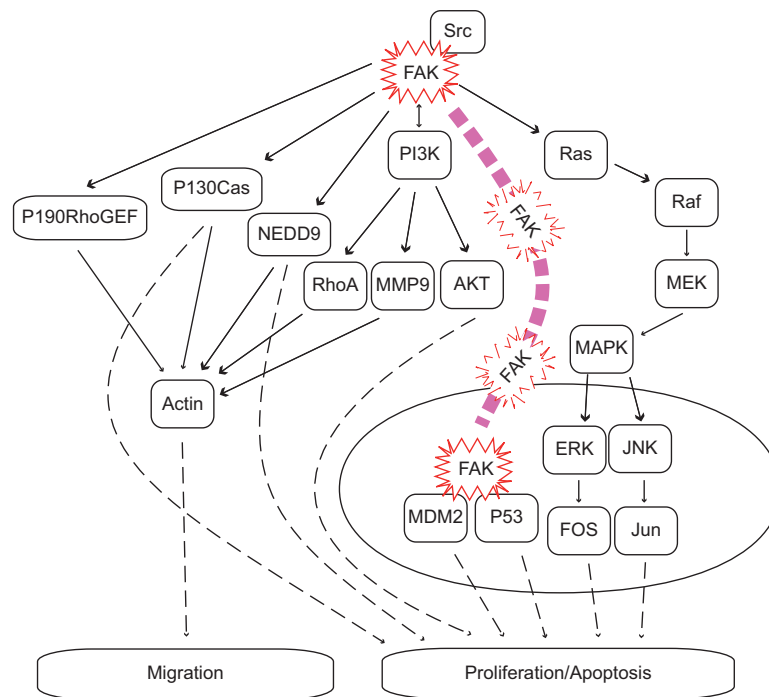


Figure 1 FAK-regulated signaling pathways related to cancer progression.

FAK regulates multiple downstream signaling pathways in cancer cells. The pathways shown are implicated in controlling tumorigenesis and/or metastasis in concert with FAK, or are intriguing candidates. These pathways control cytoplasmic and nuclear signaling events that converge to regulate cell migration, proliferation, and survival. In addition to signaling to the nucleus, e.g., through the Ras/MAPK pathway, FAK can also enter the nucleus and control p53-dependent biochemical and biological functions.

fibroblasts can grow as tumors and experiments using FAK null fibroblasts demonstrate that FAK plays a role in tumorigenesis in this model. While re-expression of wild-type FAK in these transformed null cells increases tumorigenesis by about 25%, re-expression of a Y925F mutant fails to rescue tumor formation (18). Further, expression of a Y925F FAK mutant suppresses ERK activation in B16F10 melanoma cells *in vitro* and impairs metastasis in an animal model (13). Collectively, these findings support a model where Grb2/Sos binding to FAK (at Y925) promotes ERK activation in tumor cells *in vivo* and that this signaling event is important for tumorigenesis.

CAS family

The best-characterized members of this family are p130Cas [the v-Crk-associated tyrosine kinase substrate, also known as BCAR1 (breast cancer anti-estrogen resistant locus 1)] and NEDD9 [neural precursor cell-expressed developmentally down-regulated gene 9, also known as human enhancer of filamentation 1 (HEF1)]. These scaffolding proteins localize to focal adhesions, directly interact with FAK, and are phosphorylated by FAK and Src (19).

p130Cas plays a critical role in cell signaling, motility, and proliferation in cancer cells. p130Cas is highly expressed in canine mammary tumor tissues compared with adjacent normal tissue at different stages of malignancy, and the expression level is related to tumor aggressiveness (20). In human breast cancer, p130Cas overexpression is also observed and breast cancer samples with high p130Cas and HER2 expression exhibit high Ki67-positive staining reflecting elevated proliferation (21). In a transgenic mouse model, expression of p130Cas in the mammary gland epithelium results in hyperplasia and transgenic expression of p130Cas in MMTV-neu transgenic mice results in an acceleration in the onset of tumor formation (21).

Overexpression of NEDD9 promotes cell spreading and migration in the MCF7 breast cancer cell line (22), but surprisingly inhibits the migration of normal epithelial cells (23). NEDD9 function in tumor formation and invasion was determined using NEDD9 null mice. Loss of NEDD9 expression does not affect the development of the normal mammary gland; however, loss of NEDD9 impairs early tumor development in the MMTV-Polyoma virus middle tumor antigen (MMTV-PyV MT) transgenic model of breast cancer (24). NEDD9 is also overexpressed in metastatic human melanomas (25). Primary melanocytes gain metastatic potential upon overexpression of NEDD9 and metastasis of melanoma cells is attenuated upon small interfering RNA-mediated NEDD9 knockdown (25).

The FAK/p130Cas and FAK/NEDD9 complexes are likely to play a critical role in cancer progression, although evidence in support of this hypothesis is only beginning to emerge. FAK expression is important for the formation of mammary gland tumors and metastasis in the MMTV-PyV MT breast cancer model (26–29). As described above, loss of NEDD9 expression also impairs tumor formation in this

model (24). In the absence of NEDD9, FAK phosphorylation is reduced in the primary tumors (24) and the loss of FAK results in reduced p130Cas phosphorylation when primary mammary gland epithelial cells are analyzed *in vitro* (28). Primary MMTV-PyV MT mammary gland epithelial cells lacking FAK exhibit defects in proliferation, survival, and invasion. This deficit can be rescued by re-expression of wild-type FAK, but not upon expression of a FAK variant that cannot bind p130Cas (29). These animal models of breast cancer provide evidence that interactions between FAK and Cas proteins may be biologically important in cancer; however, establishing the significance of these pathways requires additional mechanistic studies and extension of these findings to other cancers.

Phosphatidylinositol 3'-kinase

Phosphatidylinositol 3'-kinases (PI3Ks) control diverse cellular functions, including proliferation, differentiation, motility, and invasion (30–32). The class I PI3Ks, comprising a catalytic subunit and an SH2 domain containing adaptor subunit, are most relevant to this discussion. Receptor tyrosine kinases and Ras are major signaling molecules linked to the activation of class I PI3Ks (30, 33). The SH2 domain of the p85 adaptor subunit of PI3K interacts with Y397 on FAK, and PI3K might be regulated downstream of FAK. However, the relation between FAK and PI3K is complex as PI3K is reported to regulate FAK in some circumstances, e.g., in MDA-MB-231 cells (34). PI3K controls cell function by regulating levels of 3' phosphorylated phosphatidylinositols, which activate downstream effectors, e.g., protein kinase B (AKT), which in turn regulate downstream signals, e.g., through the mTOR signaling pathway (Figure 1). Deregulation of PI3K signaling is very frequent in cancer and thus the FAK/PI3K complex is potentially significant in the promotion of cancer progression by FAK.

PI3K is implicated in FAK-dependent cell migration in Chinese hamster ovary cells (35). PI3K regulates cytoskeleton organization during cancer cell migration. MCF7 motility requires Rac1-dependent actin organization, which is regulated by activation of PI3K, which in turn is regulated by FAK (36). Migration and invasion of BLM melanoma cells is also regulated by RhoA-controlled cytoskeleton organization, which is mediated by PI3K activation (37). The migration and invasion of A549 cells requires MMP-9 and RhoA activation, which is mediated through activation of FAK, Src, and PI3K (15). Increased FAK activity in A549 cells also results in inhibition of anoikis, which is mediated through PI3K/Akt signaling (38).

The interplay between FAK and PI3K is complex, potentially forming a positive feedback loop. The studies selected above illustrate that important biological events are controlled through FAK/PI3K signaling in cancer cells in culture. As FAK and PI3K are each implicated in cancer progression, it seems likely that FAK/PI3K signaling is a significant event in tumor formation and metastasis, although the experimental support for this hypothesis has yet to emerge.

Src

FAK was originally identified as an Src substrate and binding protein (39, 40). As a substrate, FAK is phosphorylated on multiple tyrosines by Src and two of these sites lie in the activation loop of FAK. Phosphorylation of these residues leads to maximal catalytic activity (41). Given these observations, it seems intuitive that FAK and Src function together in the development of human cancer.

A recent study of 108 patients with benign or malignant thyroid lesions supports the finding that elevated FAK expression correlates significantly with malignancy (42). Elevated levels of Src were also observed, although differences between benign and malignant samples did not reach significance. In colon cancer, both FAK and Src are overexpressed in both primary tumors and liver metastases. While expression of neither alone is prognostic, the combined overexpression of both FAK and Src is predictive of a short time until recurrence of disease, but is not linked to patient survival (43). Other studies have looked at tyrosine phosphorylation of FAK and Src in tumor samples, particularly the autophosphorylation sites of FAK and Src (generally indicative of activation) and sites on FAK that are substrates of Src phosphorylation (41, 44). In invasive lobular cancer of the breast, elevated levels of activated FAK are seen by immunohistochemistry and are correlated with increased levels of FAK phosphorylated on Y861 (45). In a small number of breast cancer samples, both elevated autophosphorylation of FAK (at Y397) and Src (at Y419) are seen in malignant ductal carcinoma *in situ* and invasive ductal carcinoma but not benign tissue (46). Not all studies exhibit this correlation. In 162 node-negative breast cancers, FAK overexpression was not correlated with autophosphorylation of Src, although it was correlated with Src phosphorylation at Y215 (47). Interestingly, in metastatic cancer, activated Src and phosphorylated FAK (Y576) were found in 50% and 67% of bone metastasis in patients exhibiting disease recurrence following tamoxifen treatment. These studies support the hypothesis that FAK and Src expression/activation may be linked in human disease, although there are clearly many examples of exceptions.

If FAK and Src signaling are linked in tumor cells, pharmacological perturbation of one is expected to affect the other. A number of studies using Src inhibitors have addressed this question. PH006 inhibits FAK phosphorylation at all tested sites of tyrosine phosphorylation, including Y397, in MDA-MB-435 cells grown orthotopically as tumors (48). The cross-reactivity of PH006 with FAK is unknown. A number of studies have examined the effect of AZD0530 on FAK phosphorylation. Treatment of xenograft models of lung, breast, pancreatic, and colon tumor cell lines impaired phosphorylation of FAK at Y861 (49, 50). AZD0530 also impairs Y861 phosphorylation in human pancreatic tumor specimens that were isolated and grown as tumors as xenografts in nude mice (51). In a model for tumor hypoxia, Src becomes activated and FAK phosphorylated on Y861 in hypoxic areas of pancreatic and cervical cancer xenographic models. AZD0530 impairs Src activation and FAK phosphorylation in this system (52). Dasatinib, SU11333, and CGP77675

also reduce FAK tyrosine phosphorylation in other xenograft models (53, 54). These pharmacological studies support the role of Src in the regulation of tyrosine phosphorylation of FAK in tumors.

The role of FAK as a signaling component downstream of Src in an oncogenic setting was evaluated using the v-src transformation model. Dominant-negative, RNA interference, and knockout approaches have been used. FAK null fibroblasts expressing the v-src oncogene exhibit a transformed morphology and form colonies in soft agar (55). While this study and others demonstrate FAK is dispensable for growth in soft agar (56), others suggest inhibition of FAK results in a dramatic increase in colony formation in soft agar (55, 57). The discrepancy between the results has not been resolved. The role of FAK in v-src-induced tumor formation depends on the model system. In v-src-transformed NIH3T3 fibroblasts, inhibition of FAK with a dominant-negative variant has no effect on tumor formation, but does impair experimental metastasis to the lung (56). In contrast, v-src-transformed FAK null mouse embryo fibroblasts exhibit a defect in tumor growth (18). While these findings support a role for FAK downstream of Src during tumorigenesis and metastasis, only one study addresses the possible role of Src downstream of FAK during tumor progression. Conditional knockout studies demonstrate a role for FAK in mammary gland tumorigenesis and metastasis in the MMTV-PyV MT transgenic model. This phenotype is recapitulated in an orthotopic model where mammary gland epithelial cells are isolated and re-introduced into the mammary gland fat pad of syngeneic mice (29). Re-expression of wild-type FAK in isolated mammary gland epithelial cells before orthotopic injection rescues this defect, whereas expression of a Y397F mutant does not (29). This result is supportive of a role for Src in FAK-dependent tumorigenesis; however, as the authors discuss, this mutant is defective for binding to a number of important signaling molecules, and the critical binding partner(s) has yet to be demonstrated.

Rho family of GTPases

Members of the Rho family of GTPases are important downstream components of the FAK signaling pathway. Multiple mechanisms of regulation are proposed; however, most entail recruitment of activators [guanine nucleotide exchange factors (GEFs)] or inhibitors [GTPase activating proteins (GAPs)] of these GTPases into complex. By altering localization or activity by binding or post-translation modification, the active state of Rho proteins is controlled. As critical regulators of the actin cytoskeleton, some of these pathways are important in controlling polarization and motility *in vitro* and they are presumed to be important signaling branches in FAK-promoted tumorigenesis and metastasis. In the context of tumorigenesis/metastasis, the best example may be p190RhoGEF, also known as Rgnef. This exchange factor binds the C-terminal domain of FAK, and a dominant-negative Rgnef fragment can disrupt the association of FAK with full-length Rgnef (58, 59). This Rgnef fragment impairs the ability of a

colorectal cancer cell line to form orthotopic tumors, whereas a similar Rgnef fragment that cannot bind FAK has no effect on tumorigenesis. While this result supports a role for the FAK/Rgnef complex in tumor formation, further experiments are clearly required to fully establish this molecular mechanism in promoting cancer.

As described above, miR151 is contained within *Ptrk2* intronic sequences. miR151 may also elicit biological effects by modulating the activity of Rho family proteins. RhoGDIA, Rho GDP dissociation inhibitor α , is a well-established inhibitor of Rho activity and is a target of miR151. By impairing expression of RhoGDIA, the activity of Rho family proteins is enhanced resulting in increased motility, invasion, and metastasis in hepatocellular carcinoma (5). In this model of hepatocellular carcinoma, both FAK and miR151 are proposed to act in concert to promote activation of Rho family proteins to promote invasion and metastasis.

p53

Mutation of p53 is one of the most frequent genetic alterations associated with the development of human cancer (60). p53 is a tumor suppressor that functions as a transcription factor (61). Multiple p53 mutations have been described in human tumors, and these mutations result in loss of function of p53 and frequently these can interfere with the function of wild-type p53. Normally, a labile protein, p53, is regulated by controlling protein levels through ubiquitin-mediated protein degradation.

Some evidence indicates that p53 activity is related to FAK expression levels in human cancer. Elevated expression of FAK has been correlated with mutation of p53 and elevated p53 expression in endometrial and breast cancer (62, 63). There are two p53 binding sites located in the promoter region of FAK and overexpression of wild-type p53 suppresses the expression of FAK (64). Thus, one mechanism leading to elevated expression of FAK in human tumors might be directly related to loss of function of p53.

Interestingly, the regulation of p53 and FAK is reciprocal. The FERM domain of FAK interacts with p53 directly *in vitro* and *in vivo*, and the p53-dependent regulation of p21, MDM2, and BAX can be blocked by overexpression of FAK (65, 66). FAK can localize to the nucleus and suppression of p53-induced transcription may be mediated by blocking the transcription activation function of p53 (Figure 1). Additionally, nuclear FAK also enhances p53 degradation in fibroblasts and endothelial cells by mediating the assembly of a FAK/p53/Mdm2 complex (67). Assembly of this complex promotes Mdm2-dependent ubiquitination of p53 and its subsequent degradation. Thus, elevated expression of FAK in tumors could result in enhanced p53 degradation and/or impaired p53 function, which would represent a novel mechanism of disruption of this critical signaling pathway leading to the progression of disease. It is noteworthy that pharmacological targeting of FAK may not be useful therapeutically in this scenario, as FAK-dependent p53 degradation is independent of the kinase activity of FAK.

Not surprising, the biological activities controlled by FAK/p53 are related to cell survival. Survival signals from the extracellular matrix are mediated by FAK signaling, which suppresses a p53-regulated apoptotic pathway (68). Further, expression of FAK specifically blocked p53-induced apoptosis in the SAOS-2 cell line. The function of FAK and p53 on tumor survival and apoptosis has mainly been addressed in breast or colon cancer cell lines, and these findings have yet to be extended to other types of cancers. While the link between FAK and p53 is provocative, the significance of their interaction in tumor formation and metastasis remains to be firmly established.

FAK, epithelial-to-mesenchymal transition, and cancer stem cells

Epithelial-to-mesenchymal transition (EMT) is the process whereby cells lose their epithelial phenotype, acquire a mesenchymal phenotype, disassociate, and become more motile and invasive under certain physiological or pathological conditions. Associated with these morphological and functional changes are changes in expression of protein markers of epithelial/mesenchymal cells. EMT is an important process during embryogenesis and tissue regeneration, and pathologically EMT may play an important role in cancer progression and metastasis (69). In addition to several oncogenic signaling pathways that are involved in the EMT process, such as growth factors (e.g., TGF- β), Src, and Wnt, microenvironment factors like the extracellular matrix also play a role. As several of these factors regulate FAK, one major function of FAK during carcinogenesis and cancer metastasis may be regulating EMT.

The composition of the extracellular matrix proteins plays an important role in controlling EMT. Collagen I or collagen III can induce disassembly of the E-cadherin adhesion complex, reduce E-cadherin gene expression, reduce cellular aggregation, and can promote morphological changes associated with a mesenchymal phenotype (70, 71). As FAK is activated when cells are attached to the extracellular matrix, it is a candidate for controlling collagen-induced EMT. However, there must be additional signals for specificity as FAK is also activated when cells attach to extracellular matrix components that do not promote EMT. One interesting mechanism is through engagement of additional receptors. Integrin binding to collagen activates FAK and binding of a second collagen receptor, discoidin domain receptor 1 (DDR1), activates Pyk2. Both of these signaling events are required to promote expression of N-cadherin, a mesenchymal marker, and induce EMT in a pancreatic cancer cell line (71). In a number of other scenarios, extracellular matrix proteins can cooperate with soluble ligands, e.g., TGF- β , to promote EMT. EMT can be induced in primary hepatocytes by treatment with TGF- β , but is enhanced in the presence of a collagen extracellular matrix (72). TGF- β -induced EMT in two mammary epithelial cell lines, MCF10A and NMuMG, requires the $\alpha_v\beta_5$ integrin and pharmacological inhibition of FAK activity inhibits EMT (73). One possible role for FAK in TGF- β -mediated EMT is to facilitate the assembly of an integrin β_3 /TGF- β

receptor II complex in mammary tumors (74). FAK is proposed to participate in heparin binding-epidermal growth factor-induced EMT in ovarian cancer cells by coordinating crosstalk between the growth factor receptor and integrin signaling pathways (75). FAK signaling is also required for EMT induced by TGF- β in hepatocytes (76) and renal tubular epithelial cells (77), although the role of integrin-dependent cell adhesion in these model systems is unclear.

The mechanism of regulation of EMT by FAK has not been elucidated. FAK may play a role in regulating the assembly of the E-cadherin complex (78, 79). FAK may also play a role in regulating internalization of E-cadherin. This may be directly relevant in cancer, as perturbation of FAK alters the dynamics of surface E-cadherin in A431 cells growing as tumors in animal models (80). FAK could also regulate EMT by modulating the activity/expression of transcription factors critical for EMT. KLF8 is established as a downstream target of FAK and can induce EMT in breast cancer cells (81, 82). While this is an attractive mechanism, the role of KLF8 as a transcription factor downstream of FAK signaling in promoting EMT remains to be formally tested.

Cancer stem cells are defined as a subpopulation of cells within a tumor that have the ability to initiate tumorigenesis (also known as tumor-initiating cells) by undergoing self-renewal and differentiation like other normal stem cells (83, 84). Interestingly, cancer cells that have undergone EMT share properties with cancer stem cells, including the expression of stem cell markers (85). As FAK may function in the control of EMT, it seems likely that FAK might also function in cancer stem cells. As mentioned above, deletion of FAK in the mammary gland epithelium impairs tumor formation in the MMTV-PyMT transgenic model. Isolation of mammary cancer stem cells, such as Lin⁻CD24⁺CD29⁺CD61⁺ cells or ALDH⁺ cells, reveals that FAK deletion reduces the mammary cancer stem cell population (27). FAK can promote mammary stem cell survival. IL8 signals through CXCR1, which results in FAK activation, and protects cancer stem cells from FASL/FAS-mediated cell death (86). In pancreatic cancer stem cells, CXCL12 signaling may induce the activation of FAK and PKB to promote cell growth and drug resistance (87). Ablation of FAK also leads to changes in the properties of mammary cancer stem cells, including a deficiency in tumorigenesis (27). While these recent studies support a role for FAK in controlling cancer stem cell functions, the mechanisms have yet to be determined.

FAK and cancer therapy

Several companies have developed small-molecule ATP-competitive inhibitors that impair the kinase activity of FAK. TAE226 decreases tumor size and metastasis in an ovarian carcinoma animal model. It also slows the growth of established tumors by increasing apoptosis and decreasing cell proliferation and angiogenesis. TAE226 was most effective in combination with docetaxel (88). The major shortcoming of TAE226 is its cross-reactivity with the IGF-1 and insulin receptors, significantly complicating its use therapeutically.

Pfizer has developed a series of small-molecule ATP-competitive inhibitors of FAK. PF-562,271 has an IC₅₀ of 1.5 nM on purified protein and in cell-based assays is fourfold more selective for FAK than its closest relative, Pyk2. This compound effectively inhibits tumor growth in xenograft models of prostate, breast, pancreatic, colon, glioblastoma, and lung cancers. Further, tumor regression was observed in most of these models (89). In a study investigating bone metastasis using MDA-MB-231 xenografts, PF-562,271 decreased the growth of tumor mass within the bone and after 2 weeks bone healing was observed at sites previously damaged by the tumor. Additionally, PF-562,271 improved structural parameters of bone such as thickness and cancellous bone volume in non-tumor-bearing rats (90). The effect of PF-562,271 in combination with sunitinib was examined in a hepatocellular carcinoma model. This drug combination reduced α -fetoprotein (a marker for hepatocellular carcinoma) expression fourfold and significantly decreased the growth of hepatoma cells in a subcutaneous model of tumorigenesis. Overall, the combination was significantly more effective than sunitinib alone (91). PF-562,271 has successfully completed phase 1 clinical trials and is currently undergoing phase 2 trials (92). The newest ATP-competitive inhibitor of FAK is from Pionard Pharmaceuticals. In a subcutaneous xenograft model, PND1186 prevents tumor growth by inducing apoptosis. Additionally, it inhibits ovarian carcinoma growth *in vivo*. Orthotopic breast cancer models using either 4T1 or MDA-MB-231 cells showed decreased growth and metastasis upon *ad libitum* administration of PND1186 (93).

The limitations of small-molecule ATP-competitive inhibitors include cross-reactivity with other kinases and the development of resistance through mutation. Further, FAK has both enzyme and scaffolding functions and these FAK drugs do not necessarily inhibit the latter. Thus, the development of additional drugs using different strategies to inhibit FAK function might be significant (Figure 2). There have been a number of studies describing the identification of compounds that inhibit FAK through different mechanisms. Using an *in silico* screen,

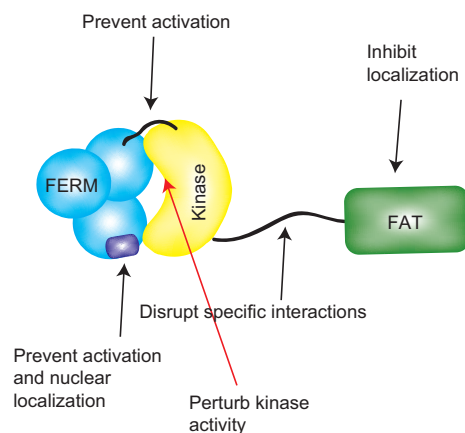


Figure 2 A cartoon representation of the FAK structure. Most inhibitors currently target the ATP binding pocket (red arrow). Other potential targets for FAK inhibitors and their possible effect on FAK function are shown.

a compound binding adjacent to Y397 and inhibiting phosphorylation was identified. This compound inhibits binding of Src and subsequently phosphorylation of the FAK activation loop, preventing full activation of FAK. This compound inhibits tumor growth in a pancreatic tumor xenograft model and has a synergistic effect with gemcitabine (94).

A combination of a phage display strategy and *in silico* screen led to the identification of a small molecule that could target the FAT domain (95, 96). This compound, chloropyramin hydrochloride [*N*-(4-chlorobenzyl)-*N*,*N*,*N*,*N*-dimethyl-*N*-pyridin-2-ylethane-1,2-diamine], binds to the FAT domain and interacts with S939 and H1025. This compound could effectively inhibit binding to paxillin as H1025 is a residue implicated in paxillin binding. While these latter compounds have not achieved the preclinical success of the small-molecule ATP-competitive inhibitors of FAK, these studies demonstrate the feasibility of alternative therapeutic strategies to target FAK.

Two important considerations in therapeutically targeting FAK in the clinic are the identification of patients most likely to benefit from FAK inhibition and appropriate biomarkers that might be used to evaluate the effectiveness of treatment. At present, with small-molecule FAK inhibitors in phase 1 trials and beginning phase 2 trials, a discussion of patients who might benefit from these compounds is speculative. Patients with elevated FAK expression could potentially benefit and tumors with elevated phosphorylation on Y397 and/or Y576/Y577, indicative of FAK autophosphorylation and catalytic activation, might be effectively treated with small-molecule FAK inhibitors (see Table 1). Triple-negative breast cancer patients might benefit from treatment with FAK inhibitors as FAK overexpression is prognostic, and effective therapies for this disease have not been discovered. Importantly, studies evaluating FAK expression and prognosis have provided insight into patients who might be harmed by FAK therapeutics, as FAK expression is indicative of a good prognosis in some types of tumors (see Table 1). The discussion thus far has considered the tumor cells as the target for FAK inhibition; however, FAK also plays important roles in normal stromal cells in the tumor microenvironment and FAK inhibition in these cells might effectively influence the growth and spread of the cancer. For example, endothelial cell FAK is important for normal and tumor-induced angiogenesis (97), and preclinical animal models of cancer have demonstrated reduced tumor angiogenesis in response to small-molecule FAK inhibitors (88, 89). Finally, the identification of biomarkers to evaluate the effectiveness of FAK inhibitors in patients is required. The direct measure of the effect of FAK inhibitors would monitor changes in FAK tyrosine phosphorylation induced by these therapeutics. Y397 is the autophosphorylation site and has been used to evaluate compound effectiveness in cells in culture in many studies in the literature. Other sites of tyrosine phosphorylation are indirect measures of inhibition of FAK catalytic activity, as autophosphorylation promotes phosphorylation at these other sites. In addition, tyrosine phosphorylation of potential downstream substrates, e.g., paxillin and p130Cas, could also be measured as an indirect readout, which is complicated by the fact that

these proteins are also substrates for other tyrosine kinases. A further challenge is how to assess effects on tyrosine phosphorylation in patients, as a surrogate tissue mimicking drug effects on FAK in the tumor has not been identified.

Outlook

The role of protein tyrosine kinase signaling pathways in controlling cancer cell carcinogenesis and metastasis is well established and they serve as effective therapeutic targets. Compelling evidence supports a role for FAK in tumorigenesis and metastasis, and it is emerging as a potential therapeutic target. However, many of the molecular details of how this enzyme functions to promote initiation and disease progression have not been rigorously defined. Many molecules have been identified as components of FAK signaling pathways; however, as signaling can be context dependent, it would seem prudent to establish which pathways are active in cells during tumor formation and metastasis. This would provide important insight into molecular mechanism, and might also be critical for the design of combinatorial therapy. As described throughout, these types of studies are slowly emerging and some signaling events downstream of FAK have been confirmed in tumor and metastatic models, and correlative evidence was observed in human tumor samples. Additional studies defining FAK-regulated signaling pathways in these contexts are one important direction for future studies.

The properties of cancer cells can change during disease progression, increasing the likelihood of spread of the disease. As a result of EMT, epithelial cells acquire a mesenchymal phenotype, become more motile and invasive, and become resistant to anoikis. Obviously, these latter properties are important for metastasis. Recent studies have focused on the role of cancer stem cells in the establishment and progression of disease. Interestingly, cancer stem cells and cells that have undergone EMT share a number of properties. FAK has been implicated in EMT and in the maintenance of cancer stem cells; however, the mechanisms remain unknown. Elucidating these mechanisms is a second important focus for future studies.

As mentioned previously, small-molecule ATP-competitive inhibitors of FAK have been developed and proven successful at inhibiting tumors and metastasis in preclinical models. FAK may be a particularly effective therapeutic target as it functions in both the cancer cells and the normal endothelial cells in the tumor microenvironment. Thus, targeting FAK may affect tumor growth by direct action on the tumor cells and indirectly by attenuating angiogenesis. There are limitations to ATP-competitive inhibitors and the development of compounds that inhibit FAK function through other mechanisms might be very beneficial. As noted above, in several studies, FAK is most effective in combination with other therapeutics. Further studies to define effective combinations of therapeutics in targeting FAK in cancer would clearly be beneficial. These types of studies are a third important direction for future research.

Highlights

- FAK regulates multiple signaling pathways that are implicated in cancer progression, including the MAPK, PI3K, and Rho family pathways.
- Evidence for these pathways in controlling tumorigenesis/metastasis downstream of FAK is only beginning to emerge.
- p130Cas and NEDD9 are important scaffolding partners that functionally interact with FAK in at least one preclinical model of breast cancer.
- Clinical and experimental evidence support the importance of FAK/Src interactions in cancer.
- FAK and p53 are reciprocally regulated and their interplay may be significant in cancer.
- Small-molecule ATP-competitive inhibitors of FAK are effective in preclinical models.
- Novel strategies to inhibit FAK function are beginning to emerge.

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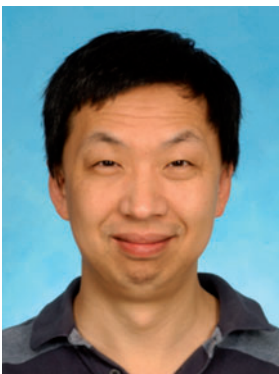
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