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REVIEW ARTICLE

FOCAL ADHESION KINASE (FAK) INHIBITION AS A POTENTIAL STRATEGY FOR ANTICANCER THERAPIES

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ABSTRACT

Focal adhesion kinase (FAK) is a multidomain nonreceptor tyrosine kinase that mediates growth factor- and adhesion-derived cell signaling. FAK plays crucial roles in cell proliferation, survival, motility and invasion, all of which are hallmarks of cancer cells. Overexpression of FAK has been observed in diverse cancer types and is used as a marker for invasion and metastasis. Furthermore, in vivo animal studies demonstrated an involvement of FAK in tumor development and malignancy. Therefore, FAK is a potential target for anticancer drug discovery. In this review, we will first present FAK and its relationship to cancer, with focus on target validation of FAK. Secondly, approaches to inhibit FAK as a potential drug target for therapeutic intervention in cancer treatment will be discussed.

INTRODUCTION

Role of focal adhesion kinase

Cellular interactions with extracellular matrix and growth factors play essential roles in tumor initiation, progression and metastasis. Focal adhesion kinase (FAK) is a 125-kDa multidomain nonreceptor protein-tyrosine kinase (PTK) that mainly localizes in the cytoplasm of cells. Upon cell adhesion on diverse extracellular matrices and/or activation by growth factors, FAK is recruited to focal adhesions (FAs), the closest contacts between the cell and the extracellular matrix, and mediates FA signaling. The complex structure of FAK results in a broad range of protein–protein interactions with other tyrosine kinases, cytoskeletal and adaptor proteins that are part of the so-called adhesome (1). FAK is known to play important roles in tumor progression and metastasis through its regulation of cancer cell migration, invasion, anchorage-dependent cell proliferation and survival. Recently, numerous in vivo studies have demonstrated the role of FAK in tumor initiation, as well as progression. In agreement with these experimental data, FAK is linked to human cancer mainly due to its overexpression and activation in a number of human tumors. Altogether these studies suggest that FAK is a potential target for drug discovery.

FAK structure and its regulation

As shown in Figure 1, FAK consists of several domains. The FERM domain (band 4.1-Ezrin-Radixin-Moesin homology) at the *N*-terminus negatively regulates the catalytic activity of FAK (2). FERM interacts with integrins and growth factor receptors, and through this domain FAK also binds the Arp2/3 complex to control actin assembly (3). In the catalytic kinase domain, autophosphorylation at the FAK Tyr397 residue recruits Src at the FA site. Furthermore, Src phosphorylates FAK at Tyr576 and Tyr577, which results in a conformational change that enhances the catalytic kinase activity. pTyr861 increases the binding affinity of p130cas to the proline-rich regions (PRRs) in the FAK *C*-terminus and is crucial to sense mechanical force (4) and H-Ras-induced transformation (5). The FA-targeting (FAT) domain at the *C*-terminal region is responsible for FAK localization to FA (6) and spatially interacts with paxillin and talin. FAK PRR binds Src homology 3 (SH3) domain-containing proteins such as p130cas, the GTPase regulator associated with FAK (GRAF) and the ARF GTPase-activating protein ASAP1. Phosphorylation of the Tyr925 residue in the FAT domain promotes GRB2 binding to FAK, which activates the mitogen-activated protein kinase (MAPK) pathway through FAK-GRB2-Ras-MEK1-ERK2. pTyr925 is also responsible for the cell survival function of FAK and mediates an MAPK-associated angiogenic switch during tumor progression (7, 8).

Recently, it was reported that pTyr407 negatively regulated kinase activity and cell migration/invasion (9, 10). Most of the tyrosine phosphorylated residues of FAK have been well studied, while the role of serine phosphorylated residues (Ser722, Ser846, Ser910) is still not very well understood (11, 12). Recently, it has been shown that phosphorylation at Ser732 in endothelial cells plays a role in the regulation of the centrosome during mitosis and this may contribute

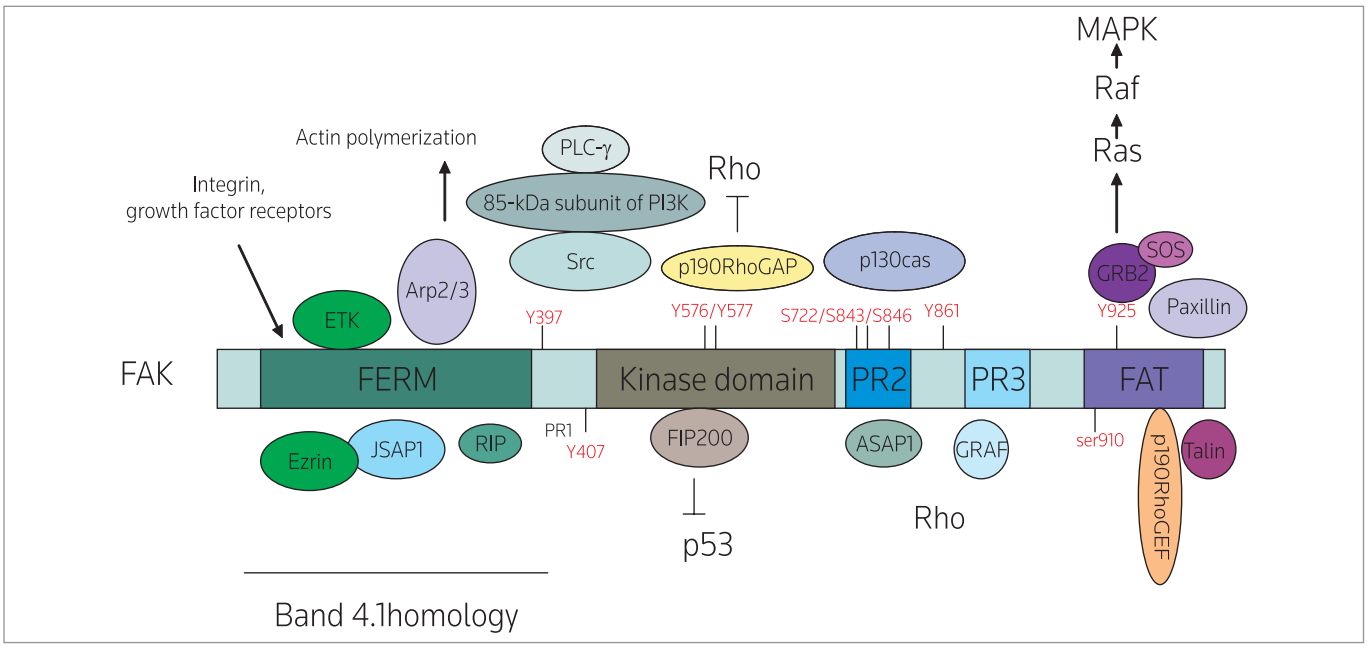

Figure 1. Focal adhesion kinase (FAK) structural features and binding partners. The kinase domain of FAK is flanked by the *N*-terminus that harbors the FERM domain and by the *C*-terminus that consists, in addition to proline-rich (PR) domains, of the FAT domain. The *N*-terminal domain has the Y397 autophosphorylation site, which is also the target site for different small-molecule inhibitors. The kinase domain has the Y576/577 tyrosines, important for the catalytic activity of FAK. The *C*-terminal part of FAK has Y861 and Y925 tyrosines. Different proteins bind to these domains and are involved in cell proliferation, motility and survival signaling.

to cell proliferation and angiogenesis (13). Ser843 is known to be phosphorylated when FAs disassemble and cells detach from the substratum, probably via inhibition of pTyr397 (14).

FAK in cellular processes

Processes related to tumor formation

FAK contributes to tumorigenesis through the promotion of cell survival and/or proliferation, which are hallmarks of cancer cells. FAK mediates survival signaling, for instance, through the PKB prosurvival pathway. FAK phosphorylation (pTyr397) is involved in doxorubicin-induced cell apoptosis in a Bcl-2- and caspase-independent manner. Indeed, inducible FAK-related non-kinase (FRNK) expression sensitizes cells to doxorubicin-induced apoptosis and inhibits doxorubicin-induced PKB activation (15). FAK also regulates cell proliferation by upregulation of some cyclins downstream of PKC, phosphatidylinositol 3-kinase (PI3K)/Akt and MAPK/ERK pathways (16-18). The FERM domain controls FAK function (19) and deletion of the FERM domain increases FAK phosphorylation and activity and affects cell cycle progression in CHO cells (2).

In addition to its role in FAs, FAK also plays a scaffolding role in the nucleus of cells under cellular stress conditions. FAK facilitates p53 degradation by its FERM domain-mediated nuclear localization and promotes cell proliferation and survival (19-21).

Processes related to tumor progression and metastasis

FAK controls FA assembly/disassembly at the leading edge of lamellipodia and disassembly at the rear of migrating cells. The role of FAK in cell motility is well documented (see reviews 6, 12, 22, 23). FAK interacts not only with integrins, but also with growth factor receptors. EGFR (erbB-1) and erbB-2 receptor signaling, which are also prognostic markers for cancer progression, regulate FA turnover, cell migration and invasion through the Src-FAK pathway (24, 25). Cellular traction forces cause dynamic conformational changes in the FERM domain that are shown to be involved in cell spreading and motility (26). A single residue, Lys38, at the subdomain F1 is important for the kinase-inhibitory effect of FERM and the mutant K38A increases FAK phosphorylation, cell cycle entry and cell migration (27). The F2 subdomain of the FAK FERM domain and phosphorylation of Tyr1349 at c-Met are critical for c-Met binding, and this interaction fully activates FAK activity and HGF-induced c-Met-mediated cell invasion (28). FRNK lacks kinase activity and has a competitive inhibitory effect on FAK at focal contacts. FRNK expression abolishes EGF- or v-Src-induced activation of downstream molecules (ERK, JNK, etc.) and cell motility, and prevents cell invasion by inhibiting matrix metalloproteinase (MMP) secretion (29, 30). Inducible ectopic expression of FRNK sensitizes the human embryonic kidney (HEK) cell line to 5-fluorouracil and decreases haptotactic mobility (31). Also, disruption of FAK by FRNK decreases cell attachment, motility and invasion in head and neck squamous cell carcinoma by downregulation of MMP-2 expression (32).

Introduction of the FAT domain, which also competes for binding of endogenous FAK at FA sites, has been reported to sufficiently inhibit cell invasion and sensitize cells to apoptotic stimuli (33). The aggressive phenotype of prostate cancer cells depends on FAK expression, which is regulated by ERK signaling (34). In addition, FAK is also involved in tumor invasion and angiogenesis by regulation of MMP and vascular endothelial growth factor (VEGF) expression (8, 29).

FAK AND CANCER

Increased FAK expression in human cancers

In agreement with the experimental data, overexpression of FAK has been reported in a wide range of cancers (reviewed in 20, 35-37), such as breast (38-42), head and neck, cervical (38), colon (43, 44), thyroid (45), prostate (46), liver (47, 48), skin, lung, bone, melanoma (49) and ovarian cancers (50). FAK apparently plays distinct roles depending on the tumor type and development stage. Similar observations have been obtained in cell lines derived from tumor and normal tissue (51, 52). These studies show that FAK overexpression plays a role in tumor formation and metastasis and could be used as a prognostic marker (38, 42, 45, 53, 54).

Role of FAK in tumor development

Many recent studies using both mouse xenograft models and conditional knockout (KO) mice have provided evidence that FAK signaling pathways can stimulate tumor initiation, as well as tumor progression and metastasis, through their regulation of cell migration, invasion, epithelial-to-mesenchymal transition and angiogenesis.

FAK in promoting tumor initiation

FAK KO animals die, and to overcome embryonic lethality conditional tissue-specific FAK deletion in vivo has been developed with Cre recombinase (Cre)/loxP strategies, including myosin light chain 2a(MLC2v)-Cre/FAK^{flox} (55), Cre-ER (estrogen receptor)/FAK^{flox} (15, 56, 57) and MLC2a mouse mammary tumor virus (MMTV)-Cre/FAK^{flox} (58). The first experimental proof using conditional FAK deletion in the epidermis demonstrated the role of FAK in skin tumorigenesis (56). The intercross of FAK$^{flox/flox}$ mice with MMTV-Cre mice resulted in the deletion of FAK in mammary epithelial cells, which disturbed mammary gland development (59). Complete deletion of FAK in both luminal and myoepithelial mammary epithelial cells disrupted mammary gland formation (van Miltenburg, In press). Using the mouse polyomavirus middle T (PyVmT) transgenic breast cancer model, conditional floxed FAK was introduced. In the MMTV-Cre/PyVmT mice, the mammary epithelium-specific disruption of FAK altered the FAK-related signal cascades and retarded tumor initiation and progression, as well as lung metastasis formation (60). FAK deletion also reduced the population of mammary cancer stem/progenitor cells (61) and disrupted the transition of premalignant hyperplasias to carcinomas and subsequent metastasis (58).

200 kDa FAK family kinase-interacting protein (FIP200), also known as RB1-inducible coiled-coil 1, was identified as an inhibitor of FAK and Pyk2 by interaction with their kinase domains (62, 63). FIP200 inhibits FAK/Pyk2-related cellular functions and interacts with RB1 and p53 (64). Large truncation deletion of the *RB1CC1* gene has been observed in 20% of primary breast cancers and this indicates a possible role for FIP200 as a tumor suppressor (65).

FAK in promoting tumor progression and metastasis

Several studies in mouse xenograft models provide evidence that FAK is required for both tumor progression and metastasis. Overexpression of FAK in human malignant astrocytoma cells contributed to an increase in tumor volume due to enhanced cell proliferation (66). After tail vein injection of v-Src-transformed NIH/3T3 fibroblasts (30) or B16F10 mouse melanoma cells (67), stable expression of FRNK results in a reduction of experimental lung metastases. Recently, our group demonstrated that FRNK sensitizes rat breast cancer MTLn3 cells to doxorubicin in vitro and in vivo via inhibition of doxorubicin-induced PKB activation and downregulation of FRA-1 (a member of the activator protein-1 complex) (15). Inducible expression of FRNK inhibits cell spreading and migration in vitro, as well as primary tumor formation and the early phase of metastasis, but not the outgrowth of macrometastases (68). In agreement with these results, by using FAK-null or knockdown cells, others have confirmed that the catalytic activity of FAK is required for metastatic breast cancer progression (8, 69, 70) and that FAK signaling is critical for erbB-2/erbB-3 receptor cooperation for oncogenic transformation and invasion (71) and for Ras- and PI3K-dependent breast tumorigenesis in mice (72). Pharmacological inhibition of FAK in wild-type mice suppressed angiogenesis, which is a key element in tumor progression and metastasis (57).

STRATEGIES FOR FAK INHIBITION IN CANCER THERAPY

In vitro and in vivo studies, as well as clinical studies, support the fact that FAK is a potential target for the treatment of cancer. Currently, inhibition of FAK signaling is under investigation for its beneficial effects in cancer treatment. FAK signaling can be disturbed by affecting, in practice, either FAK expression or FAK activation.

Agents inhibiting FAK expression

Interfering RNAs

FAK short interfering RNA (siRNA) shows high efficacy in gene expression downregulation in in vitro studies (73, 74). Inhibition of FAK with short hairpin RNAs (shRNAs) prevents FAK function in cell adhesion, migration and proliferation in the highly invasive human prostate cancer cell line PC-3M and the mouse breast cancer cell line 4T1 in vitro, and suppresses tumor growth in heterotopic/orthotopic mouse models in vivo (75). Moreover, knockdown of FAK and the family member Pyk2 extended survival in orthotopic glioma xenograft models in mice (76). However, because of its fast degradation, it is difficult to deliver siRNA very efficiently in vivo, questioning the clinical practicability of using siRNA. A modified polyethylenimine (PEI) gene carrier can be used to deliver FAK shRNA in vitro and in vivo to study the effect of FAK inhibition as a melanoma therapeutic (77). Recently, a neutral lipid liposome, 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC), has been reported to introduce FAK siRNA successfully and silencing of FAK showed antitumor and antiangiogenic effects; specifically, it improved the chemosensitivity to docetaxel or cisplatin in an orthotopic ovarian carcinoma model (78). This type of lipid liposome improved intratumoral penetration, delivery efficacy and toxicity. If the efficiency of specific RNA delivery can be improved, the strategy of inhibiting FAK expression is promising for cancer treatment.

(In)direct pharmacological modulation of FAK activity

Receptor tyrosine kinase inhibitors

FAK phosphorylates downstream effectors, and the use of kinase inhibitors consequently disturbs signaling transduction. The EGFR

inhibitor gefitinib (Iressa®) and the PTK inhibitor genistein are already used in the clinic. Gefitinib was shown to decrease FAK phosphorylation and inhibit the metastasis of oral squamous cancer cells to the lymph nodes (79). Genistein increased the adhesion of prostate cancer cells by modulating FAK activity (80). In addition, herbimycin A, another PTK inhibitor, decreased FAK phosphorylation and resulted in decreased migration of oral squamous cancer cells (81). Trastuzumab (Herceptin®), which binds to the juxtamembrane region of the erbB-2 receptor, was shown to inhibit Src and FAK activities, thereby inhibiting FA turnover, which was evident by increased FA stability and reduced cell invasion (24). Bosutinib (SKI-606), a novel Src kinase inhibitor, inhibits the phosphorylation of FAK and suppresses the migration and invasion of human breast cancer cells (82). Dasatinib (BMS-354825) inhibits the migration and invasion of non-small cell lung cancer and head and neck squamous cell carcinoma cells. The effects on migration and invasion correlated with the inhibition of Src and downstream mediators of adhesion such as FAK, p130cas and paxillin (83).

Small-molecule inhibitors

Several FAK inhibitors have been developed by pharmaceutical companies (see Table I), the most promising of which are PF-562271 (Pfizer) and TAE-226 (Novartis) (26). These are ATP analogues and effectively inhibit the kinase activity of FAK. Treatment of cells with these inhibitors results in a decrease in Tyr397 phosphorylation, which is associated with inhibition of cell migration (84, 85).

PF-562271 has a dual effect on FAK and Pyk2, with a nanomolar IC_{50} in vitro (86). In vivo maximal inhibition of pFAK (78%) is obtained 1 h after a dose of 33 mg/kg p.o. in tumor-bearing mice. After a single dose, > 50% inhibition of FAK phosphorylation lasts for over 4 h. Furthermore, dose-dependent tumor growth inhibition and regression were observed in a broad range of human s.c. xenograft models, including prostate, breast, pancreatic, colon, lung and glioblastoma tumors, with no observation of weight loss, morbidity or death.

The in vivo inhibitory mechanisms of PF-562271 rely on anoikis/apoptosis and reduction of microvascular density (86). Recently, in a rat bone model of human cancer, this compound was shown to inhibit breast cancer MDA-MB-231 growth in the bone without altering normal bone formation (87). Initial phase I data on PF-562271 from patients with different types of cancer revealed that the compound appears to be performing well (88).

TAE-226 is another dual inhibitor specific for both FAK and insulin-like growth factor 1 receptor (IGF-I receptor), so a direct effect due to FAK inhibition cannot be determined. Nevertheless, it is a novel bis-anilinopyrimidine inhibitor that is reported to efficiently inhibit FAK signaling, arrest tumor growth and invasion, and prolong the life-span of mice with glioma or ovarian tumor implants (84, 89, 90). TAE-226 induces an intermediate conformation of the kinase activation loop (91), which inhibits the phosphorylation of FAK and the downstream molecules Akt and ERK. TAE-226 also decreases cell proliferation, adhesion, migration and invasion in glioma cells (84). It has significant activity in ovarian carcinoma and inhibits pTyr397, pTyr861 and cell growth in a time- and concentration-dependent manner. Moreover, in tumor-bearing mice it shows a synergistic effect with docetaxel on reduction in cell growth and tumor burden and increase in survival (89). TAE-226 is also promising in the therapy of imatinib-resistant gastrointestinal stromal tumors (92). A phase I clinical trial study is under way.

In November 2008, Pfizer initiated a phase I clinical study with another inhibitor, PF-04554878, in patients with advanced non-hematological malignancies.

The primary mechanisms of action of FAK inhibitors in vivo are apoptosis/anoikis, antiangiogenesis and reduced invasion. However, the role of specific FAK inhibitors in vivo and their possible long-term side effects should be investigated.

Table I. *FAK inhibitors in cancer research.*

Inhibitor	In vitro/in vivo effects	Ref.
PF-562271* (FAK/Pyk2)	Xenograft models and patients (*antiangiogenic, antitumor activity*) Phase I clinical trial (Pfizer)	(86, 88) http://clinicaltrials.gov/ct2/show/NCT00666926
TAE-226* (FAK/IGF-I receptor)	Breast cancer, glioblastoma, gastrointestinal stromal tumors, ovarian carcinoma, esophageal adenocarcinoma (*cell proliferation/growth, cell cycle, cell migration, chemoresistance to docetaxel in vitro and in vivo*) Phase I clinical trial (Novartis)	(78, 84, 88, 90, 92, 98)
PF-573228* (FAK)	Human tumor cell lines (*cell migration*)	(85, 96)
TAC-544* (FAK/Pyk2)	Animal study (*angiogenesis*)	(58)
1,2,4,5-Benzenetetraamine tetrahydrochloride* (FAK)	Breast cancer (*cell adhesion, tumor regression*)	(99)
PF-04554878# (FAK/Pyk2)	Nonhematological malignancies Phase I clinical trial (Pfizer)	http://clinicaltrials.gov/ct2/show/NCT00787033

*ATP analogues that inhibit FAK kinase activity; #unknown inhibitory mechanism.

CONCLUSIONS AND PROSPECTIVE RESEARCH

FAK, a central protein in FA sites, regulates various cellular processes, including cell proliferation, survival, migration and invasion, which are crucial steps in tumorigenesis and metastasis formation. The implications of FAK overexpression in cancer suggest that FAK inhibition is a potential target for anticancer therapy. More and more evidence shows that FAK inhibition alone and in combination with other traditional therapies is a promising strategy in cancer treatment (74, 93-97). This review summarizes the latest studies and findings on genetic, functional and pharmaceutical inhibition of FAK. Future work should focus on the development and clinical evaluation of new inhibitors, as well as modulation of target-specific delivery.

DISCLOSURE

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