Cellular Characterization of a Novel Focal Adhesion Kinase Inhibitor

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Focal adhesion kinase (FAK) is a member of a family of non-receptor protein-tyrosine kinases that regulates integrin and growth factor signaling pathways involved in cell migration, proliferation, and survival. FAK expression is increased in many cancers, including breast and prostate cancer. Here we describe perturbation of adhesion-mediated signaling with a FAK inhibitor, PF-573,228. In vitro, this compound inhibited purified recombinant catalytic fragment of FAK with an IC\textsubscript{50} of 4 nM. In cultured cells, PF-573,228 inhibited FAK phosphorylation on Tyr\textsuperscript{397} with an IC\textsubscript{50} of 30–100 nM. Treatment of cells with concentrations of PF-573,228 that significantly decreased FAK Tyr\textsuperscript{397} phosphorylation failed to inhibit cell growth or induce apoptosis. In contrast, treatment with PF-573,228 inhibited both chemotactic and haptotactic migration concomitant with the inhibition of focal adhesion turnover. These studies show that PF-573,228 serves as a useful tool to dissect the functions of FAK in integrin-dependent signaling pathways in normal and cancer cells and forms the basis for the generation of compounds amenable for preclinical and patient trials.

The ability of cells to respond appropriately to environmental cues is critical to maintaining cellular, tissue, and organism homeostasis. One such environmental cue is derived from cellular adhesion to the extracellular matrix. The loss of adhesion-dependent cellular regulation can lead to increased cellular proliferation, decreased cell death, changes in cellular differentiation status, and altered cellular migratory capacity, all of which are critical components of carcinogenesis and metastatic progression. The FAK\textsuperscript{4} family kinases (which include FAK and Pyk2) regulate cell adhesion, migration, and proliferation in a variety of cell types (for review see Refs. 1–3). Adhesion of cells to the extracellular matrix is mediated by heterodimeric transmembrane integrin receptors located within sites of close opposition to the underlying matrix called focal adhesions. Integrin engagement and clustering stimulates FAK phosphorylation on Tyr\textsuperscript{397}, creating a high affinity binding site for Src and Src family kinases. The FAK-Src complex phosphorylates many components of the focal adhesion, resulting in changes in adhesion dynamics and the initiation of signaling cascades. In addition to FAK catalytic activity, FAK also functions as a scaffold to organize structural and signaling proteins within focal adhesions.

The importance of FAK as a regulator of normal cellular function is underscored by the number of cancers reported to have alterations in FAK expression and/or activity, including colon, breast, thyroid, prostate, cervical, ovarian, head and neck, oral, liver, stomach, sarcoma, glioblastoma, and melanoma (4, 5). Additionally, alterations in FAK expression and/or activity have been associated with tumorigenesis and increased metastatic potential (4, 5). Currently, it is unclear how the catalytic and/or scaffolding function of FAK contributes to tumor progression. To date studies of FAK function have relied on the expression of dominant interfering mutants or elimination of FAK expression by genetic knock-out, antisense oligonucleotide expression, or small interfering RNA.

Herein, we report the biochemical and cellular characterization of a novel small molecule inhibitor, PF-573,228 (here after referred to as PF-228), that targets FAK catalytic activity. The inhibitor interacts with FAK in the ATP-binding pocket and effectively blocks the catalytic activity of recombinant FAK protein or endogenous FAK expressed in a variety of normal and cancer cell lines. Treatment of cells with PF-228 blocked FAK phosphorylation on Tyr\textsuperscript{397} and concomitantly reduced the tyrosine phosphorylation of paxillin, a recognized downstream effector of FAK signaling. Drug treatment of normal and cancer cells resulted in decreased cell migration and inhibited adhesion turnover, biological activities previously ascribed to FAK. Interestingly, inhibition of FAK activity had little effect on normal or cancer cell growth or apoptosis in culture. PF-573,228 provides an appropriate tool to dissect the role of FAK in regulation of cell adhesion signaling and the regulation of adhesion dynamics.

**EXPERIMENTAL PROCEDURES**

**Chemical Synthesis**—PF-573,228 was identified through a combination of high throughput screening, structure based
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drug design, and conventional medicinal chemistry approaches (38).

PF-573,228 was prepared according to the procedures described in a patent (35). The structure and inhibitory activity are shown in Fig. 1.

Recombinant Kinase Assay—Purified activated FAK kinase domain (amino acids 410–689) was reacted with 50 μM ATP, and 10 μg/well of a random peptide polymer of Glu and Tyr (molar ratio of 4:1), poly(Glu/Tyr) in kinase buffer (50 mM HEPES, pH 7.5, 125 mM NaCl, 48 mM MgCl₂) for 15 min. Phosphorylation of poly(Glu/Tyr) was challenged with serially diluted compounds at ½-log concentrations starting at a top concentration of 1 μM. Each concentration was run in triplicate. Phosphorylation of poly(Glu/Tyr) was detected with a general anti-phospho-tyrosine (PY20) antibody, followed by horseradish peroxidase-conjugated goat anti-mouse IgG antibody. The standard horseradish peroxidase substrate 3,3’,5,5’-tetramethylbenzidine was added, and Optical Density readings at 450 nm were obtained following the addition of stop solution (2M H₂SO₄). The IC₅₀ values were determined using the Hill slope model. Broad kinase selectivity profiling was performed using the KinaseProfiler™ selectivity screening service available through Upstate Biotechnology, Inc. For more information please see: www.upstate.com/discovery/services/kp_overview.q

Cellular Kinase Assays—Using the GeneSwitch inducible system from Invitrogen, stable A431 epithelial carcinoma clones were generated to express either wild type V5-tagged FAK protein or mutant FAK Y397F V5-tagged protein under the inducible regulation of mifepristone (36). Stable clones were grown in Dulbecco’s modified Eagle’s medium, 10% fetal bovine serum, 750 μg/ml Zeocin, and 50 μg/ml Hygromycin. One day prior to running the FAK cell ELISA, A431-FAKwt cells were seeded at 1.2 × 10⁶ cells/ml in growth medium in 96-well U-bottom plates. After 4–6 h at 37°C, 5% CO₂, FAK expression was induced with 0.1 μM mifepristone. Uninduced controls were included. Goat anti-mouse or anti-rabbit plates were subsequently coated with either anti-V5 or anti-FAK (1.0 μg/ml) or an irrelevant antibody control in Superblock Tris-buffered saline buffer. Anti-V5- or anti-FAK-coated plates were blocked in 3% bovine serum albumin, 0.5% Tween for 1 h at room temperature. The cells were treated with ½-log serial dilutions starting at a top concentration of 1 μM for 30 min at 37°C, 5% CO₂. Lysates from cells treated with indicated concentrations of compound were prepared in P-lysis buffer (50 mM Tris-HCl, pH 7.4, 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM Na₃VO₄, 1 mM NaF, and protease inhibitors) and transferred to the anti-V5- or anti-FAK-coated plates to capture total induced or total FAK protein. Anti-phosphospecific FAK[Y397] was used to detect auto-phosphorylated FAK Tyr³⁹⁷, followed by secondary reporter antibody. Horseradish peroxidase substrate was added, and plates were read at 450 nm. The IC₅₀ values were determined using the Hill slope model. For Western blot analysis, REF52 cells were treated with the indicated concentrations of inhibitor for the indicated periods of time prior to lysis in CH buffer (50 mM HEPES, 0.15 mM NaCl, 2 mM EDTA, 1% Nonidet P-40, and 0.5% sodium deoxycholate, pH 7.2) containing 1 mM phenylmethylsulfonyl fluoride, 100 mM leupeptin, and 0.05 TIU/ml aprotinin, 1 mM Na₃VO₄, 40 mM NaF, and 10 mM Na₄P₂O₇. For suspension/plating experiments, REF52 cells were suspended in serum-free medium in the presence or absence of the indicated concentrations of inhibitor and were allowed to reattach to plates coated with 5 μg/ml FN for 30 min in the continued presence or absence of inhibitor. The cells were lysed in CH-buffer, and Western blot analysis of whole cell lysates was performed using 25–50 µg of protein.

Cell Growth and Apoptosis—Growth assays were performed by seeding 1 × 10⁴ REF52 or PC3 cells/well of a 24-well plate in triplicate 24 h prior to daily treatment with the indicated concentrations of each inhibitor for 3 days. Subsequently, the cells were harvested and counted. Apoptosis assays were performed using a cell death detection ELISA (Roche Applied Science) according to the manufacturer’s instructions. Briefly, REF52, PC3 or MDCK cells were treated for 24 h (16 h for MDCK) with the indicated concentrations of each inhibitor prior to lysis. Cells suspended for 16–24 h in serum-free medium served as a positive control. The cell lysates were incubated in duplicate in the ELISA system. The data represent the means ± standard deviation of one of three experiments performed in duplicate.

Cell Migration Assays—Cell migration was assessed using modified Boyden chambers (tissue culture-treated, 6.5-mm diameter, 10-mm thickness, 8-mm pores; BioCoat; Fisher) as described previously (6). Chemokinesis was determined to be the number of cells migrating to the lower side of the chamber in serum-free medium. For growth factor-mediated chemotaxis, 10% fetal bovine serum was included in the lower chamber. To assess FN-stimulated haptotaxis, the underside of the membrane was coated with 1.5 μg/ml FN suspended in PBS in the lower chamber, and PBS was placed in the upper chamber. After incubating the chambers overnight at 4°C, the FN solutions were removed, and Dulbecco’s modified Eagle’s medium without serum was added to each chamber. For inhibitor studies, 1 × 10⁵ REF52 cells were pretreated with each inhibitor for 30 min prior to harvest and addition to the transwell. The inhibitor remained in both chambers of each well for the duration of the migration assay. The cells were allowed to migrate for 6 h at 37°C. Nonmigrating cells on the upper side of the membrane were removed with a cotton swab. The cells that had migrated to the underside of the membrane were washed twice in PBS, fixed with 4% paraformaldehyde at room temperature for 20 min, washed twice with PBS, and stained with crystal violet. Migration was assessed by counting four 320 × fields using a Zeiss Axiosvert 135TV inverted fluorescence microscope. The data presented represent the means ± standard deviation of one of three experiments performed in triplicate normalized to the untreated control. Each experimental group was analyzed using single-factor analysis of variance. If the global F test for differences among any one of the groups was significant at the 5% level, we proceeded to investigate which treated sample(s) differed from untreated controls using Student’s t tests assuming unequal variance. Statistical significance was defined as p ≤ 0.05.

Wound Healing Assay—Confluent REF52 monolayers on 35-mm Bioptechs delta-T dishes (Fisher) were wounded with a 10-μl pipette tip, and cells migrating into the wound were filmed for 9 h at 37°C by time lapse microscopy. PF-573,228
RESULTS

Inhibition of FAK Kinase Activity in Vitro and in Vivo—PF-573,228, (6-[(4-((3-(methylsulfonyl)benzyl)amino)-5-trifluoromethyl)pyrimidin-2-yl)amino]-3,4-dihydro-1H-quinolin-2-one) is a competitive inhibitor of ATP with specificity for FAK family protein-tyrosine kinases (Fig. 1A). PF-573,228 was one of several compounds evaluated for biochemical and cellular activities against FAK and was chosen for additional studies based on its specificity for FAK, minimal activity against other kinases tested, and utility for in vitro evaluation.

Using a catalytically active fragment of FAK, the inhibitory properties of PF-228 were assessed initially by measuring the phosphorylation of poly(Glu-Tyr) by enzymatically active recombinant FAK protein. PF-228 inhibited catalytic activity in this assay with an IC$_{50}$ of 4 nM (Fig. 1B and Table 1). In a parallel assay, PF-228 inhibited Pyk2 with an IC$_{50}$ of 1 µM (Table 1). PF-228 was screened against a commercially available panel of recombinant enzymes (Upstate Biotechnology, Inc.; (7)) at 1 µM of Tyr397 phosphorylation in PC3 (prostate carcinoma), SKOV-3 (ovarian carcinoma), L3.6p1 and F-G (pancreatic carcinomas), and MDCK.

Two cell-based assays were used to assess the activity of PF-228. First, A431 epithelial carcinoma cells expressing epitope-tagged FAK under the inducible regulation of mifepris- tone were incubated with increasing concentrations of PF-228, and the phosphorylation of FAK Tyr$_{397}$ was measured in a quantitative ELISA (see “Experimental Procedures”). PF-228 inhibited FAK phosphorylation in A431 cells with IC$_{50}$ of 11 nM (Fig. 2A).

To assess the ability of PF-228 to block endogenous FAK activity, the phosphorylation of FAK Tyr$_{397}$ was measured in cultured REFS2 cells (a nontransformed, immortalized rat fibroblast cell line). Treatment of cells with increasing concentrations of PF-228 for 60 min revealed half-maximal inhibition of Tyr$_{397}$ phosphorylation of ~100 nM. Greater than 75–80% inhibition was routinely achieved with PF-228 concentrations of 0.3–3 µM (Fig. 2B). PF-228 also blocked FAK Tyr$_{397}$ phosphorylation in PC3 (prostate carcinoma), SKOV-3 (ovarian carcinoma), L3.6p1 and F-G (pancreatic carcinomas), and MDCK.
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TABLE 1
Kinase selectivity profile of PF-228

| Kinase     | PF-228 IC_{50} (nM) |
|------------|---------------------|
| FAK        | 4                   |
| Ptk2       | >1000               |
| Abi(h)     | 8                   |
| ACK        | 29                  |
| AKT        | 0                   |
| ASK        | 1                   |
| Btk        | 11                  |
| c-Raf(h)   | 0                   |
| CaMKIV(h)  | 0                   |
| CDK1(cyclinB(h)| 77| 486|
| CDK2(cyclinA(h)| 50| |
| CDK2(cyclinE(h)| 44| |
| CDK3(cyclinE(h)| 44| |
| CDK3/p35(h)| 0                   |
| CDK6(cyclinD3(h)| 15| |
| CDK7(cyclinH/MAT1(h)| 86| 197|
| CHK1       | 13                  |
| cSrc(h)    | 1                   |
| EGFb(h)    | 0                   |
| EphA2      | 15                  |
| EphB2      | 11                  |
| Fyn        | 28                  |
| GSK3b(h)   | 64                  |
| Jnk1a1(h)  | 4                   |
| c-kit      | 25                  |
| Lck        | 0                   |
| MAPK1(h)   | 48                  |
| MEK1(h)    | 20                  |
| Mkk         | 48                  |
| Mkk6       | 16                  |
| PDGFR-β    | 2                   |
| P70S6K(h)  | 31                  |
| PI 3-Ky(h) | 0                   |
| Pka(h)     | 0                   |
| PkBo(h)    | 13                  |
| PkCo(h)    | 12                  |
| Pkc-θ      | 36                  |
| Rock-Il(h) | 8                   |

*Percentage of inhibition.

controls (Table 2). Treatment of cells with 1 μM PF-228 failed to inhibit cell growth, although this concentration of PF-228 was sufficient to inhibit FAK phosphorylation by greater than 80–90%. Growth of REF52 and PC3 cells was significantly inhibited at the higher concentration of 10 μM PF-228 (Table 2). To assess whether the inhibition of growth observed at higher concentrations was perhaps due to off target effects of PF-228, FAK-deficient fibroblasts (11) were treated with increasing concentrations of PF-228. Inhibition of growth was observed using 10 μM PF-228 (data not shown), indicating that prolonged treatment of cells at high concentrations of drug may inhibit cell growth in a FAK-independent manner.

To examine whether inhibition of FAK catalytic activity induced apoptosis, REF52, PC3, or MDCK cells were treated with the indicated concentrations of PF-228 for 24 h, and apoptosis was assessed using an ELISA assay, which measures the presence of free histones in lysates (Fig. 3). As previously reported suspension of MDCK cells for 16 h readily induced apoptosis (Fig. 3) (12). Similarly, suspending REF52 cells for 24 h also induced apoptosis, although to a lesser extent than the apoptosis induced in MDCK cells. PC3 cells did not undergo apoptosis in suspension. Treatment of attached REF52, PC3, or MDCK cells with PF-228 failed to induce histone accumulation at concentrations shown to significantly block FAK Tyr^{397} phosphorylation.

Effects of FAK Inhibition on Cell Spreading and Migration—Cell adhesion to extracellular matrix components such as FN stimulates FAK activity and cell spreading (1, 2). To assess the ability of PF-228 to block adhesion-dependent FAK activation, REF52 cells were suspended in serum-free medium containing increasing concentrations of PF-228 or vehicle control for 1 h and plated on FN-coated dishes for 30 min in the continued presence or absence of inhibitor (Fig. 4A). Treatment of cells with 1–3 μM PF-228 reduced FN-stimulated FAK Tyr^{397} phosphorylation by ~65–85% (Fig. 4A). In addition, treatment of cells with PF-228 also reduced the tyrosine phosphorylation of paxillin on Tyr^{31} in a dose-dependent manner (data not shown).

To examine the effects of PF-228 on the morphology of cell spreading, REF52 cells were suspended in the presence of the inhibitor and then plated on 5 μg/ml FN for 60 min in the continued presence of inhibitor. Immunostaining showed a maximal inhibition of FAK phosphorylation and a loss of paxillin organization in focal adhesions with 3 μM PF-228 (Fig. 4B).

A role for FAK catalytic activity in cell migration was assessed in transwell assays using 10% serum or FN as chemoattractants. REF52 cells were pretreated with the indicated concentrations of PF-228 for 30 min and allowed to migrate toward 10% serum or FN for 6 h in the continued presence of inhibitor (Table 3). Treatment of cells with 1.0 μM PF-228 (a concentration that reduces FAK phosphorylation by 80%) had no significant effect on random migration of REF52 cells (Table 3). However, at this concentration of PF-228, both FN and serum-stimulated migration were significantly inhibited. Treatment of cells with 10 μM PF-228 blocked random migration and also efficiently blocked serum and FN-stimulated migration (Table 3).
Inhibition of cell migration by PF-228 was also assessed in wound assays performed with REF52 cells (supplemental Fig. S1). Treatment of cultures with 1 μM PF-228 significantly reduced the rate of movement of individual cells into the wound, supporting the results from the transwell assay (Table 3).

Cell migration requires the coordinated formation and disassembly of focal adhesions, a phenomenon known as focal adhesion turnover (13). The effects of PF-228 treatment on focal adhesion turnover were analyzed using a wound assay in NIH-3T3 cells stably expressing green fluorescent protein-paxillin. Focal adhesions in cells at the edge of the wound were analyzed by TIRF microscopy. The lifetime of the adhesions was measured by monitoring the fluorescence intensity of single adhesions over time. Untreated cells exhibited active adhesion assembly and disassembly (Fig. 5A, arrowheads), and the average adhesion lifetime was 21.9 ± 11.3 min (Fig. 5B). In contrast, all of the adhesions in the cells pretreated with PF-228 showed a decrease in the rate of disassembly (Fig. 5A, arrowheads) and exhibited significantly longer lifetimes than the adhesions in the untreated cells (Fig. 5B, average adhesion lifetime of 33.1 ± 12.9 and 42.0 ± 14.4 min for 1 and 10 μM PF-228, respectively). Treatment with PF-228 did not inhibit the assembly of new adhesions (Fig. 5A, arrow). Interestingly, whereas the adhesion turnover (13). The effects of PF-228 treatment on focal adhesion turnover were analyzed using a wound assay in NIH-3T3 cells stably expressing green fluorescent protein-paxillin. Focal adhesions in cells at the edge of the wound were analyzed by TIRF microscopy. The lifetime of the adhesions was measured by monitoring the fluorescence intensity of single adhesions over time. Untreated cells exhibited active adhesion assembly and disassembly (Fig. 5A, arrowheads), and the average adhesion lifetime was 21.9 ± 11.3 min (Fig. 5B). In contrast, all of the adhesions in the cells pretreated with PF-228 showed a decrease in the rate of disassembly (Fig. 5A, arrowheads) and exhibited significantly longer lifetimes than the adhesions in the untreated cells (Fig. 5B, average adhesion lifetime of 33.1 ± 12.9 and 42.0 ± 14.4 min for 1 and 10 μM PF-228, respectively). Treatment with PF-228 did not inhibit the assembly of new adhesions (Fig. 5A, arrow). Interestingly, whereas the
adhesions in the cells treated with PF-228 showed a lack of turnover, they exhibited a pattern of movement toward the center of the cell (see supplemental Movies S1 and S2).

DISCUSSION

In this study we characterize the biochemical and cellular properties of a novel and specific small molecule inhibitor of FAK, PF-228. The inhibitor effectively blocked recombinant
FAK catalytic activity and FAK Tyr\(^{397}\) phosphorylation in cultured cells. Treatment of cultured cells with concentrations of the inhibitor that significantly inhibited FAK autophosphorylation of Tyr\(^{397}\) failed to inhibit growth or apoptosis. However, similar treatment of cells with PF-228 resulted in inhibition of serum or FN-directed migration and decreased focal adhesion turnover. Because increased FAK expression and activity are hallmarks of many cancers and the observations that increased cell migration often characterizes tumor cells with increased metastatic potential, FAK inhibitors may have therapeutic potential for the treatment of cancer.

PF-228 inhibited recombinant FAK kinase activity with an IC\(_{50}\) of 4 nM, 50–250-fold lower than any other kinase tested. In cultured cells, PF-228 inhibited FAK phosphorylation to varying degrees. ELISA measurements of FAK phosphorylation in A431 cells engineered to overexpress FAK produced an IC\(_{50}\) of 11 nM compared with ~100 nM in REF52 cells measured by Western blot (Fig. 2). A survey of inhibitory activity of PF-228 in four additional cancer cell lines using Western blotting revealed a range of IC\(_{50}\) of 30–500 nM. Although it is possible that the discrepancy could be due to differences in sensitivity between ELISA and Western, ELISA measurements of FAK phosphorylation in REF52 cells generated similar results to the Western analysis (data not shown). Rather, it is likely that the discrepancy reflects differences in the cell systems. The A431 cells overexpress FAK at levels significantly higher than endogenous. The degree of overexpression is expected to alter the subcellular localization and activity of FAK, which could make it more susceptible to inhibition by PF-228. If this is the case, tumors that overexpress FAK would be expected to be more sensitive to FAK inhibition than normal cells, consistent with nonclinical in vivo studies (data not shown).

FAK functions have been implicated in the adhesion-dependent signaling events that regulate cell growth, death, and migration (1, 2, 4, 5). The data presented here implicate FAK kinase activity in the regulation of cell migration, but interestingly concentrations of PF-228 that efficiently inhibit FAK activity do not block cell growth or induce apoptosis in vitro. In contrast, several studies have implicated FAK in the regulation of both processes (14–18). Expression of the C-terminal domain of FAK, which inhibits FAK function, induces apoptosis in some cell systems (18, 19). Cre-mediated loss of FAK expression in a mouse model of skin cancer suppresses tumor formation, which is accompanied by increased keratinocyte cell death (16). This study and others examining a role for FAK in apoptosis rely on the loss of FAK or the use of dominant interfering mutants of FAK and implicate the scaffolding function of FAK in the regulation of apoptosis. Inhibition of FAK kinase activity with PF-228 was not sufficient to induce apoptosis in vitro in three cell types examined (Table 2). This may be due to the conditions in which cells are grown and the pathways that are activated when attached to plastic substrates or due to the fact that the scaffolding function of FAK rather than its kinase activity is important for the regulation of apoptosis.

Adhesion-dependent signaling events have been shown to effect cellular proliferation by preventing the transition from the G\(_1\) phase to the S phase. A role for FAK in this process is somewhat controversial. Overexpression of wild type FAK has been shown to stimulate adhesion-independent growth of a variety of tumors (4, 5), which in fibroblasts was demonstrated to reflect an increase in cell cycle progression through G\(_1\) to S phase (20). Likewise, overexpression of the dominant negative C-terminal domain of FAK inhibits growth (4, 5), and dominant interfering mutants decreased G\(_1\) to S transition (20). However, mesodermal cells derived from FAK null embryos display no impairment in cell growth (11). Only treatment of REF52 and PC3 cells with high concentrations of PF-228 for 3 days blocked cell growth (Table 2). However, this concentration and duration of PF-228 treatment also blocked growth of FAK null fibroblasts. Moreover, elevated concentrations of PF-228 inhibited many cyclin-dependent kinases (Table 1). Therefore, the growth inhibitory effects reported here at the high concentration of PF-228 likely involve inhibition of off target kinases in addition to FAK.

The regulation of cell migration is a very well studied aspect of FAK function on cellular activity. Antisense oligomers, RNA interference, FAK knock-out, and expression of dominant interfering mutants all block cell migration (11, 21–24). Furthermore, migration is restored in FAK null cells expressing wild type FAK but not kinase-deficient FAK, the autophosphorylation mutant of FAK or the C-terminal domain of FAK, implicating both the scaffolding function and kinase activity of FAK in the regulation of cell migration (25, 26). The observation that PF-228 inhibited cell migration in a dose-dependent manner supports a role for FAK kinase activity in the regulation of cell migration. Moreover, inhibition of FAK kinase activity with PF-228 decreased focal adhesion turnover, a necessary component of cell migration (13). However, although the adhesions in the PF-228-treated cells failed to turn over efficiently, they did exhibit a coordinated movement toward the center of the cell. This movement is consistent with the rearward flow of actomyosin in stationary cells (27, 28). Thus, it is possible that inhibition of FAK may uncouple adhesions from the substrate while still allowing them to be connected to the mobile actin network in the cell. This uncoupling would lead to defects in the generation and/or maintenance of tension at sites of adhesion, which would affect cell migration and tumor metastasis (29, 30).

Recently, Shi and colleagues reported the effects of a FAK inhibitor from Novartis, TAE226, on glioma cell function (31). Similar to the observations reported here, Shi et al. observed an inhibition of cell migration. However, unlike PF-228, TAE226 also induced apoptosis and inhibited growth of glioma cells in culture. These differences could be due to cell type or more likely specificity of the inhibitors. Notably, TAE226 is a diazino-pyrimidine with indistinguishable activity against IGF-1R and FAK and modest activity in cell-based kinase assays with an IC\(_{50}\) range of 100–300 nM (32). PF-228 has substantially greater potency in cell-based kinase assays and significant selectivity relative to IGF-1R and many other kinases (Table 1), allowing for greater understanding of FAK inhibition and resulting cellular effects. The effects of TAE226 on other kinases was not reported (31).

PF-228 and similar compounds provide useful tools to assess the role of adhesion signaling in regulation of cellular proliferation, apoptosis, and migration and may prove to have impor-
tand therapeutic applications in the prevention and/or treatment of cancer. Although other small molecules with FAK inhibitory activity have been described, they are either nonselective, thereby confounding cellular data interpretation and utility, or have not been evaluated in cellular systems (33, 34). The careful evaluation of pharmacokinetics and pharmacodynamics in vivo will be required to fully understand the role of FAK inhibition in the regulation of tumorigenesis and metastatic progression. The importance of FAK kinase activity in the regulation of cell migration implicates these inhibitors as potential metastasis suppressors. Importantly, bioavailable FAK inhibitors demonstrate substantial anti-tumor activity in multiple murine models of human disease corresponding to a concomitant decrease in tumor or vessel-associated phosphorylated FAK (37). Moreover, a FAK inhibitor has recently entered human clinical testing for the treatment of cancer (37).

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