# Differential requirement for focal adhesion kinase signaling in cancer progression in the transgenic adenocarcinoma of mouse prostate model

Jill K. Slack-Davis,[1] E. Daniel Hershey,[1] Dan Theodorescu,[2] Henry F. Frierson,[3] and J. Thomas Parsons[1]

Departments of [1]Microbiology, [2]Molecular Physiology and Biological Physics, and [3]Pathology, University of Virginia Health System, Charlottesville, Virginia

## Abstract

Increasing evidence indicates that adhesion signaling plays an important role in the tumor microenvironment, contributing to cancer progression, invasion, and metastasis. Focal adhesion kinase (FAK) is a nonreceptor protein tyrosine kinase that regulates adhesion-dependent cell signaling and has been implicated in mediating steps in cancer progression and metastasis in many human cancers, including prostate. We have investigated the role of FAK in the appearance of adenocarcinoma (atypical epithelial hyperplasia of T antigen) and neuroendocrine carcinomas in the transgenic adenocarcinoma of mouse prostate (TRAMP) model using either Cre-mediated recombination to genetically ablate FAK expression or pharmacologic inhibition of FAK activity with the small-molecule inhibitor, PF-562,271. We provide evidence that loss of FAK or its inhibition with PF-562,271 does not alter the progression to adenocarcinoma. However, continued FAK expression (and activity) is essential for the androgen-independent formation of neuroendocrine carcinoma. These data indicate that integrin signaling through FAK is an important component of cancer progression in the TRAMP model and suggest that treatment modalities targeting FAK may be an appropriate strategy for patients with castrate-resistant cancer. [Mol Cancer Ther 2009;8(8):2470–7]

Requests for reprints: J. Thomas Parsons, Department of Microbiology, University of Virginia, 1300 Jefferson Park Avenue, Charlottesville, VA 22908. Phone: 434 924-5395; Fax: 434 982-1071. E-mail: jtp@virginia.edu

## Introduction

The tumor microenvironment plays a critical role in cancer invasion and metastasis (1–3). In particular, the loss of adhesion-dependent cellular regulation is a hallmark of cancer, and factors that regulate cell-extracellular matrix interactions and signaling have been implicated in tumorigenesis and metastatic progression. Focal adhesion kinase (FAK) is a nonreceptor protein tyrosine kinase that regulates adhesion-dependent cell signaling (4). There is abundant evidence that FAK plays a role in cancer progression and metastasis including that for prostate cancer (4). First, FAK expression is increased in prostate cancer cell lines (5), and increased expression correlates with enhanced motility and tumorigenicity (6). In human prostate cancer, FAK is present in high-grade prostatic intraepithelial neoplasia, primary adenocarcinomas, and metastatic lesions (7). The inhibition of FAK activity by dominant interfering mutants or small interfering RNAs decreases prostate cancer cell migration (8) and inhibits the growth of prostate tumor cells in immunocompromised mice. Finally, small-molecule inhibitors, developed specifically for FAK family members (FAK and PYK2), inhibit prostate cancer cell migration, anchorage-independent growth, and growth of prostate cancer xenografts (9, 10).

Transgenic adenocarcinoma of mouse prostate (TRAMP) is a well-studied mouse model of prostate cancer in which SV40 T-antigen is expressed in prostate secretory epithelial cells under the control of the androgen-responsive minimal rat probasin promoter (11, 12). In this model, mice develop progressive, multifocal, and heterogenous disease, characterized by atypical epithelial hyperplasia of T antigen (referred to hereafter as adenocarcinoma) and a pronounced shift to neuroendocrine carcinomas in late-stage disease (13). Castration of animals at 12 to 15 weeks of age results in the appearance of androgen-independent neuroendocrine carcinomas (14). Transplantation studies indicate that the neuroendocrine carcinomas develop from bipotential progenitor cells during an early stage of SV40 T-antigen–driven tumorigenesis (13).

We have investigated the role of FAK in the appearance of adenocarcinoma and neuroendocrine carcinomas in the TRAMP model using either Cre-mediated recombination to genetically ablate FAK expression or pharmacologic inhibition of FAK activity with the small-molecule inhibitor PF-562,271 (7, 8). We provide evidence that loss of FAK or its inhibition with PF-562,271 does not alter the progression to adenocarcinoma. However, we observe that continued FAK expression (and activity) is essential for the androgen-independent formation of neuroendocrine carcinoma. These data indicate that integrin signaling through FAK is an important component of cancer progression in the

TRAMP model and suggest that treatment modalities targeting FAK may be an appropriate strategy for patients with castrate-resistant cancer.

## Materials and Methods

### Mice

Mice expressing SV40 T antigen from the probasin promoter (TRAMP; ref. 12; The Jackson Laboratory), Cre recombinase (*ARR2Probasin-Cre* transgenic line, PB-Cre4; NCI), ROSA26 lox-stop-lox LacZ (ref. 15; The Jackson Laboratory), or floxed FAK (16) were bred onto a C57Bl/6 background. TRAMP and Pb-Cre alleles were maintained on the same individual as heterozygotes; LacZ and floxed FAK alleles were maintained as homozygotes. TRAMP/Pb-Cre$^+$ mice were bred with LacZ/FAK$^{fl/fl}$ to generate TRAMP$^{+/-}$, Pb-Cre$^{+/-}$, FAK$^{fl/fl}$, LacZ$^{+/+}$ mice. Pb-Cre was maintained only in males; all other transgenes were carried on either sex.

### Genotyping

Mice were genotyped using tail DNA isolated from 10- to 14-d-old pups and the Purelink genomic DNA kit (Invitrogen). Transgene identification was done by PCR using the following primer sets: TRAMP, 5′-CAGAGCAGAATTGTG-GAGTGG-3′, 5′-GGACAAACCACAACTAGAATG-CAGTG-3′; Pb-Cre, 5′-TTCCCGCAGAACCTGAAGATG-3′, 5′-CGCCGCATAACCAGTGAAAC-3′; FAK$^{fl/fl}$, 5′-GA-GAATCCAGCTTTGGCTGTTG-3′, 5′-GAATGCTACAG-GAACCAAATAAC-3′; Rosa26-LacZ #1, 5′-AAAGTCGCTCTGAGTTGTTAT-3′, rosa26 #2, 5′-GCGAA-GAGTTTGTCCTCAACC-3′, rosa26 #3, 5′-GGAGCGGGA-GAAATGGATATG-3′.

TRAMP sequences were amplified using 1 cycle 95°C 5 min; 10 cycles 94°C 10 s, 53°C 30 s, 68°C 3 min; 20 cycles 94°C 10 s, 53°C 30 s, 68°C 3 min 20 s; and 1 cycle 68°C 7 min to generate a 474-bp product. The remaining transgene sequences were amplified using 1 cycle 95°C 5 min; 10 cycles 94°C 10 s, 57°C 30 s, 68°C 3 min; 20 cycles 94°C 10 s, 57°C 30 s, 68°C 3 min 20 s; and 1 cycle 68°C 7 min to produce 166-bp Pb-Cre, 400-bp FAK$^{fl/fl}$ (290-bp wild-type), and 300-bp LacZ (650-bp wild-type) fragments.

To detect sequence alterations in the FAK gene as a result of Cre induction, DNA from prostate or tumor tissue was extracted and DNA was amplified using 1 cycle 95°C 5 min; 35 cycles 94°C 30 s, 62°C 30 s, 72°C 2 min; 1 cycle 72°C 6 min, using the following primers: 5′-GAATGCTACAGGAAC-CAAATAAC-3′ and 5′-GACCTTCAACTTCTCATTTCTCC-3′. The floxed FAK allele is 1,800 bp; wild-type allele 1,690 bp; and recombined FAK 300 bp. All PCR products were resolved on 2% agarose gels containing ethidium bromide.

### Castration and Drug Treatment

Radical orchiectomy was done on mice 15 to 16 wk of age anesthetized with i.p. injection of 0.018 mL/g 2.5% tribromoethanol. A transverse incision was made through the shaved abdominal skin and wall just superior to the preputial glands. Testicles were everted one at a time and removed by cauterization. The abdominal wall was sutured and the skin glued, stapled, or sutured. Analgesia (bupivicane) was administered at the incision. Following recovery from surgery (4–5 d), mice were treated for 4 wk p.o, bd with 33 mg/kg PF-562,271 suspended in paraffin oil vehicle or vehicle alone.

### Immunohistochemistry and β-Galactose Staining

Following euthanasia, the total urogenital tract was removed and weighed, and the prostate and/or tumor was isolated, weighed, and prepared for immunohistochemistry or β-galactose staining. Additional organs including lymph node, kidney, liver, and lung were examined grossly for metastasis, removed, and prepared for histology. Prostates and tumors were washed twice in phosphate buffered saline (PBS) and then fixed for 30 min at room temperature in 0.1 mol/L sodium phosphate (pH 7.3), 20 mmol/L Tris (pH 7.3), 5 mmol/L EGTA, 2 mmol/L MgCl$_2$, 0.25% glutaraldehyde, and 1% formaldehyde. Fixed tissues were washed twice in PBS and stained for β-galactose activity in 0.1 mol/L sodium phosphate (pH 7.3), 20 mmol/L Tris (pH 7.3), 2 mmol/L MgCl$_2$, 5 mmol/L potassium ferrocyanate, 5 mmol/L potassium ferricyanate, 0.1% deoxycholate, 0.2% NP40, and 1 mg/mL 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (in dimethylformamide) 16 h at room temperature in the dark. Tissues were destained following several PBS washes and either cleared for whole mount by incubating for 16 h in 2:1 benzylbenzoate/benzyl alcohol followed by methanol dehydration for 48 h or fixed for histology. Selected tissues were fixed in zinc formalin, embedded in paraffin, sectioned, and stained with H&E. Immunohistochemistry was done on additional sections for FAK (1:200, Santa Cruz), T antigen (1:200, BD Pharmingen), synaptophysin (1:50, BD Pharmingen), and E-cadherin (1:25, DAKO). Before staining, deparaffinized, hydrated sections were placed in citrate buffer (10 mmol/L sodium citrate, pH 6.0) for microwave antigen retrieval followed by incubation in 3% H$_2$O$_2$ for 5 min at room temperature. FAK immunohistochemistry was done using ABC Vectastain (Vector Laboratories), and slides were stained with 3,3′-diaminobenzidine and then counterstained with hematoxylin. For the remaining antibodies, tissues were blocked using the M.O.M. kit (Vector Laboratories), stained with 3,3′-diaminobenzidine, and counterstained with hematoxylin.

### Western Blot Analysis

Prostate or tumor tissue was homogenized and lysed in supplemented radioimmunoprecipitation assay buffer (50 mmol/L HEPES, 0.15 mol/L NaCl, 2 mmol/L EDTA, 0.1% NP40, and 0.05% sodium deoxycholate, pH 7.2) containing EDTA-free protease inhibitor cocktail (Roche), 1 mmol/L Na$_3$VO$_4$, 40 mmol/L NaF, and 10 mmol/L Na$_4$P$_2$O$_7$. Proteins from 25 to 50 μg of whole-cell lysates were resolved on 8% SDS-PAGE, transferred onto nitrocellulose, and blotted for FAK (clone 4.47, Millipore), β-actin (Millipore), or β-galactosidase (Invitrogen).

## Results

### Disruption of FAK Expression in the Prostate of Normal and TRAMP Mice

To assess the role of FAK in prostate cancer progression in TRAMP mice, normal and TRAMP mice were interbred

with conditional FAK knockout mice (FAK$^{fl/fl}$) and further interbred with the *ARR2Probasin-Cre* transgenic line PB-Cre4, in which the Cre recombinase is under the control of a modified rat prostate-specific probasin promoter (Pb-Cre). To have a readily detectable marker for Cre activity, we introduced the GTRosa26 Cre-inducible β-galactosidase reporter (15) into the normal and TRAMP genetic backgrounds. The resulting mice carried the TRAMP allele, FAK$^{fl/fl}$, Rosa26-LacZ, and either expressed Pb-Cre (Pb-Cre$^+$) or lacked Pb-Cre expression (Pb-Cre$^-$). All mice were maintained on a C57Bl/6 background.

PCR analysis revealed that recombination of FAK$^{fl/fl}$ was readily detectable in the prostate tissue of normal and TRAMP mice expressing Pb-Cre, whereas no recombination was observed in other tissues (e.g., kidney; Fig. 1A). Normal mice expressing Pb-Cre and FAK$^{fl/fl}$ were fertile and phenotypically normal up to 40 weeks of age; no detectable alteration in prostate architecture was observed and robust β-galactosidase activity was present in 60% to 80% of the individual glands (Fig. 1B–D; ref. 17).

### Appearance of Lesions in FAK$^{fl/fl}$ Mice

As expected, TRAMP/FAK$^{fl/fl}$ mice that did not express Pb-Cre developed adenocarcinoma as early as 7 weeks after birth. These lesions, as described by others (12, 13), were characterized by multifocal atypical hyperplastic lesions within the lining epithelial cells. As illustrated in Fig. 2 and tabulated in Table 1, by 20 weeks of age, 49% of the TRAMP animals exhibited adenocarcinoma. Between 11 and 20 weeks, about 16% of tumor-bearing animals exhibited neuroendocrine carcinomas or a mix of neuroendocrine and adenocarcinoma (Table 1; refs. 12, 13). By 21 to 30 weeks, all animals except one exhibited adenocarcinoma alone, neuroendocrine tumors alone, or both, approximately 21%, 7%, and 5% respectively (Table 1). Neuroendocrine tumors were typically large and grew rapidly; they were locally invasive and exhibited metastasis to lymph nodes, kidney, and lung (data not shown). Immunohistochemical

analysis revealed that virtually all adenocarcinomas were T antigen positive, E-cadherin positive, and synaptophysin negative. Neuroendocrine tumors characteristically were T antigen positive, E-cadherin negative, and synaptophysin positive at this stage of development (Fig. 3). FAK staining was weak in normal prostate epithelial cells but was clearly enhanced in both adenocarcinoma and neuroendocrine tumors (Fig. 3). TRAMP/FAK$^{fl/fl}$ mice that expressed Pb-Cre showed no significant differences in time to appearance of adenocarcinoma or neuroendocrine tumors compared with Pb-Cre$^-$ mice (Table 1). The relative number of animals with adenocarcinoma was not statistically different between Pb-Cre$^+$ and Pb-Cre$^-$ mice. In addition, there was no difference in the percent of mice exhibiting neuroendocrine or mixed adenocarcinoma/neuroendocrine tumors in the Pb-Cre$^+$ cohort. No statistically significant differences in urogenital weights were observed at early, intermediate, or late times after transgene expression (Fig. 2).

### FAK Recombination and Expression in TRAMP/FAK$^{fl/fl}$ Mice

PCR genotyping of lesions revealed significant recombination of the FAK$^{fl/fl}$ allele in prostates with adenocarcinoma, whereas no recombination was observed in normal kidney tissue (Fig. 4A, *left*). In contrast, neuroendocrine tumors showed little evidence of recombination of the FAK$^{fl/fl}$ allele (Fig. 4A, *right*). These data were consistent with observations based on Western blotting of multiple adenocarcinoma and neuroendocrine lesions (Fig. 4B), in which there was readily detectable expression of FAK irrespective of the age of the animal or the type of lesion. The expression of β-galactosidase was detectable in all normal prostate samples as well as in prostates with adenocarcinomas (Fig. 4B, *left*). Loss of β-galactosidase expression was typically observed in late-stage adenocarcinoma (e.g., animals >20 weeks of age). Interestingly, neuroendocrine tumors were always negative for β-galactosidase expression, irrespective of age. These data support the observations from PCR
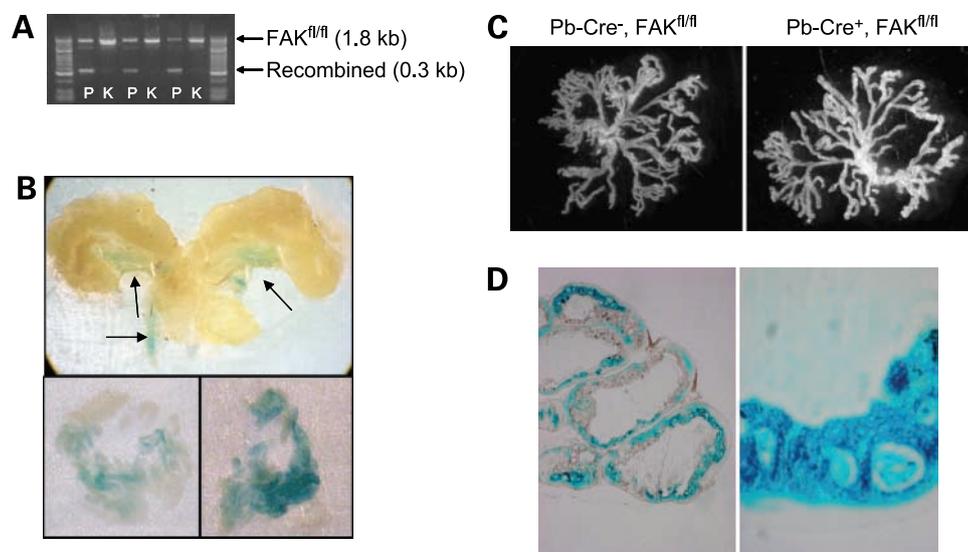


**Figure 1.** Prostate development in Pb-Cre$^+$, FAK$^{fl/fl}$ mice. **A,** genomic DNA isolated from prostates (*P*) and kidneys (*K*) was subjected to PCR amplification of the *FAK* locus to identify floxed (1,800 bp) and recombined (300 bp) FAK alleles. **B,** urogenital tract (*top*) and individual prostate glands (*bottom*) were isolated and stained for β-galactosidase activity (*blue*). *Arrows*, prostate glands. **C,** individual prostate glands from Pb-Cre$^+$ and Pb-Cre$^-$ mice were dissected to reveal the ductal tree. **D,** sections of prostate from Pb-Cre$^+$, FAK$^{fl/fl}$ mice stained for β-galactosidase activity. Secretory epithelial cells from 60% to 70% of the glands stained positively (*blue*). Magnification, ×400.

In figure: FAK$^{fl/fl}$ (1.8 kb); Recombined (0.3 kb); P K P K P K; **C** Pb-Cre$^-$, FAK$^{fl/fl}$   Pb-Cre$^+$, FAK$^{fl/fl}$

**Figure 2.** Tumor distribution in FAK$^{fl/fl}$ mice. Schematic representation of the onset and duration of well-differentiated adenocarcinoma (*AD*) and neuroendocrine (*NE*) tumors in the TRAMP model (*top*). **A,** percentage of mice with adenocarcinoma (*dark gray*), neuroendocrine (*light gray*), or no tumor (*black*) in Pb-Cre$^+$, FAK$^{fl/fl}$ (*Pb +*) or Pb-Cre$^-$, FAK$^{fl/fl}$ (*Pb−*) mice. **B,** weight of the urogenital tract including seminal vesicles, prostate, and any tumor from TRAMP$^-$ (*Tr−*), TRAMP$^+$, Pb-Cre$^+$, FAK$^{fl/fl}$ (*Tr+, Pb +*), or TRAMP$^+$, Pb-Cre$^-$, FAK$^{fl/fl}$ (*Tr+, Pb−*) mice. Statistical significance was determined using the Kruskal-Wallis test followed by Dunn's multiple comparison post-test. The numbers above each graph represent the number of mice analyzed in each group.
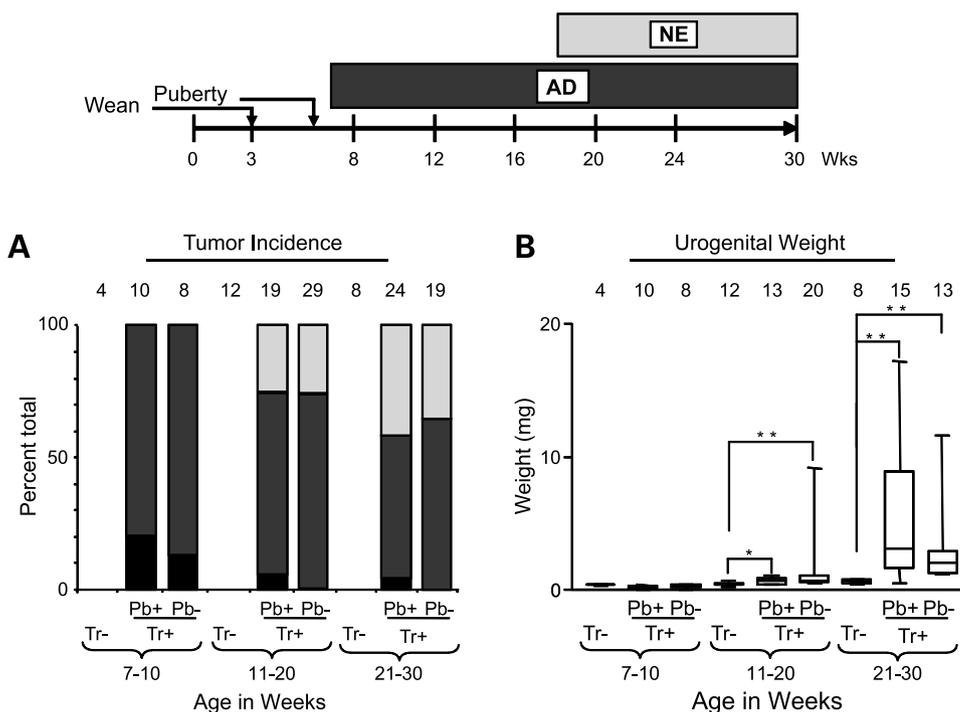


genotyping and indicate that neuroendocrine tumors develop from cells that fail to undergo Cre-mediated recombination at the floxed *FAK* or the floxed *Rosa26* locus. In accordance with these observations, we also failed to observe β-galactosidase staining in tissue sections from neuroendocrine carcinomas (data not shown). In contrast, typically 60% to 70% of glands exhibiting adenocarcinoma were positive for β-galactosidase (Fig. 3). These data indicate that deletion of FAK and loss of FAK expression does not prevent the outgrowth of adenocarcinoma. However, the fact that virtually all neuroendocrine tumors had FAK expression and were derived from cells that seemed to fail to undergo Cre-mediated recombination indicates that sustained FAK expression may be required for the efficient outgrowth of neuroendocrine carcinomas.

### Appearance of Neuroendocrine Tumors in TRAMP Mice Treated with the FAK Inhibitor PF-562,271

Castration of TRAMP mice at 12 to 14 weeks of age leads to regression of adenocarcinomas and the efficient outgrowth of neuroendocrine tumors (14, 18). To test whether FAK was required for the outgrowth of neuroendocrine carcinomas, mice were castrated at 15 to 16 weeks of age, a time in which adenocarcinoma and/or neuroendocrine lesions were observed in more than 95% of the TRAMP mice. Following recovery from surgery, castrated mice were assigned to either a treatment group that received the FAK inhibitor PF-562,271 at a dose of 33 mg/kg, bd, p.o. for 4 weeks or a group treated in a parallel fashion with drug carrier only. Control groups included non-TRAMP mice treated with PF-562,271 as indicated above. This treatment regimen has been shown to efficiently inhibit the growth of several different human tumor cell lines implanted s.c. in immunocompromised mice as well as reduce the level of FAK phosphorylation on tyrosine397 (9). Control non-TRAMP mice, as expected, did not get lesions and were healthy and viable following 4 weeks of PF-562,271 treatment (data not shown). Castrated animals that did not receive PF-562,271 developed large, highly invasive

**Table 1.** Tumor incidence by age and genotype

| Age (wk) | | Normal | | AD | | AD/NE | | NE | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pb-Cre status | | − | + | − | + | − | + | − | + | − | + |
| <10 | n (%) | 1 (2) | 2 (4) | 7 (13) | 8 (15) | | | | | 8 (14) | 10 (19) |
| 11–20 | n (%) | | 1 (2) | 20 (36) | 14 (26) | 6 (11) | 4 (8) | 3 (5) | | 29 (52) | 19 (36) |
| 21–30 | n (%) | | 1 (2) | 12 (21) | 12 (23) | 4 (7) | 2 (4) | 3 (5) | 9 (17) | 19 (34) | 24 (45) |
| Total | | 1 | 4 | 39 | 34 | 10 | 6 | 6 | 9 | 56 | 53 |

NOTE: AD, animals in which only adenocarcinoma was detected; NE, animals with only neuroendocrine tumors; AD/NE, animals with both adenocarcinoma and neuroendocrine tumors.

neuroendocrine carcinomas; six of eight mice developing neuroendocrine tumors exhibited lymph node metastases, three of which also had metastases within the kidney or lung, with one of the three having metastases in both the kidney and the lung. Castrated mice treated with PF-562,271 developed fewer neuroendocrine carcinomas, and those that did arise were considerably smaller (Fig. 5B and C). Lesions developing in the castrated mice that were not treated with PF-562,271 exhibited the typical neuroendocrine phenotype, as they were T-antigen positive and synaptophysin positive (data not shown). Nonneoplastic glands present in castrated, PF-562,271–treated animals showed evidence of atrophy with dilated glands that were

lined by flattened epithelial cells with minimal secretory cytoplasm (data not shown) The above observations confirm a role for FAK in the outgrowth of neuroendocrine carcinomas in the setting of androgen independence.

## Discussion

Our studies point to several important roles for FAK in the progression of cancer in the TRAMP model. First, loss of FAK expression by targeted deletion in prostate epithelial cells did not alter the time to appearance or the frequency of well-differentiated adenocarcinoma, indicating that FAK expression is not necessary for the development of these
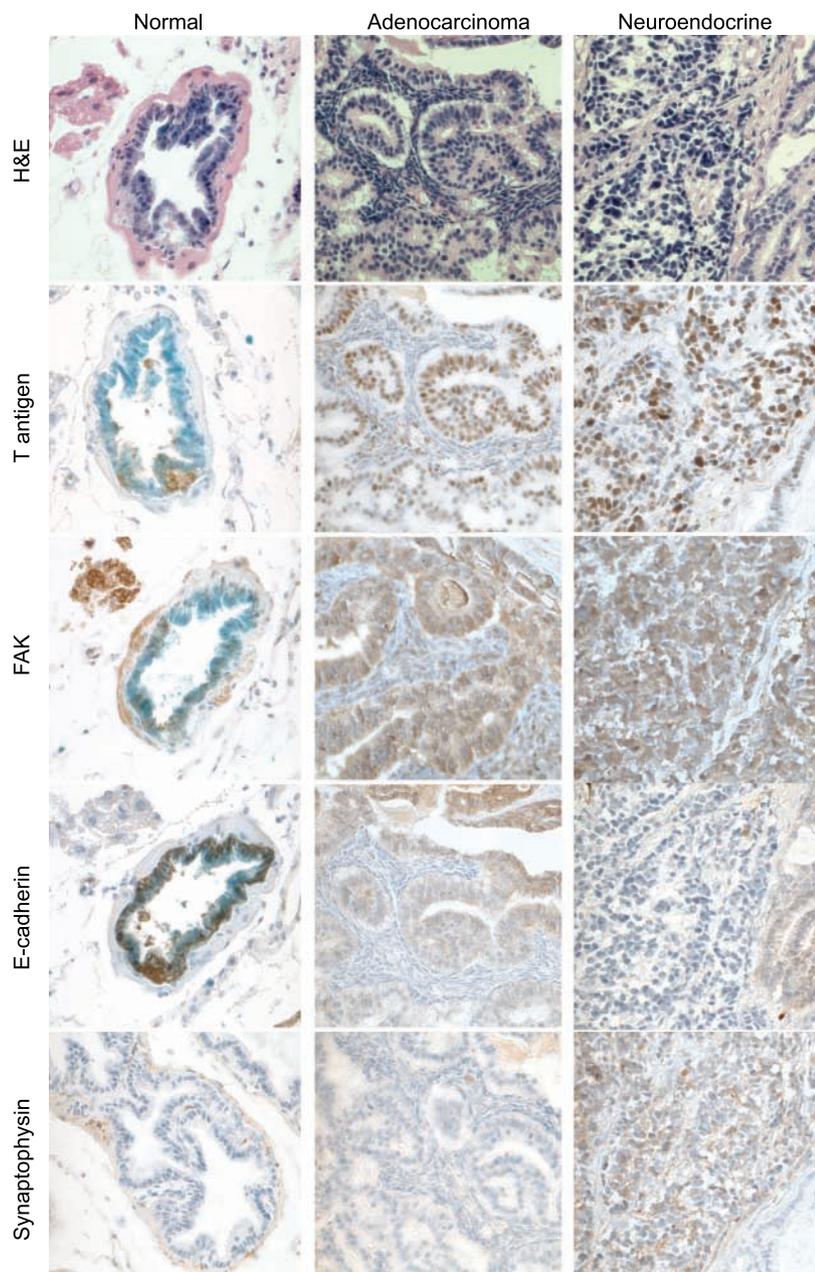


**Figure 3.** Immunohistochemical analysis of mouse prostates. Representative sections of TRAMP⁻ prostates (*Normal*) or TRAMP⁺ adenocarcinoma and neuroendocrine tumors were stained with H&E to reveal tissue architecture. Sections were stained for T antigen, FAK, E-cadherin, and synaptophysin as indicated.

**Figure 4.** Incidence of Cre-mediated recombination in mouse prostate tumors. **A,** genomic DNA isolated from prostate tumors [*P*; adenocarcinoma (*left*) or neuroendocrine (*right*)] or kidney (*K*) was subjected to PCR amplification of the *FAK* locus to identify floxed (1,800 bp) and recombined FAK (300 bp). **B,** Western blot analysis was done on protein isolated from prostates of Pb-Cre⁺ mice. Lysates were blotted for β-galactosidase (*β-gal*), total FAK, or actin (loading control). TRAMP and FAKᶠˡ/ᶠˡ status is indicated. *N*, normal tissue.
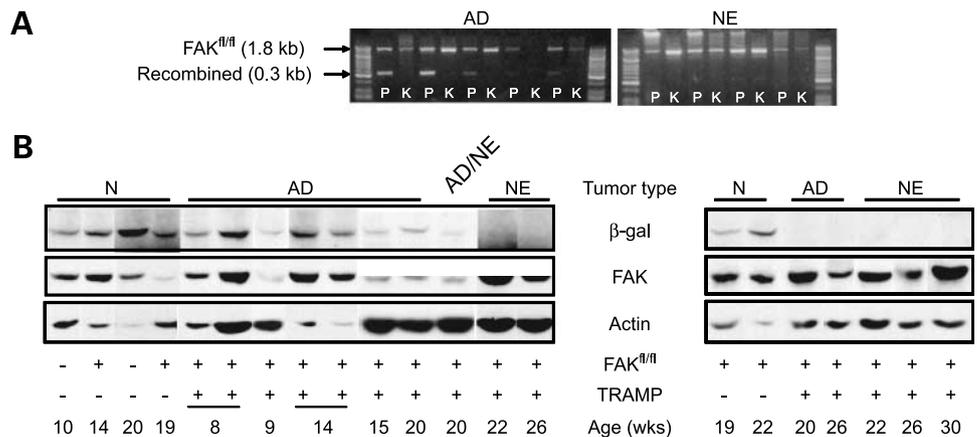


of early intraepithelial lesions. In contrast, all neuroendocrine tumors expressed FAK and seemed to have escaped Cre-mediated recombination at both the *FAK* and the *Rosa26-LacZ* loci. These observations suggest a role for FAK function in progression to the more aggressive neuroendocrine tumors and a less important role in development of early *in situ* lesions. It is possible that the early progenitor cells that give rise to neuroendocrine tumors become transformed by expression of T-antigen but fail to express Cre, thus accounting for the observed expression of FAK and the lack of expression of β-galactosidase. To test this possibility, we used the pharmacologic inhibitor of FAK, PF-562,271, and found that this inhibitor significantly limited the outgrowth of neuroendocrine tumors following castration of mice at 15 weeks of age. These data suggest that integrin signaling through FAK is an important component of cancer progression in the TRAMP model and that treatment

modalities targeting FAK should be considered as a therapeutic strategy for patients with castrate-resistant cancer.

The prostate epithelium in the mouse is composed of luminal, basal, and neuroendocrine cells (12). Recent studies suggest that in the TRAMP model, neuroendocrine carcinomas arise independently from atypical hyperplasias/adenocarcinomas or other epithelial lesions in a population of bipotential progenitor cells that express both markers of epithelial (E-cadherin) and neuroendocrine (synaptophysin) lineages (13). In our studies, we observed that neuroendocrine tumors expressed T antigen and synaptophysin, but were deficient in the expression of E-cadherin. Neuroendocrine tumors from Pb-Cre⁺ mice expressed FAK and lacked β-galactosidase expression, indicating that Cre-mediated recombination had failed to take place in these tumor cells. These observations would suggest that oncogenic transformation and/or progression of tumors

**Figure 5.** Pharmacologic inhibition of FAK inhibits growth of castrate-resistant tumors. **A,** schematic representation of prostate cancer onset in the TRAMP model and the treatment strategy. Mice were castrated at 16 wk of age and treated with PF-562,271 for 4 wk starting 5 d after castration. **B,** percentage of mice with prostate tumors after castration and treatment with PF-562,271 (*PF-271*; 10 mice) or vehicle (9 mice). TRAMP⁻ (*Normal*) mice were castrated and treated with PF-562,271 (10 mice) or vehicle. **C,** the weight of prostates, including tumor if present, was determined from TRAMP⁻ mice, those castrated and treated with PF-271, or mice castrated and treated with vehicle. Statistical significance was determined using the Kruskal-Wallis test followed by Dunn's multiple comparison post-test. *, $P < 0.05$, compared with normal; **, $P < 0.05$, compared with vehicle.
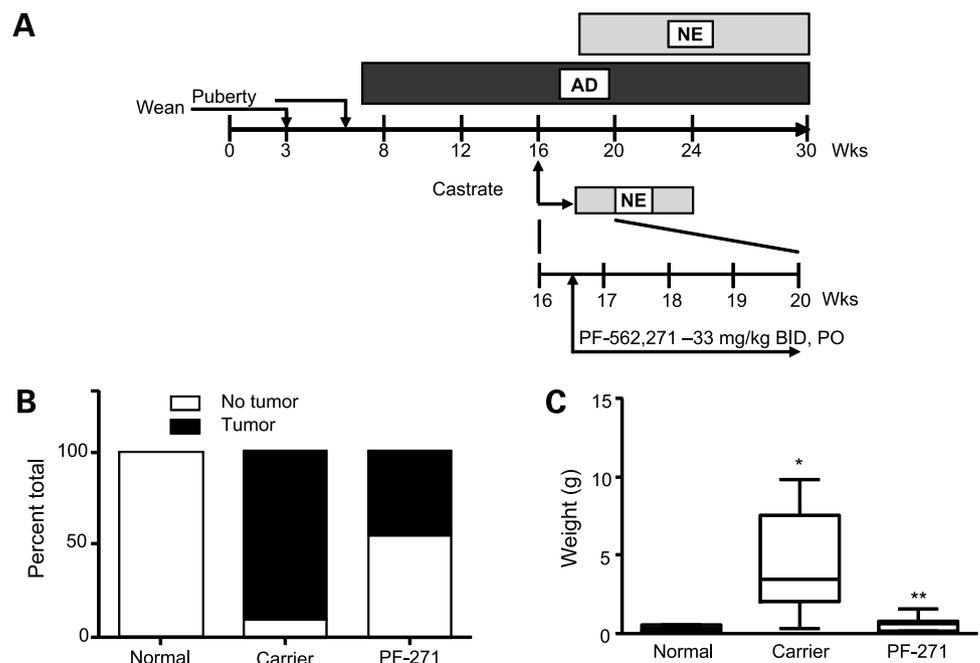
with the neuroendocrine phenotype occurs only in mice that have escaped FAK deletion and, hence, have FAK activity. In contrast, the oncogenic transformation and outgrowth of epithelial cells that give rise to the atypical hyperplastic phenotype characteristic of early adenocarcinomas do not seem to be influenced by the loss of FAK expression. This is consistent with our observations that loss of FAK expression in the normal prostate does not seem to influence normal glandular structure or function. Thus, maintenance of the epithelial character of early T-antigen–induced hyperplasia seems to bypass the requirement for FAK signaling. It is possible that in these glandular structures, PYK2 may compensate for the loss of FAK expression, although we observe no clear increase in PYK2 expression in prostates of FAK-deficient mice (data not shown).

Androgen seems to play an important role in regulating the progression of cancer in the TRAMP models, mimicking to some degree its role in human cancers. Maintenance of T-antigen–induced adenocarcinoma requires androgen because castration results in the loss of these lesions (14). The relative synchronous outgrowth of neuroendocrine tumors following castration indicates their androgen-independent progression. At this time, it is not clear whether FAK is required for the growth of neuroendocrine tumors or is important for some aspect of transdifferentiation of epithelial cell progenitors to the neuroendocrine tumor phenotype. The treatment of TRAMP mice with PF-562,271 for 10 to 14 weeks did not block the appearance of adenocarcinomas, consistent with the genetic ablation studies.

Genetic approaches using Cre-mediated recombination to delete FAK have been used to assess the role of FAK in several cancers (19–22). Targeted disruption of FAK in the mammary epithelium impairs mammary tumor development in the mouse mammary tumor virus-polyoma virus middle T-antigen mouse tumor model. In these studies, FAK expression was required for the transition of premalignant hyperplasias to carcinomas and their subsequent metastases. Mice that lacked functional FAK expression developed mammary tumors, yet there was a decrease in the number of hyperplasic lesions. Interestingly, the late-stage tumors and lung metastases arising in homozygously floxed FAK mice did not exhibit Cre-mediated recombination, consistent with a role for FAK function in mammary tumor progression in this model. Similar observations have been reported by Provenazano et al. (20). In these studies, FAK was not required for tumor initiation but was required for tumor progression. Interestingly, late-stage tumors that lacked FAK did not show evidence of invasion, suggesting that FAK is required for progression to the infiltrative phenotype. A role for FAK has been confirmed in breast cancer models driven with other oncogenes. Pylayeva et al. (21) showed that on silencing of FAK in mouse mammary tumor cells transformed by activated Ras, cells became senescent and lost their invasive ability. Loss of FAK expression in *Neu*-transformed cells induced growth arrest and apoptosis, albeit more efficiently if integrin $\beta_4$–dependent signaling was also inactivated. These observations in mouse breast cancer models parallel the observations in previous reports showing that FAK deletion in papillomas blocks conversion to squamous cell carcinomas (22) and are consistent with the finding described above.

The preclinical data indicating a role for FAK in breast and prostate cancers, as well as preclinical data with PF-562,271, suggest that FAK inhibitors may prove efficacious in the clinical setting. Preliminary reports of data from a phase I clinical trial provide evidence that PF-562,271 is well tolerated in cancer patients on extended p.o. administration, with some patients showing durable stable disease (23). Future trials in breast and prostate patients will provide new information about the effectiveness of FAK inhibitors for treating late-stage cancers.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### References

1. Langley RR, Fidler IJ. Tumor cell-organ microenvironment interactions in the pathogenesis of cancer metastasis. Endocr Rev 2007;28:297–321.

2. Radisky DC, Bissell MJ. Cancer. Respect thy neighbor!. Science 2004; 303:775–7.

3. Fidler IJ, Kim SJ, Langley RR. The role of the organ microenvironment in the biology and therapy of cancer metastasis. J Cell Biochem 2007; 101:927–36.

4. Parsons JT, Slack-Davis J, Tilghman R, Roberts WG. Focal adhesion kinase: targeting adhesion signaling pathways for therapeutic intervention. Clin Cancer Res 2008;14:627–32.

5. Lacoste J, Aprikian AG, Chevalier S. Focal adhesion kinase is required for bombesin-induced prostate cancer cell motility. Mol Cell Endocrinol 2005;235:51–61.

6. Slack JK, Adams RB, Rovin JD, Bissonette EA, Stoker CE, Parsons JT. Alterations in the focal adhesion kinase/Src signal transduction pathway correlate with increased migratory capacity of prostate carcinoma cells. Oncogene 2001;20:1152–63.

7. Rovin JD, Frierson HF, Jr., Ledinh W, Parsons JT, Adams RB. Expression of focal adhesion kinase in normal and pathologic human prostate tissues. Prostate 2002;53:124–32.

8. Slack-Davis JK, Eblen ST, Zecevic M, et al. PAK1 phosphorylation of MEK1 regulates fibronectin-stimulated MAPK activation. J Cell Biol 2003;162:281–91.

9. Roberts WG, Ung E, Whalen P, et al. Antitumor activity and pharmacology of a selective focal adhesion kinase inhibitor, PF-562,271. Cancer Res 2008;68:1935–44.

10. Slack-Davis JK, Martin KH, Tilghman RW, et al. Cellular characterization of a novel focal adhesion kinase inhibitor. J Biol Chem 2007;282: 14845–52.

11. Greenberg NM, DeMayo F, Finegold MJ, et al. Prostate cancer in a transgenic mouse. Proc Natl Acad Sci U S A 1995;92:3439–43.

12. Kaplan-Lefko PJ, Chen TM, Ittmann MM, et al. Pathobiology of autochthonous prostate cancer in a pre-clinical transgenic mouse model. Prostate 2003;55:219–37.

13. Chiaverotti T, Couto SS, Donjacour A, et al. Dissociation of epithelial and neuroendocrine carcinoma lineages in the transgenic adenocarcinoma of mouse prostate model of prostate cancer. Am J Pathol 2008;172: 236–46.

14. Huss WJ, Gray DR, Tavakoli K, et al. Origin of androgen-insensitive

poorly differentiated tumors in the transgenic adenocarcinoma of mouse prostate model. Neoplasia 2007;9:938–50.

**15.** Soriano P. Generalized lacZ expression with the ROSA26 Cre reporter strain. Nat Genet 1999;21:70–1.

**16.** Tilghman RW, Slack-Davis JK, Sergina N, et al. Focal adhesion kinase is required for the spatial organization of the leading edge in migrating cells. J Cell Sci 2005;118:2613–23.

**17.** Wu X, Wu J, Huang J, et al. Generation of a prostate epithelial cell-specific Cre transgenic mouse model for tissue-specific gene ablation. Mech Dev 2001;101:61–9.

**18.** Johnson MA, Iversen P, Schwier P, et al. Castration triggers growth of previously static androgen-independent lesions in the transgenic adeno-carcinoma of the mouse prostate (TRAMP) model. Prostate 2005;62: 322–38.

**19.** Lahlou H, Sanguin-Gendreau V, Zuo D, et al. Mammary epithelial-specific disruption of the focal adhesion kinase blocks mammary tumor progression. Proc Natl Acad Sci U S A 2007;104:20302–7.

**20.** Provenzano PP, Inman DR, Eliceiri KW, Beggs HE, Keely PJ. Mammary epithelial-specific disruption of focal adhesion kinase retards tumor forma-tion and metastasis in a transgenic mouse model of human breast cancer. Am J Pathol 2008;173:1551–65.

**21.** Pylayeva Y, Gillen KM, Gerald W, Beggs HE, Reichardt LF, Giancotti FG. Ras- and PI3K-dependent breast tumorigenesis in mice and humans requires focal adhesion kinase signaling. J Clin Invest 2009;119:252–66.

**22.** McLean GW, Brown K, Arbuckle MI, et al. Decreased focal adhesion kinase suppresses papilloma formation during experimental mouse skin carcinogenesis. Cancer Res 2001;61:8385–9.

**23.** Siu LL, Burris HA, Mileshkin L, et al. 2007 ASCO Annual Meeting Pro-ceedings Part I. J Clin Oncol 2007, Vol 25, No. 18S (June 20 Supple-ment): 3527.

# Molecular Cancer Therapeutics

## Differential requirement for focal adhesion kinase signaling in cancer progression in the transgenic adenocarcinoma of mouse prostate model

Jill K. Slack-Davis, E. Daniel Hershey, Dan Theodorescu, et al.

| | |
|---|---|
| **Updated version** | Access the most recent version of this article at:<br>doi:10.1158/1535-7163.MCT-09-0262 |

| | |
|---|---|
| **Cited articles** | This article cites 22 articles, 9 of which you can access for free at:<br>http://mct.aacrjournals.org/content/8/8/2470.full#ref-list-1 |
| **Citing articles** | This article has been cited by 5 HighWire-hosted articles. Access the articles at:<br>http://mct.aacrjournals.org/content/8/8/2470.full#related-urls |

| | |
|---|---|
| **E-mail alerts** | Sign up to receive free email-alerts related to this article or journal. |
| **Reprints and Subscriptions** | To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org. |
| **Permissions** | To request permission to re-use all or part of this article, use this link<br>http://mct.aacrjournals.org/content/8/8/2470.<br>Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC)<br>Rightslink site. |