

Focal adhesion kinase as potential target for cancer therapy (Review)

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Abstract. Focal adhesion kinase (FAK) is a 125-kDa non-receptor and non-membrane protein tyrosine. FAK can function with integrins and growth factor receptors to promote cell survival dependent kinase activity and nuclear FAK promotes cell proliferation and survival through FERM (FAK, ezrin, radixin, moesin) domain-enhanced p53 degradation independent kinase activity. Many previous studies have indicated that FAK plays a critical role in the biological processes of normal and cancer cells and FAK has been proposed as a potential target in cancer therapy. Small molecule inhibitors (PF-573,228; PF-562,271 and NVP-226) for use as potential cancer therapies have been developed. However, the detailed mechanism of the role for FAK in tumor cell generation and progression remain unclear, so future work is needed to explore these issues. New inhibitors that can be effectively inhibit the function of FAK still need to be explored due to the low specificity, and resistance.

3. FAK signaling and function
4. FAK and cancer
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1. Introduction

Focal adhesion kinase (FAK) is a 125-kDa non-receptor protein tyrosine which was originally identified in chicken embryo cells transformed by v-Src (1) and BALB/c3T3 fibroblasts (2) and was shown to localize to focal adhesions at the same time. The FAK gene is located on human chromosome 8q24 and mouse chromosome 15 (3). Many previous studies have indicated that FAK plays a critical role in the biological processes of normal and cancer cells and FAK has been proposed as a potential target in cancer therapy.

2. FAK structure

FAK is a non-receptor and non-membrane associated protein tyrosine kinase (PTK), which does not contain Src homology 2 (SH2) or SH3 protein interaction domains (4). FAK related PTK have been isolated and are known as cell adhesion kinase β (CAK β), protein tyrosine kinase 2, (PYK2), related adhesion focal tyrosine kinase (RAFTK), calcium-dependent tyrosine kinase (CADTK), and focal adhesion kinase 2 (FAK2) (5-8). FAK contains three main domains: the centrally located catalytic kinase domain flanked by a large N-terminal domain comprising the FERM region and a C-terminal domain harboring the focal adhesion targeting (FAT) (4,9,10). At least 6 tyrosine sites (Tyr-397, -407, -576, -577, -861 and -925) need to be phosphorylated during the activation of FAK (11,12). Tyr-576 and Tyr-577 are within the kinase activation loop; Tyr-861 and Tyr-925 are within the C-terminal domain (4). Growth factors or clustering of integrins facilitates the rapid phosphorylation of FAK at Tyr-397 and this in turn would recruit Src-family PTKs, resulting in the phosphorylation of Tyr-576 and Tyr-577 in

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1. Introduction
2. FAK structure

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Abbreviations: FAK, focal adhesion kinase; FERM, FAK-ezrin-radixin-moesin homology; FRNK, FAK-related non-kinase; FAT, focal adhesion targeting; PTK, protein tyrosine kinase; FAs, focal adhesions; GTPase, guanosine triphosphatase

Key words: focal adhesion kinase, FAK-related non-kinase, FERM, FAK inhibitor

the FAK activation loop and full catalytic FAK activation (4,10).

C-terminal domain of FAK. The C-terminal non-catalytic domain of FAK is rich in protein-protein interaction sites, comprising two regions, one region containing two proline-rich sequences proximal to the catalytic domain; the other region is the focal adhesion targeting (FAT) sequence (4,13). The FAT sequence directs FAK to newly formed and existing adhesion complexes (13). Fusion of this 15.5-kDa fragment to other proteins is sufficient for the localization at focal adhesions (14). X-ray crystallography and nuclear magnetic resonance (NMR) analysis showed that the FAT domain is comprised of four helical bundles that resemble structures present in other adhesion proteins, including vinculin, Crk-associated substrate (Cas) and α -catenin (15-17). The FAT is also the binding site for the adhesion associated proteins paxillin and talin (4,10). Paxillin is an adaptor protein containing different interaction domains such as a proline-rich site for SH3 domain binding, four zinc-finger LIM domains which are important for paxillin targeting to focal contacts (18). The adapter protein paxillin interacts with its LD2 domain to helix 1 and with its LD4 domain to helix 3 of the FAT domain (19). Talin is a structural protein that can associate with β -integrin cytoplasmic tails. Binding analyses showed that the talin binding region localized at residues 965-1012 of the FAK FAT domain (20).

The entire C-terminal, non-catalytic domain of FAK (FAK related non-kinase-FRNK) is autonomously expressed in some cell types, and has been utilized as a dominant negative mutant to elucidate FAK function (21-24). Vascular smooth muscle cells have an elevated expression of FRNK and it appears that vascular injury also causes upregulation (25). In most cells, forced overexpression of FRNK inhibits cell spreading, cell migration and growth-factor mediated signals to MAP kinase (25-27).

N-terminal domain of FAK. The N-terminal domain of FAK contains an autophosphorylation site, Tyr-397, and a region of approximately 300 amino acids that has shown sequence homology with the band 4.1 protein/ERM proteins within a region known as the FERM domain (4,28,29). FERM domains have been indicated to mediate both protein-protein interactions and protein-membrane interactions (30). The cytoplasmic tail of β -integrins and growth factor receptors, which can promote cell motility and signaling, is the best-known interaction partner of the FAK FERM domain (31,32). The FAK FERM structure reveals a three-lobed (F1-F3) architecture characteristic (33). Some studies showed that truncation of the FERM domain of FAK resulted in an increase in phosphorylation of FAK and its kinase activity (34,35), suggesting a negative regulatory role in FAK activation. The FERM domain of FAK can interact with the kinase domain of FAK *in vitro* and *in vivo*, proposing a direct autoinhibitory working model of FAK regulation (30). The crystal structure of FAK residues 31-686 containing the FERM and kinase domains is in accord with this model (36). The autoinhibition is a result of an interaction between the FERM domain and the kinase C-lobe and the disruption of this interaction activates the FAK kinase. The FERM domain directly binds the kinase

domain, blocking access to the catalytic cleft and protecting the FAK activation loop from Src phosphorylation (36).

Some studies indicated that the FERM domain of FAK interacts with p53, regulating the signaling of the tumor cell's survival (37-39). FAK FERM functioned as a scaffold with the binding of p53 and murine double minute-2 (Mdm2) to different lobes of the FERM domain. p53 inactivation by FAK required FAK FERM F1 lobe binding to p53, FERM F2 lobe-mediated nuclear translocation, and FERM F3 lobe for connections to Mdm2 and proteasomal degradation (39). Importantly, it was found that the FERM-mediated survival pathway is a FAK kinase-independent event and needs FAK nuclear localization (39). Within FAK FERM, the nuclear localization motif is localized to the F2 lobe and is made up of a basic residue cluster (K190, K191, K216, K218, R221 and K222) contiguous at the distal tip of the F2 lobe but separated by a primary sequence (39).

3. FAK signaling and function

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 tumor cells (40).

Integrin related signaling. The crucial step of cell cycle is integrin-mediated cell attachment to the extracellular matrix (41) and FAK is the canonical mediator of integrin signals (10). FAs are large integrin-based multiprotein complexes that mediate strong cell-substrate adhesion and transmit information in a bidirectional manner between extracellular molecules and cytoplasm (42). FAs comprise integrins, integrin-associated adaptor and signaling proteins such as FAK, Src, Grb2, p130Cas paxillin, vinculin, tensin, growth factor receptors and their related downstream targets (42,43). FAK is primarily recruited to FAs by interactions between its C-terminal domain and integrin-associated proteins paxillin (44) and talin (20), resulting in autophosphorylation at Tyr-397 of FAK and total FAK activation (11,45,46).

Integrin-stimulated FAK phosphorylation at Tyr-397 makes a high-affinity binding for Src-homology 2 (SH2) domain of Src-family PTKs (SFKs), leading to the conformational activation of SFKs and the formation of a transient FAK-Src signaling complex in fibroblasts and epithelial cells (46,47). In turn, the binding and subsequent activation of the Src kinase results in the phosphorylation of FAK at its 'downstream' residues, Tyr-576 and Tyr-577, and maximal kinase activation (11). The adaptor proteins paxillin and p130Cas are the two main phosphorylation targets of the FAK-Src complex (46). Phosphorylation of both paxillin (residues Y31 and Y118) and p130Cas (within the Cas substrate domain) create binding sites for the SH2 domain of the Crk adaptor protein (48), activating small GTPase, such as Rac1 or Cdc42 and c-Jun N-terminal kinase (JNK), and the subsequent promotion of membrane protrusion and cell migration (49-51).

Integrin-stimulated FAK auto-phosphorylation at Tyr-397, Src-family PTK binding to FAK Tyr-Y397, and the formation of a FAK-Src signaling complex promote cell motility and cell survival (46,52). There are several survival signaling mediated by FAK. The first is related with PI3K-Akt and FAK could

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upstream of such survival pathways (32,53). Extracellular signal via the FAK activation leads to the sequential activation of PI3K and Akt (53). Activated Akt phosphorylates BAD (member of the Bcl-2 family) Ser-136 and finally promote survival (32,54). The second is extracellular signal-FAK-JNK survival pathways, requiring Cas and Ras. ECM activated the formation of FAK-p130Cas complex and this complex activates C-JunNH2-terminal kinase (JNK) through a Ras/Rac1/Pac1/MAPK kinase 4. Activated JNK finally promote the survival of fibroblasts cultured on fibronectin (55). There is still a third FAK-mediated survival due to the interaction of the N-terminal of FAK with p53 and will be discussed below in detail.

FAK FERM and p53 survival signaling. It is estimated that at least 50% of human cancers is due to the ablation or dysfunction of the p53 gene (56). As a transcription factor, p53 has been implicated in the regulation of numerous tumor suppressor genes. In normal conditions, p53 expression is maintained at low levels by poly-ubiquitination and proteasomal degradation (57) and Murine double minute-2 (Mdm2) is one of several ubiquitin E3 that regulate p53 levels in cells (58). FAK and tumor suppressor gene p53 were reported to functionally connect with each other. The researchers showed that p53 controls survival signals from the extracellular matrix transduced by FAK in anchorage-dependent cells (59). In squamous cell carcinoma cells, a link between p53-mediated anoikis and FAK was demonstrated (60). A correlation between FAK and p53 overexpression was indicated via immunohistochemical analysis of 115 endometrial carcinoma samples (61). As previously described by Golubonskaya *et al* the N-terminal fragment of FAK directly interacts with the N-terminal transactivation domain of p53, suppressing p53-mediated apoptosis and inhibiting the transcriptional activity of p53 (37).

Recently the structure of the FAK FERM was elucidated (36) and Lim *et al* proposed a novel FAK FERM-mediated survival pathway which is different from canonical FAK functions in two ways: it is a FAK kinase independent event and the direct regulation of p53 requires FAK nuclear localization (39). The authors demonstrated that FAK inhibits p53 via FAK FERM nuclear translocation, FERM-mediated binding to p53, and FERM-enhanced Mdm2-dependent p53 ubiquitination (39). They further stated that nuclear FAK accumulation was associated with a loss of FAK at focal contacts which is in accordance with the notion that there is a cytoplasmic pool of FAK, freely shuttling in and out of the nucleus. They proposed a model whereby under conditions of cellular stress or reduced integrin signaling, the cytoplasmic pool of FAK is elevated, leading to increased FAK nuclear accumulation, which acts to enhance cell survival by facilitating p53 turnover. It is interesting to note that some transformed cell types can survive under suspension conditions and that this associated with a reduced p53 activation (62). FAK levels are elevated in many tumor cells (63), and FAK can form a complex with p53 in both normal and tumor cells (39). However, whether FAK FERM-mediated regulation of p53 might act in a kinase-independent manner to promote tumor progression still needs to be studied further.

Proliferation. FAK also plays an important role in regulating cell cycle progression, requiring Erk activity, cyclin D1 transcription, and the cyclin-dependent kinase (cdk) inhibitor p27^{kip1} (52). Cyclin D1 is a key regulator of G1 to S phase progression the cell cycle and the transition from G1 to S phase is regulated by many cyclin-dependent kinase which are controlled by phosphorylation, cyclins, and cdk inhibitors (64-66). FAK signaling regulated cyclin D1 expression at the transcription level. This regulation depends on integrin-mediated cell adhesion and is likely through its activation of Erk signaling pathway (66). FAK overexpression upregulates cyclin D3 and enhances cell proliferation through the PKC and PI3K-Akt pathways (66,67).

Migration and invasion. FAK also plays an important role in cell adhesion and migration (68,69) its autophosphorylation and complex formation with paxillin (adhesion) and Src (migration/invasion) is necessary during these processes (32). Several FAK-mediated signaling pathways have been proposed leading to migration. One is the FAK/CAS signaling pathway: Crk-associated substrate (CAS) proteins are phosphorylated by cellular Src binding to FAK, resulting in a Crk family adaptor molecule activation of a small GTPase, for instance Rac1 or Cdc42 and c-Jun N-terminal kinase (JNK), and the subsequent promotion of membrane protrusion and cell migration (49-51,70); also a role for PI3K in the facilitation of FAK-mediated cell migration was reported and it was proposed that the binding of Src and p130Cas to FAK may not be sufficient for the migration (71); FAK controlled actin assembly via interaction with Arp2/3 complex also likely contribute to cell migration (72). FAK-deficient cells spread more slowly on extracellular matrix proteins, exhibit an increased number of prominent focal adhesions and migrate poorly in response to chemotactic signals (31,73-75). When esophageal adenocarcinoma cells were treated with TAE226, which is a potent ATP competitive inhibitor of FAK, cell proliferation and migration were greatly inhibited with an apparent structural change of actin fiber and a loss of cell adhesion (76).

4. FAK and cancer

Numerous studies have reported FAK overexpression in various tumor cells, including neuroblastoma, pancreatic, ovarian, cervical, osteosarcoma, kidney, lung, prostate, brain, melanoma, thyroid, oral, head and neck, colon cancer, and acute myeloid leukemia (40) and its expression correlates with increased tumor malignancy. FAK mRNA levels have been shown to be increased in premalignant adenomatous tissues and invasive and metastatic tumors (77,78). Increased FAK mRNA expression was demonstrated in adenomatous tissues, invasive tumors, and metastatic tumors (77,79). Real-time PCR analysis of matched samples of normal colon mucosa, colorectal carcinoma, and liver metastases demonstrated increased FAK mRNA and protein levels in tumor and metastatic tissues versus normal tissues (79). Increased regional expression of FAK is found at the invasive tumor edge, implicating FAK in tumor invasion (80). Additionally, FAK is expressed in the microvascular endothelial cells of human glioma tumor biopsies and U251MG glioma xenografts, which links FAK to glioma angiogenesis (81).

Conditional inactivation of FAK is used to study the role of FAK signaling in the processes of tumorigenesis and progress. In tumor cells, attenuation of FAK expression induces detachment and apoptosis (82), suggesting that a FAK-dependent signal is required for tumor cell growth. Furthermore, an activated form of FAK leads to resistance to anoikis (83), and FAK degradation is associated with apoptosis (84,85). Overexpression of wild-type FAK in SF767 or G112P glioma cells increased cell proliferation (86,87), while antisense oligonucleotides targeting FAK decreased the proliferation of U251MG cells (88). The overexpression of the C-terminal FAK carboxy-terminal domain that is produced from a separate transcript in avian cells can disrupt FAK function (21), and this protein (FRNK for FAK-related non-kinase) inhibits cell spreading and the phosphorylation of FAK, the focal adhesion protein paxillin, and, to a lesser extent, tensin (89). Researchers have exogenously expressed an analogous fragment of human FAK, which is called FAK-CD (FAK carboxy-terminal domain). They have found that FAK-CD causes cell rounding, loss of adhesion, and apoptosis in tumor cells, but not in normal cells (90-92). Other groups have also reported that a dominant-negative inhibitor, FAK-CD, did not induce apoptosis in normal cells (93). Thus, FAK-CD provides a convenient means to inactivate FAK function and dissect the signaling requirements for FAK in tumor cells. When FAK expression was selectively inhibited in skin keratinocytes, a loss of FAK suppressed chemically-induced skin tumor formation (94). However, increased keratinocyte cell death was observed after FAK deletion *in vitro* and *in vivo*. Elevated cell apoptosis also occurs upon conditional FAK deletion in endothelial cells (95), but not necessarily in neuronal cells (96). RNAi-mediated knock-down of FAK in aggressive breast carcinoma cells did not affect proliferation or apoptosis in culture, but carcinoma cells lacking FAK did not exhibit invasive activity *in vitro* or spontaneous mammary-to-lung metastasis *in vivo* (46).

Together elevated FAK expression and activity is associated with malignancy in a variety of cancer cells, indicating that FAK play a critical role in tumor progression. Given the role of FAK in processes important in tumorigenesis and metastasis and the link to prominent oncogenes, FAK might be a promising target in the ongoing search for anti-cancer drugs.

5. FAK inhibitors

FAK play a critical role in the biological processes of cancer cells, so FAK has been proposed as a potential target in cancer therapy and small molecule inhibitors for use as potential cancer therapies have been developed. Compounds: PF-573,228; PF-562,271 and NVP-226 have been recently generated by two groups. These compounds are ATP analogs and effectively inhibit the kinase activity of FAK (97,98).

TAE226 is a novel ATP-competitive tyrosine kinase small-molecule inhibitor designed to target FAK, and can effectively prevent FAK phosphorylation, extracellular signal-related kinase (ERK), S6 ribosomal protein phosphorylation and downstream signal transduction, as determined by decreased AKT. TAE226 inhibits insulin receptor (InsR) and insulin-like growth factor-I receptor (IGF-IR), albeit, 10-fold

less potently (IC₅₀=44 nM for InsR and IC₅₀=140 nM for IGF-IR), and is a potent inhibitor of FAK (IC₅₀=5.5 nM) (99). TAE226 displays otherwise good selectivity against a panel of 30 kinases (99). TAE226 treatment potently disrupted glioma cell proliferation, attachment, migration, and invasion (99). TAE226 was shown to induce apoptosis in breast cancer cell lines (100). Notably, the compound efficiently increased survival rates for glioma xenografts (99) or ovarian tumor cell implants in animals (101).

PF-573,228 inhibited phosphorylation of FAK and its downstream effector paxillin, finally affected cell migration and adhesion turnover (97). But PF-573,228 had little inhibitory effect on the growth and apoptosis of normal and cancer cell and this may indicate that the FAK kinase activity is not essential for cell growth-proliferation, which is mediated through FAK FERM regulation of P53 (39,97).

PF-562,271 is a newly developed diaminopyrimidine-type compound that inhibits FAK and Pyk2 and exhibits high degree of selectivity in the inhibition of PTKs (102). PF-562,271 have inhibited the tumor growth of prostate, pancreatic, colon, glioblastoma, and H460 lung xenotropic tumor models (102). PF-562,271 blocked bFGF-stimulated blood vessel angiogenesis as performed in chicken chorio-allantoic membrane assays and low level administration of PF-562,271 potently blocked blood vessel sprouting without detectable changes in vascular leakage (39). PF-562,271 has since moved on to clinical trials, where it has thus far shown minimal toxicity along with some tumor regression (103). FAK inhibitors show a good application prospect for cancer therapy.

However, there are still some problems in the development of drugs that obstruct FAK function. Two of the possibilities for the developments of an inhibitor of FAK are already well explained in a review by Nimwegen and Water (32). The first possibility is inhibition of the kinase domain, thereby preventing the activation of downstream signaling cascades. But since FAK kinase activity itself may not be absolutely essential for its signaling functions, some problems have emerged. Lim *et al* reported that FAK FERM-mediated nuclear localization of FAK promotes enhanced cell survival through the inhibition of tumor suppressor p53 independent of its kinase activity (39). Another problem with the first possibility is the specificity of the kinase inhibitor, because kinase domains of a range of different proteins show a high degree of amino acid conservation in the catalytic domains (32). The second possibility is blocking the adaptor function of FAK, such as preventing the binding of proteins to one or multiple tyrosines, to the proline rich domains or by preventing localization of FAK to the focal adhesions (32). The prevention of the association of FAK to certain binding partners will lead to a blockade of specific downstream signaling pathways, but whether it is possible to achieve this specificity *in vivo* still unknown because other signaling cascades might compensate for the function of FAK, resulting in the activation of the intended signaling pathways (32). Another problem within the second possibility is as to which protein-interactions should be targeted. However, interfering with the localization of FAK at the focal adhesions by the expression of the splice variant of FRNK can prevent its active role in focal adhesion signaling and focal adhesion



103). However, targeting FAK kinase domain and its function still provides a prospective approach for cancer therapy.

6. Conclusions

FAK is overexpressed in a variety of cancer cells, indicating that FAK plays a critical role in tumor progression and FAK has been proposed as a potential target in cancer therapy. FAK can function with integrins and growth factor receptors to promote cell survival-dependent kinase activity and nuclear FAK promotes cell proliferation and survival through FERM-enhanced p53 degradation independent kinase activity. However, whether FAK signaling within the tumor or stroma is the key event in promoting tumor progression and the detailed mechanism of the role for FAK in tumor cell generation and progression remain unclear, so future work is needed to explore these issues.

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