Focal Adhesion Kinase: Targeting Adhesion Signaling Pathways for Therapeutic Intervention

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Abstract The tumor microenvironment plays a central role in cancer progression and metastasis. Within this environment, cancer cells respond to a host of signals including growth factors and chemotactic factors, as well as signals from adjacent cells, cells in the surrounding stroma, and signals from the extracellular matrix. Targeting the pathways that mediate many of these signals has been a major goal in the effort to develop therapeutics.

Integrin Signals and Cancer

Integrins constitute the primary set of receptors for extracellular matrix (ECM) components within the tumor microenvironment, and integrin expression is often modulated during cancer progression and metastasis (1–6). Integrins are heterodimeric transmembrane receptors that link the cell to the ECM by direct binding to the actin cytoskeleton through a scaffold of several actin-binding proteins (7). Thus, they serve as sites of contact between the cell and the ECM. In adherent cells such as fibroblasts, these sites of contact are termed focal adhesions (8). Focal adhesions serve as nodes to transmit environmental signals initiated by soluble growth factors, ECM, and surrounding mechanical forces to regulate cell growth and migration (9–11). Within tumors, cancer cells respond to changes in the expression and organization of matrix proteins such as fibronectin and collagen. In addition, tumor cells secrete proteases that cleave ECM molecules (metalloproteases) providing both growth-promoting remodeling of the tumor microenvironment and escape of tumor cells through the basement membrane (12).

Tyrosine protein kinases play a key role in regulating signals mediated by integrin receptors (13, 14). A central regulator of integrin signaling is focal adhesion kinase (FAK), a ubiquitously expressed nonreceptor tyrosine protein kinase (15). FAK expression is required for many normal cellular functions (16) and its expression is up-regulated in a variety of late-stage cancers (16–19). Here we review the role of FAK in promoting integrin and growth factor signals and its link to cancer progression. We also discuss experiments validating FAK as a therapeutic target, summarize the current state of development of small-molecule inhibitors of FAK, and report progress in assessing such inhibitors in human clinical trials.

FAK structure and regulation. FAK is recruited to focal adhesions and activated upon integrin engagement with the ECM (15, 16). The overall molecular structure of FAK is consistent with its function as a protein scaffold, in addition to its function as a tyrosine kinase. The central catalytic domain is flanked upon the NH2 terminus by a FERM domain and COOH-terminal proline-rich and focal adhesion targeting domains (Fig. 1A). The FERM domain has been reported to serve as a site of interaction with a variety of molecules including integrin cytoplasmic domains, ezrin, Arp2/3, p53, and membrane receptors (13, 15). The COOH-terminal domain functions as a scaffold with two proline-rich protein interaction domains (PR-I, PR-II) and a focal adhesion targeting domain, a tetrahedral structure, which is required for the localization of FAK to focal adhesions likely through binding the focal adhesion protein, paxillin (20). The proline-rich regions provide binding sites for several SH3-domain containing proteins including p130Cas, GRAF, and ASAP1 (21–23).

FAK is maintained in an inactive and autoinhibited state by the binding of the FERM domain to the kinase domain (ref. 24; Fig. 1A). Engagement of integrins with the ECM leads to signals that presumably relieve autoinhibition, resulting in stimulation of FAK catalytic activity and the concomitant phosphorylation at tyrosine 397 (Y397). The phosphorylation of Y397 provides a canonical binding site for the SH2 domain of c-Src (and other Src-family kinases; refs. 15, 25). Binding of the of Src-SH2 domain to FAK relieves the autoinhibition of Src kinase and thus sustains both Src and FAK in their activated states, thereby creating a functional bipartite kinase complex (14, 24, 25). Activated c-Src phosphorylates other sites on FAK and other focal adhesion proteins such as paxillin and p130Cas (26, 27). The phosphorylation of these proteins has been implicated in the activation of downstream kinases, p21-activated kinase and mitogen-activated protein kinase/extracellular signal–regulated kinase kinase/extracellular signal–regulated kinase, the small GTPases, Rac and Rho, as well as other signaling pathways (28–30).

FAK expression in human cancers. There is now abundant evidence for the increased expression of FAK in a broad range of human cancers (Fig. 1B). Using a variety of methods, including Western blotting, immunohistochemistry, Northern blotting, and quantitative PCR, increased expression of FAK has been
Fig. 1. A, proposed activation pathway for FAK. FAK resides in an inactive conformation via an interaction of the FERM domain and the catalytic domain. Integrin clustering leads to the juxtaposition of FAK molecules and FAK is activated through a putative mechanism that alters the conformation of the FERM domain (FERM*). Phosphorylation of FAK on Y397 provides a binding site for Src and leads to Src activation. The bipartite FAK/Src complex phosphorylates FAK on additional tyrosines and phosphorylates other focal adhesion proteins such as paxillin and Cas. B, cancer progression and the altered regulation of FAK expression and signaling in cancer cells. Alterations in growth factor signaling, genetic alterations/mutations, and changes in the microenvironment drive the increase in FAK expression observed in late-stage cancers. Two candidate regulators of FAK expression, p53 and nuclear factor-κB (NF-κB), have been directly implicated in tumor progression and their role in regulation of FAK gene expression remains to be clarified. The overexpression of FAK is proposed to augment signaling pathways that contribute to cell proliferation, cell survival, and cell migration leading to cancer progression and metastasis.
reported in tumors of the breast, colon, thyroid, prostate, oral cavity, liver, stomach, skin, head and neck, lung, kidney, pancreas, bone, and ovary (17–19). In addition, increased FAK expression has been reported in a variety of tumor-derived cancer cell lines (19). Whereas increased expression of FAK protein and/or mRNA is well documented, the molecular mechanisms underlying this increased expression are poorly understood. FAK amplification within chromosome q8.24.3 has been reported in a subset of primary hepatocellular carcinomas (31). Elevation of FAK protein in cell lines derived from invasive squamous cell carcinomas as well as frozen sections from such tumors has been reported to be accompanied by gains in copy number of the fak gene (32). In addition, increased fak copy number was observed in numerous cell lines derived from human tumors of lung, breast, and colon (32). In a study of more than 200 head and neck carcinomas, FAK expression was increased in most cancers; however, elevated FAK expression did not correlate with increased fak gene copy number or amplification of the fak gene (33). These studies point to the possibility that epigenetic mechanisms serve as the primary trigger for increased FAK gene expression and increased protein expression.

The analysis of the human FAK promoter reveals an ∼600-bp region required for maximal promoter activity. Interestingly, this region contains putative binding sites for several transcription factors, including two nuclear factor κB and p53 binding sites (ref. 34; Fig. 1B). Coexpression experiments reveal that FAK promoter activity was stimulated by nuclear factor κB and repressed by p53 (17, 34). These observations suggest the interesting possibility that cytokine and growth factor signals and p53 status of tumors may directly influence the levels of FAK expression.

Validation of FAK as a Therapeutic Target

Attempts to validate FAK as a therapeutic target in human cancers have centered on the use of three strategies: interfering with FAK function by overexpressing dominant negative mutants of FAK, reduction of FAK expression using either antisense oligonucleotides or small interfering RNAs (siRNA), or genetic manipulation of the fak locus in mice to extinguish FAK expression.

Dominant negative mutants of FAK. Dominant negative mutants of FAK are based on the observation that the COOH-terminal domain of FAK is expressed in some cells as an ∼43-kDa protein termed FAK-related nonkinase (FRNK; refs. 35–37). Forced expression of FRNK in a variety of normal and cancer cells inhibits activation of endogenous FAK and blocks a number of FAK-dependent processes including migration and cell survival (38). In cancer cells, expression of FRNK using adenovirus delivery blocks the growth of prostate and breast cancer cells in cell culture and in immunodeficient mice (3). Inhibition of FAK activity with FRNK in MTLn3 cells (rat mammary carcinoma cells) decreased proliferation in culture and decreased primary tumor growth in syngeneic rats (39). Similarly, adenovirus-mediated expression of dominant negative FAK in human breast cancer cells caused the loss of adhesion and degradation of endogenous FAK and induced apoptosis (40). These experiments support a role for FAK in tumor cell growth and survival. However, as a cautionary note, the overexpression of FRNK may affect other critical pathways because this domain is well documented to interact with numerous proteins that potentially regulate pathways controlling serine/threonine kinases and small GTPases (15, 16).

FAK-specific antisense oligonucleotides and siRNAs. Antisense oligonucleotides and siRNAs have served as useful tools to reduce the expression of endogenous proteins in both normal and cancer cells. Treatment of A549 human adenocarcinoma cells with FAK antisense oligonucleotides significantly reduced FAK expression and the concomitant signaling to c-Jun NH2-terminal kinase; cell migration and invasion through Matrigel were also inhibited (41). Treatment of melanoma cell lines with FAK antisense oligonucleotides resulted in the decreased expression of FAK and a 2.5-fold increase in cell death compared with treatment with control oligonucleotides (42).

More recently, the use of siRNAs to FAK has proved informative in dissecting FAK functions in a variety of cell types (43). In several different human cancer cell lines, FAK-specific siRNA reduced FAK protein levels by ∼70%; however, there was no clear evidence of apoptosis. FAK siRNA treatment of lung cancer cells significantly decreased colony formation in soft agar and migration in response to serum or epidermal growth factor (44). In the murine 4T1 breast carcinoma cells, inhibition of FAK expression using FAK-specific small hairpin RNAs failed to inhibit 4T1 proliferation in culture. However, 4T1 cells with reduced FAK expression exhibited reduced invasion through Matrigel and, in mice, fewer metastasis to the lung and enhanced survival (45).

The therapeutic potential of FAK siRNAs has been assessed recently using FAK siRNAs in the neutral liposome 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC). A single dose of FAK siRNA-DOPC was shown to be highly effective in reducing in vivo FAK expression and tumor weight in three different human ovarian cancer cell lines grafted into nude mice (46).

Whereas siRNAs and antisense reagents seem to be relatively efficient in suppressing FAK expression, it is clear that reduction/suppression of FAK expression does not induce a common set of phenotypes. This may indicate that the biological setting of the tumor (e.g., microenvironment) may play an important role in governing phenotypic responses on loss of FAK expression. In addition, partial loss of FAK expression due to incomplete inhibition of action with siRNA or antisense strategies may also complicate the in vivo observations of tumor cell behavior.

Germ-line deletion of FAK in murine tumors. The availability of mice with conditionally regulated alleles of FAK (floxed fak) has made it possible to selectively knock out FAK expression in tumor models driven by carcinogen treatment or transgenic expression of strong oncogenes. In mice heterozygous for FAK expression, papilloma formation resulting from the application of carcinogens to the skin was reduced compared with wild-type mice (47). More recently, analysis of mice bearing a floxed fak allele and Cre recombinase under the control of the keratin-14-estrogen receptor promoter revealed that hydroxy-tamoxifen treatment induced fak deletion in the epidermis and suppressed...
chimically induced skin tumor formation (48). Deletion of fak after benign tumors had formed served to inhibit malignant progression.

A similar approach has been used to examine the polyoma virus middle T-antigen transgenic mouse model of breast cancer. Mice bearing floxed fak and mouse mammary tumor virus (MMTV)–driven Cre exhibited fewer premalignant lesions and the transition of premalignant hyperplasias to carcinomas and their subsequent metastases was significantly reduced. This block in tumor progression was further correlated with impaired mammary epithelial proliferation, indicating that FAK plays a critical role in mammary tumor progression. Analogous studies show that the mammary gland–specific deletion of FAK leads to significant suppression of tumor initiation, growth arrest and apoptosis of middle-T–transformed mammary epithelial cells, and inhibition of the ability of such cells to undergo metastasis.5

The role of FAK in prostate tumor progression has been examined in a parallel fashion using transgenic adenocarcinoma of the mouse prostate mice (in which the prostate-specific promoter for probasin drives SV40 T-antigen expression) bearing floxed fak with or without probasin-Cre. As in the murine MMTV-midleT mice, Cre expression leads to deletion of FAK in the prostate, but there is little inhibition of early malignant transformation of prostate epithelial cells. However, there is a strong correlation between FAK expression and late-stage tumorigenesis, indicating that FAK is required for progression of early tumors to late-stage adenocarcinoma.6

Thus, in two transgenic mouse models for cancer progression, the sustained expression of FAK seems to correlate with progression to late-stage disease. These observations parallel observations in β1-integrin–deficient, MMTV-middle T antigen–initiated breast cancer wherein continued β1-integrin expression is critical for both the initiation of mammary tumorigenesis in vivo and maintenance of the proliferative capacity of late-stage tumor cells (49). Thus, these mouse studies underscore the importance of the integrin-FAK signaling pathway in cancer progression.

Clinical-Translational Advances

Small-molecule inhibitors of FAK. Several recent reports describe the properties FAK inhibitors both in cell culture and animal studies. PF-573,228, an early prototype ATP-competitive inhibitor of FAK, inhibits recombinant FAK activity with an IC50 of 3 to 4 nmol/L. PF-573,228 blocks FAK autophosphorylation in normal fibroblasts and human tumor cells and inhibits cell migration by blocking adhesion turnover. Interestingly, PF-573,228 has no effect on the growth of normal or cancer cells in culture and does not induce apoptosis in fibroblasts (50). A second compound, PF-562,271 (with bioavailability suitable for preclinical animal and human studies), is a potent inhibitor of both FAK and the related kinase Pyk2. PF-562,271 exhibits >100-fold selectivity for FAK and Pyk2 when assayed against a panel of non-target kinases. In preclinical animal models, PF-562,271 inhibited FAK phosphorylation in vivo in a dose-dependent fashion in several human s.c. xenograft models, including PC-3M (prostate), BT474 (breast), BxPc3 (pancreatic), LoVo (colon), U87MG (glioblastoma), and H460 (lung).7 Treatment of cultured fibroblasts, epithelial cells, or prostate, ovarian, and pancreatic cancer cells with PF-562,271 resulted in a dose-dependent decrease in FAK autophosphorylation and maximal inhibition at concentrations of 100 to 300 nmol/L.8 Treatment with PF-562,271 inhibited ligand-stimulated cell migration of most cancer cell lines tested. PF-562,271 treatment of normal fibroblasts or epithelial cells and cancer cell lines with maximal inhibitory doses failed to inhibit cell growth in tissue culture or induce apoptosis of adherent cells when used at concentrations that effectively inhibited FAK activity. However, when tumor cells were placed under conditions of anchorage-independent growth, PF-562,271 effectively blocked tumor cell growth, mimicking the effects of this drug in xenograft models. Thus, cell culture and animal studies provide strong support for the use of PF-562,271 for the treatment of human malignancies.

TAE226 is a dual-specificity inhibitor, exhibiting specificity for FAK and insulin-like growth factor I receptor. The activity of TAE226 has been assessed in gliomas and breast cancer cell lines (51–53). In glioma cell lines, TAE226 inhibits cell migration, induces apoptosis, and inhibits cell growth. TAE226 inhibits ECM-induced phosphorylation of FAK on Y397 and insulin-like growth factor I–induced phosphorylation of insulin-like growth factor I receptor as well as inhibits putative downstream target genes such as mitogen-activated protein kinase and Akt. TAE226 treatment significantly increased animal survival in an intracranial glioma xenograft model (51). Treatment of breast cancer cell lines with TAE226 induces a dose-dependent detachment and apoptosis of cells (53). TAE226 inhibition of FAK Y397 phosphorylation correlated with the activation of poly(ADP-ribose) polymerase and caspase-3 proteins. Because TAE226 is an effective inhibitor of both FAK and insulin-like growth factor I receptor, its activity against gliomas and breast cancer cells likely reflects its dual inhibition of growth-promoting and adhesion pathways.

Therapeutic trials in humans. The FAK inhibitor PF-562,271 is currently in phase I clinical trials (54). A preliminary report describes treatment of 32 patients with colorectal, breast, neuroendocrine, lung, gastric, small-cell, and ovary cancers. Adverse events related to PF-562,271 treatment (Common Toxicity Criteria grades 1–2) were observed in ~10% of patients and included nausea, vomiting, fatigue, anorexia, abdominal pain, diarrhea, headache, sensory neuropathy, rash, constipation, and dizziness. Doses >15 mg twice daily produced steady-state plasma concentrations exceeding levels of the drug required to inhibit FAK as predicted from preclinical models. In one patient with ovarian cancer, positron emission tomography scans showed significant (46%) decline in tumor.

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mass and improved tumor-related symptoms. Four patients undergoing more than six cycles of treatment had stable disease. These preliminary studies provide evidence that PFK-562,271 is well tolerated in cancer patients on extended oral administration, with some patients showing durable stable disease. The study is ongoing at this time.

**Prospect.** The role of integrin in sensing the extracellular environment has long been appreciated (6). An exciting prospect is that knowledge of the connection between integrins and intracellular signaling pathways is being used to identify and validate targets for therapeutic intervention. As discussed above, FAK is one such target under intense investigation, and with inhibitory compounds currently in clinical trial, it should be clear within the next year or two whether inhibition of the FAK pathway is efficacious for the treatment of human cancer. However, for cancer biologists, many questions remain. How is FAK expression up-regulated in cancer? The possible role of p53 and NF-κB suggested that loss of tumor suppressors and stimulation of proinflammatory signals may play an important role in modulating the adhesion response of tumor cells during cancer progression. In addition, it is likely that the adhesion signaling pathway will yield additional targets for therapeutic intervention in the next decade.

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**References**

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