Differential transformation capacity of Src family kinases during the initiation of prostate cancer

Houjian Cai\textsuperscript{a}, Daniel A. Smith\textsuperscript{b}, Sanaz Memarzadeh\textsuperscript{c,d}, Clifford A. Lowell\textsuperscript{e}, Jonathan A. Cooper\textsuperscript{f}, and Owen N. Witte\textsuperscript{a,d,g,1}

\textsuperscript{a}Department of Microbiology, Immunology, and Molecular Genetics, \textsuperscript{b}Molecular Biology Institute, \textsuperscript{c}Department of Obstetrics and Gynecology, and The David Geffen School of Medicine, \textsuperscript{d}El and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, and \textsuperscript{g}The Howard Hughes Medical Institute, University of California, Los Angeles, CA 90095; \textsuperscript{e}Department of Laboratory Medicine, University of California, San Francisco, CA 94143; and \textsuperscript{f}Fred Hutchinson Cancer Research Center, Seattle, WA 98109

Contributed by Owen N. Witte, March 10, 2011 (sent for review January 27, 2011)

Src family kinases (SFKs) are pleiotropic activators that are responsible for integrating signal transduction for multiple receptors that regulate cellular proliferation, invasion, and metastasis in a variety of human cancers. Independent groups have identified increased expression of individual SFK members during prostate cancer progression, raising the question of whether SFKs display functional equivalence. Here, we show that Src kinase, followed by Fyn kinase and then Lyn kinase, exhibit ranked tumorigenic potential during both paracrine-induced and cell-autonomous–initiated prostate cancer. This quantitative variation in transformation potential appears to be regulated in part by posttranslational modification of Src family kinases with respect to prostate tumorigenesis. We demonstrate that individual SFK members are differentially used during FGF10-induced prostate cancer. Epithelial deficiency of Src kinase blocks FGF10-induced tumorigenesis and diminishes the heightened expression of epithelial AR normally associated with paracrine FGF10 signaling, whereas knockout of Lyn kinase partially inhibits transformation, and loss of Lyn kinase had no effect. We further demonstrate that SFKs have distinct roles in cell-autonomous initiation of prostate cancer. Ectopic expression of constitutively activated Src, Fyn, and Lyn kinases exhibit differential capacities for transformation of prostate epithelium. Src kinase presents the strongest oncogenic phenotype, followed by Fyn and then Lyn. Palmitoylation plays an essential role in mediating the distinct functions of Src and Lyn kinases with respect to prostate tumorigenesis. Gain of palmitoylation in Src kinase inhibits tumorigenesis induced by constitutively active Src kinase, whereas loss of palmitoylation of Fyn, but not Lyn kinase accelerated tumorigenesis. These data collectively demonstrate that SFK members exhibit distinct intracellular functions and differential response to paracrine signals in the initiation of prostate cancer.

Results

Selective Loss of SFKs Inhibits Paracrine FGF10-Induced Prostatic Intraepithelial Neoplasia (PIN) and Carcinoma. Aberrant paracrine signaling from the tumor microenvironment can act as a driving factor in tumorigenesis (16). FGF/fibroblast growth factor receptor (FGFR) paracrine signaling is one of many important pathways in the initiation of numerous cancers (17, 18). We have previously shown that chronic exposure to paracrine FGF10 leads to murine PIN (mPIN) and adenocarcinoma (14) with lesions exhibiting enhanced levels of phosphorylated SFK protein.

Author contributions: H.C. and O.N.W. designed research; H.C. and S.M. performed research; H.C., D.A.S., C.A.L., J.A.C., and O.N.W. analyzed data; and H.C., D.A.S., and O.N.W. wrote the paper.

The authors declare no conflict of interest.

Freely available online through the PNAS open access option.

1To whom correspondence should be addressed. E-mail: owenwitte@mednet.ucla.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1103904108/-/DCSupplemental.

www.pnas.org/cgi/doi/10.1073/pnas.1103904108
and CK5+ basal cells and exhibited typical AR expression patterns in murine prostate tissue constrained with Src, Fyn, and Lyn (Fig. S1A). This evidence, combined with studies reporting that Src kinase can mediate FGF signaling with analogous sites in SFK members, indicates functional utilization of SFK members in FGF10-induced transformation.

To assess differential utilization of SFK members during paracrine-induced prostate adenocarcinoma, we used mice bearing targeted knockouts of Src, Fyn, or Lyn. Prostate epithelium from Src−/−, Fyn−/−, Fyn−/−, or Lyn−/− knockout mice and wild-type (WT) littermates were combined with FGF10- or GFP-transduced urogenital sinus mesenchymal cells (FGF10-UGSM or GFP-UGSM). In a normal prostate regeneration system, UGSM provides an inductive environment for the regeneration of prostate tissues from knockout mice combined with GFP-UGSM. GFP-UGSM displayed normal prostate tubules containing CK8+ luminal cells and CK5+ basal cells (Fig. 1A) and exhibited well-differentiated prostate adenocarcinoma, characterized by maintenance of CK5+ cells (Fig. 1B). Similar to WT epithelium, prostate tissues regenerated from Lyn−/− epithelium combined with FGF10-UGSM displayed neoplastic growth (Fig. 1C and D). Tissues from Fyn−/− epithelial cells with FGF10-UGSM primarily exhibited mPIN lesions, characterized by an expansion of the CK8+ luminal population with maintenance of CK5+ cells (Fig. 1C and D). In striking contrast, regenerated tissues from Src−/−, Fyn−/− epithelium combined with FGF10-UGSM presented normal histology, indicated by normal glandular structures with CK8+ luminal and CK5+ basal epithelial layers (Fig. 1C and D). Collectively, our results indicate that the oncogenic effects of FGF10 are largely mediated by Src and Fyn kinases in prostatic epithelium.

Selective Loss of SFKs Leads to a Diminution of Epithelial AR in Response to Paracrine FGF10. Our previous study identified that the expression of epithelial AR increases in response to paracrine FGF10 signaling (14). To investigate modulation of AR expression in the context of paracrine FGF10 and selective loss of SFK members, we examined the expression of AR in regenerated tissues from SFK knockout tissue by immunohistochemistry (IHC). Expression of AR in grafts derived from Src−/−, Fyn−/−, Lyn−/− epithelial cells with control UGSM was similar to grafts derived from WT littermates (Fig. S3). Selective loss of Lyn did not alter the expression pattern of AR and cyclin D1 in FGF10 grafts compared with WT prostate epithelia, whereas expression of AR and cyclin D1 was decreased to a lesser extent in tissues regenerated from Fyn−/− epithelium (Fig. 2). In contrast, the epithelial expression of AR and cyclin D1 was downregulated in tissues regenerated from Src−/−, Fyn−/− epithelium combined with FGF10-UGSM cells compared with WT prostate epithelia. Collectively, the data indicate that loss of Src kinase,
and to a certain extent loss of Fyn but not Lyn kinase, modulate expression of AR and cyclin D1 in response to paracrine FGF10.

Inhibition of Src Family Kinase Signaling by a Dominant Negative Src Kinase Mutant Attenuates FGF10-Induced Adenocarcinoma. To support that Src family kinases mediate FGF10 signaling, we blocked SFK signaling by ectopic expression of Src(Y529F/K298M), an open conformation, kinase dead mutant of Src kinase (21). Dissociated prostate epithelial cells were transduced with either control vector or Src(Y529F/K298M) and combined with FGF10-UGSM cells. Both control and Src(Y529F/K298M) vectors contained an RFP fluorescent reporter driven by a separate CMV promoter. Tubules infected with control vector exhibited FGF10-induced adenocarcinoma (Fig. 3), with transformed tubules presenting increased AR expression. In contrast, tubules infected with dominant negative Src kinase were phenotypically normal and expressed low amounts of AR compared with neighboring RFP negative tubules (Fig. 3). As the dominant negative Src kinase could inhibit signaling through multiple SFK members, these data do not indicate hierarchical significance. However, they do strongly support a role for SFK signaling, mediating transformation and increased epithelial AR expression in response to chronic FGF10 signaling.

Constitutively Active SFK Members Exhibit Differential Oncogenic Potential in Primary Prostate Cells. Our results indicate that SFK members are not functionally equivalent in the context of paracrine-induced carcinoma; therefore, we asked whether this pattern was conserved in cell-autonomous transformation by SFK members. Independent laboratories have reported increased expression and activation of wild-type Src, Fyn, and Lyn with prostate cancer progression (22–24). We generated lentiviruses bearing constitutively active Src(Y529F), Fyn(Y528F) or Lyn(Y508F) kinase with an RFP reporter (Fig. 4A) and confirmed expression by Western analysis (Fig. S1B). Although rarely observed in human cancers, constitutively active mutants phenocopy the synergy of c-Src and AR in prostate cancer and chronic SFK activation by signal transduction pathways (13).

To assess differential cell-autonomous transformation in primary cells, dissociated prostate epithelial cells were transduced with Src(Y529F), Fyn(Y528F), or Lyn(Y508F) kinase and combined with WT UGSM. Src(Y529F) tumors lacked glandular structure and were predominantly CK8+ luminal cells, characteristic of poorly differentiated invasive adenocarcinoma (Fig. 4B). Tubules overexpressing Fyn(Y528F) exhibited mPIN lesions with stratified layers of CK8+ luminal cells (Fig. 4B). In contrast, overexpression of Lyn(Y508F) resulted in phenotypically normal regeneration (Fig. 4B). Collectively, these in vivo results clearly demonstrate that cell-autonomous expression of constitutively active SFKs in naive adult prostate epithelium results in dramatically different phenotypes.

Alteration of Palmitoylation Sites Change Oncogenic Potential of Constitutively Active Src and Fyn Kinases in Prostate Cancer. To investigate potential mechanisms for the observed differences in transformation between SFK members, we asked whether alteration of the palmitoylation status of SFK members modulates transformation capacity. Segregation of SFK members into lipid rafts by palmitoylation could further enhance preferential interactions with receptors and determine functional specificity (25, 26). To assess the role of palmitoylation of SFKs in determining oncogenic potential, wild-type and constitutively active Src and Fyn kinases, were respectively mutated at predicted palmitoylation sites (Fig. 5A) (25). We transduced Src−/−Yes−/− Fyn−/− (SYF) fibroblasts with control or palmitoylation mutant
Src and Fyn kinases and assessed in vitro colony formation in soft agar. Compared with controls, the Src(S3C/S6C) or Src(Y529F/S3C/S6C) palmitoylation mutants exhibited reduced colony formation and attenuated Src activation, whereas Fyn(C3S/C6S) or Fyn(Y528F/C3S/C6S) palmitoylation mutants exhibited dramatically increased colony formation or size of colony (Fig. S4). In addition, gain of palmitoylation sites in Src(S3C/S6C) or Src(Y529F/S3C/S6C) mutants attenuated expression of phospho-Src kinase (Fig. S4).

We then assessed transformation activity of Src palmitoylation mutants in the prostate regeneration assay. Src(Y529F) grafts displayed solid tumors without glandular structure (Fig. 5B) and exhibited primarily CK8+ but not CK5+ cells, both E-cadherin and vimentin expression, elevated phospho-Src(Y416), and phosphotyrosine. Additionally, both Src(Y529F/S3C/S6C) and Src(Y529F) regenerations displayed similar levels of total Src kinase (Fig. 5C). Finally, Src(Y529F) grafts also exhibited increased expression of phospho-ERK and phospho-FAK, but not Cbp and phospho-AR, compared with Src(Y529F/S3C/S6C) tissue (Fig. 5B).

We next examined whether loss of predicted palmitoylation sites in Src kinase would likewise alter prostate transformation efficiency in vivo. In contrast to the mPIN lesions induced by Fyn(Y528F), lesions induced by Fyn(Y529F/C3S/C6S) presented as poorly differentiated invasive carcinoma and resembled transformation by Src(Y529F) (Fig. 5B and C). The transformed tissues exhibited CK8+ but not CK5+ cells, vimentin but not E-cadherin expression, and highly elevated levels of pSrc(Y416) and phosphotyrosine (Fig. 5C). Fyn expression was assessed using a Src kinase antibody that exhibits cross-reactivity for other SFK members. The total Fyn expression was elevated in Fyn(Y529F/C3S/C6S)-transformed tissues compared with Fyn(Y529F) (Fig. 5C). In addition to changing how Fyn is trafficked within the cell, Fyn palmitoylation mutants could also exhibit higher stability, leading to more efficient expression (27, 28).

Additionally, the expression of phospho-FAK was increased in Fyn(Y529F/C3S/C6S)-transformed tissue, but not the expression of Cbp, phospho-ERK, and phospho-AR (Fig. S5). Finally, expression of Lyn(Y508F) loss-of-palmitoylation mutants resulted in phenotypically normal regenerations (Fig. S6). Collectively, our studies suggest that palmitoylation modification of the SH4 domain modulates tumorigenic potential of constitutively active Src and Fyn kinases by regulating downstream signaling.

**Discussion**

Despite separate lines of evidence that indicate Src, Fyn, and Lyn kinases are each up-regulated in prostate cancer (22–24), our findings indicate that (i) individual SFK members differently mediate paracrine FGF10 signal transduction and transformation and (ii) exhibit differential capacity for cell-autonomous transformation. SFKs have been considered as potential drug targets in prostate cancer. Dasatinib (Sprycel; Bristol Myers-Squibb), saracatinib (formerly AZD0530; AstraZeneca), and bosutinib (previously SKI-606; Wyeth) represent three inhibitors of Src kinase being used in the clinical trials (3). Dasatinib has high affinity for Src and BCR/ABL, but also targets other SFK members, c-KIT, PDGFR, and ephrin A2. Similarly, saracatinib can effectively inhibit Src and other SFK members with activity against ABL and activated mutant forms of EGF receptor, whereas bosutinib is a dual Src/ABL kinase inhibitor that also targets other SFK members without inhibition of KIT or PDGFR (3). Although these inhibitors exhibit clinical efficacy, reports have identified toxic effects, including centrosomal and mitotic spindle defects to normal cells, reduced tubular secretion of creatinine, and cardiac toxicity (4, 29, 30). Several adverse clinical symptoms such as renal failure, nausea, fatigue, lethargy, anorexia, proteinuria, vomiting, and diarrhea are also associated with treatment (3). Although the mechanisms leading to these adverse symptoms are unknown, given the functional differences of SFKs observed in our study, it becomes prudent to investigate whether selective, rather than broad, inhibition of SFKs could represent an effective treatment strategy and potentially reduce adverse effects.

The transformation capacity of SFK members is directly related to their differential localization within plasma membrane micro-
domains, which is determined in part by N-terminal lipid modification (25, 31). With respect to Src kinase, activity is seemingly dependent upon its distribution between plasma membrane microdomains that sequester inhibitory factors and substrate access outside of these domains (26). By enhancing the association of Src kinase with hydrophobic microdomains by artificial palmitoylation, its oncogenic activity is likely inhibited by endogenous regulatory mechanisms (26, 31). In contrast, loss of palmitoylation mutation in Fyn kinase results in gain of function that phenocopies regulatory mechanisms (26, 31). In contrast, loss of palmitoylation, its oncogenic activity is likely inhibited by endogenous regulatory mechanisms (26, 31).

Supporting this hypothesis, enhancement of FGF10/FGFR signaling can modulate cell-autonomous transformation activity, it remains to be seen whether this plays a role in the human disease.

Our results support previous studies that FGF10-induced prostate adenocarcinoma exhibits elevated AR expression in epithelial cells (14). Over 80% of castration-resistant prostate cancer cases express high levels of AR and androgen-responsive genes (35). Our study suggests that specific SFK members are critical in mediating FGF10-induced transformation and the subsequent increase in AR expression, offering an in vivo mechanism linking FGF10 signal transduction and AR expression. Supporting a role for SFK members in modulating AR expression, a study by DaSilva et al. (36) identified that stabilization of AR by parathyroid hormone-related protein (PTHrP)/EGFR signaling is mediated by Src kinase. In our study, epithelial loss of Src kinase presented the greatest inhibitory effect on transformation and up-regulation of AR, indicating the greatest functional significance. Supporting this hypothesis, enhancement of FGF10/FGFR→Src kinase→AR signaling pathway by coexpression of wild-type Src kinase and AR in prostate epithelium results in a potent synergistic transformation phenotype (13). Collectively, these results imply that targeting this signaling pathway represents an important route for treating prostate tumorigenesis.

Materials and Methods

Plasmids. Control FUCGW and FGF10-FUCGW vectors were prepared as described (14). The ORFs of murine Src and Fyn kinases were amplified by PCR from cDNA of mouse spleen or thymus using primer pairs of Src(F)-Gene and Src(R)-Gene, and Fyn(F)-Gene and Fyn(R)-Gene, respectively. The ORF of human Lyn kinase was PCR amplified from a plasmid generated as described (37). PCR products were cloned into the Xba1 and EcoR1 sites of the FUCRW lentivector, in which RFP is constitutively expressed by the CMV promoter (Fig. 2A). Constitutively active Fyn and Lyn kinase mutants were generated by PCR amplification using primer pairs Fyn(F)-Gene/Fyn(Y529F)-R encoding for phenylalanine at residue 529. Constitutively active Fyn and Lyn kinase mutants were generated by PCR amplification using primer pairs Fyn(F)-Gene/Fyn(Y529F)-R and Lyn(F)-Gene/ Lyn(Y508F)-R with substituted nucleotides encoding phenylalanine at residue 529. Our results support previous studies that FGF10-induced prostate adenocarcinoma exhibits elevated AR expression in epithelial cells (14). Over 80% of castration-resistant prostate cancer cases express high levels of AR and androgen-responsive genes (35). Our study suggests that specific SFK members are critical in mediating FGF10-induced transformation and the subsequent increase in AR expression, offering an in vivo mechanism linking FGF10 signal transduction and AR expression. Supporting a role for SFK members in modulating AR expression, a study by DaSilva et al. (36) identified that stabilization of AR by parathyroid hormone-related protein (PTHrP)/EGFR signaling is mediated by Src kinase. In our study, epithelial loss of Src kinase presented the greatest inhibitory effect on transformation and up-regulation of AR, indicating the greatest functional significance. Supporting this hypothesis, enhancement of FGF10/FGFR→Src kinase→AR signaling pathway by coexpression of wild-type Src kinase and AR in prostate epithelium results in a potent synergistic transformation phenotype (13). Collectively, these results imply that targeting this signaling pathway represents an important route for treating prostate tumorigenesis.
dyes 528 and 508, respectively. The tyrosine-to-phospholipid mutation in 5Fk members allows adoption of an open conformation of the catalytic domain, leading to constitutive activation (38). Palmitoylation mutants of Src, Fyn, and Lyn kinases were generated by PCR amplification using primer pairs palm-Src-F5Fc(R)-Gene or Src(Y529F)-R, palm-Fyn-FYF5Fc(R)-Gene or Fyn(Y528F)-R, palm-lyn-F5Fc(Lyn)-Gene, or Lyn(Y508F)-R. Primer sequences are listed in Table S1 with underlined nucleotides indicating point mutations.

Mouse Strains. Mouse strains used in this study include: (i) Fynfi−/−, Fynfi+/− and wild-type littermates on a BL6/129S7 mixed genetic background and were purchased from Jackson Labs; and (ii) Lynfi−/− and wild-type littermates on a BL6/129S7 mixed genetic background and were purchased from Jackson Labs; and (iii) Lynfi−/−

Regeneration and Transduction of Prostate Epithelium. The regeneration process, lentivirus preparation, steering, and transduction of dissociated prostate cells were performed under University of California, Los Angeles safety regulations for lentivirus use as described previously (20). Lab animal housing, maintenance, and all surgical and experimental procedures were undertaken in compliance with the regulations of the Division of Laboratory Animal Medicine of the University of California, Los Angeles. Prostate regenerations were prepared as described (20). In brief, dissociated prostate cell suspensions were collected from 6- to 10-wk-old male mice. A total of 1–2 × 10^6 dissociated prostate cells were transduced with lentivirus carrying the gene of interest at a multiplicity of infection (MOI) of 50. Transduced cells were mixed with 1–2 × 10^5 UGSM cells and suspended in collagen. Grafts were implanted under the kidney capsule in SCID mice and allowed to regenerate for 6–8 wk.

Immunohistochemistry and Western Analysis. Following regeneration, hosts were killed and grafts were recovered via surgical resection of the kidney. Transilluminated and fluorescent images were taken using a dissecting microscope. Grafts were fixed in 10% buffered formalin overnight, embedded in paraffin, and sectioned at 4 μm. Sections were stained with hematoxylin and eosin (H&E) for representative histology. IHC stains were visualized using the Envision+ system (Dako). Primary antibodies for Src kinase (1:250; Cell Signaling), phospho-Src-family (Tyr416) (1:50; Cell Signaling), AR (1:200; Santa Cruz Biotechnology), phospho-AR (Ser213/210; 1:50; ImageX, Cpb(ab14989; 1:200; Abcam), phospho-FAK(ab4803; 1:150; Abcam), and phospho-Erk (4376; 1:50; Cell Signaling) were used. For immunofluorescence analysis, sections were incubated with primary antibodies against vimentin (1:250; Abcam), E-cadherin (1:250; BD Transduction Laboratories), C5 (Covance; 1:1,000), or ErbB2 (Cell Signaling; 1:1,000) and visualized by Alexa-488- or Alexa-594-conjugated secondary antibodies (Molecular Probes; 1:1,000). For biotinylated secondary antibodies, sections were incubated with FITC-conjugated streptavidin (Molecular Probes; 1:250). Sections were counterstained with DAPI (Vector Laboratories). Primary antibodies for phospho-Src family (Tyr416) (1:1,000; Cell Signaling), Erk2 (1:15,000; Santa Cruz Biotechnology) were used in Western analysis.

Acknowledgments. We thank Li Xin, Deanna Janzen, Yang Zong, Andrew Goldstein, Tanya Stoyanova, and Justin Drake for technical help and scientific discussions and Esther Jhingan and Moham M. Ansari for maintaining the Src knockout mice colony. We thank the Tissue Procurement Laboratory at University of California, Los Angeles (UCLA) for assistance on tissue processing and H&E staining. This work was supported by funds from the Army Medical Research and Material Command Grant W81XWH-08-1-0329 (to H.C.) and the Prostate Cancer Foundation (PCF) Challenge Award (to C.B.). A.C.A. and O.N.W. are supported by the PCF Young Investigators Award, a Building Interdisciplinary Research Careers in Women’s Health (BIRCWH) Grant (National Institutes of Health/National Institute of Child Health and Human Development S1 K12 HD001400), a UCLA Jonsson Comprehensive Cancer Center Foundation Seed Grant, The Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research Award, and the Scholars in Translational Medicine gift. D.A.S. is supported by the UCLA Tumor Biology Program Health and Human Services Ruth L. Kirschstein Institutional National Research Service Award T32 CA009056. O.N.W. is an investigator of the Howard Hughes Medical Institute.

Supporting Information

Cai et al. 10.1073/pnas.1103904108

SI Materials and Methods

Soft Agar Assay and Western Analysis of SFKs Expression. SYF fibroblast or 293T cells were transduced with either control or lentivirus carrying wild type, constitutively active, or their palmitoylation mutants of Src, Fyn, or Lyn. Transduced RFP+ cells were sorted by FACS (FACSVantage; BD Biosciences). The soft agar assay was performed according to the manufacturer-supplied protocol (Millipore; ECM570). Briefly, 5,000 cells from each condition were plated in 60-mm tissue culture dishes and incubated for 21 d. Colonies larger than 500 μm in diameter were counted and representative photos for each condition were taken.

For Western analysis, sorted SYF cells or 293T cells from each condition were cultured in DMEM supplemented with 10% FBS. At ~80% confluence, cells were harvested and protein lysates were prepared using lysis buffer (20 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 5% glycerol, 2% N-octyl-beta-D-glucoside) supplemented with phosphatase inhibitor mixture 1 and 2 (P5726 and P2850; Sigma). Expression of SFK members were assessed by Western analysis with ERK1/2 as a loading control. Primary antibodies for Src kinase (1:1,000; Cell Signaling), Fyn kinase (1:1,000; Cell Signaling), Lyn (1:1,000; Cell Signaling), phospho-Src family (Tyr416) (1:1,000; Cell Signaling), and Erk2 (1:5,000; Santa Cruz Biotechnology) were used in Western analysis.

Fig. S1. Expression analysis of SFK members Src, Fyn, and Lyn in primary cells, cell lines, and transduced cells. (A) SFKs are expressed in normal murine prostate tissues. Src, Fyn, and Lyn kinase expression patterns in normal murine prostate tissue, human prostate cancer cell lines LNCap and PC3, and murine spleen (control) were determined by Western analysis. (B) Transduction into 293T cells leads to robust expression of Src, Fyn, and Lyn kinases. To confirm over-expression, 293T cells were transduced with lentivirus bearing Src, Fyn, or Lyn kinase. Lysates were prepared 48 h posttransduction and expression was detected by Western analysis.

Fig. S2. Prostate epithelial cells from inbred (BL6), mixed genetic background (BL6/129S7), and Fyn+/− (BL6/129S7) mice respond similarly to control GFP-UGSM and paracrine FGF10-UGSM. Histological features show normal glandular structure in controls with development of multifocal adenocarcinoma in response to FGF10.

Cai et al. www.pnas.org/cgi/content/short/1103904108
**Fig. S3.** Loss of SFK members Src, Fyn, or Lyn in prostate epithelium does not alter AR expression in grafts regenerated with normal UGSM. AR expression was determined by IHC analysis of regenerated tissues derived from primary prostate cells of WT, Src\(^{-/-}\)/Fyn\(^{+/+}\), Fyn\(^{-/-}\), and Lyn\(^{-/-}\) combined with GFP-UGSM.

**Fig. S4.** Mutation of palmitoylation sites modulates transformation potential of Src and Fyn kinases in vitro. (A and C) SYF cells were transduced with Src, Src\((Y529F)\), or palmitoylation mutants (A); Fyn, Fyn\((Y528F)\), or palmitoylation mutants (C). Only colonies above 500 \(\mu\)m in diameter were counted. Whereas the difference in colony number between Fyn\((Y528F)\) and Fyn\((Y528F/C3S/C6S)\) was not significant, the percentage of large colonies (>1,000 \(\mu\)m) in Fyn\((Y528F/C3S/C6S)\) was significantly higher than Fyn\((Y528F)\) (C, Insert). (B and D) Representative images of colonies formed by SYF cells overexpressing Src and Fyn kinases or palmitoylation mutants. Colonies in the Src\((Y529F/C3S/C6S)\) or Src\((C3S/C6S)\) conditions were smaller than respective Src\((Y529F)\) or Src. Colony size was not significantly different between Fyn and Fyn\((C3S/C6S)\). (E) Western analysis of Src kinase, phospho-Src, and Erk1/2 loading control in SYF cells overexpressing Src kinase or palmitoylation mutant derivatives.
Fig. S5. Expression of phospho-FAK, phospho-ERK, phospho-AR, and Cbp in constitutively active Src and Fyn palmitoylation mutants. IHC staining of phospho-FAK, phospho-ERK, phospho-AR, and Cbp in regenerated tissues derived from primary prostate cells infected with Src(Y529F), Src(Y529F/S3C/S6C), Fyn(Y528F), and Fyn(Y528F/C3S/C6S). (Scale bar, 100 μm.)

Fig. S6. Loss of palmitoylation at C3 of constitutively active Lyn kinase, Lyn(Y508F/C3S), does not modulate tumorigenic potential. (A) The cysteine 3 of Lyn (Y508F) was mutated to serine, preventing palmitoylation of constitutively active Lyn(Y508F) kinase. (B) Regenerated prostate tissues were derived from $2 \times 10^5$ prostate cells transduced with Lyn(Y508F) or Lyn(Y508F/C3S). (C) H&E staining (Low magnification bar, 800 μm; high magnification bar, 100 μm.), RFP signal, and IHC analysis of Lyn expression in regenerated tissues derived from primary prostate cells transduced with Lyn(Y508F) and Lyn(Y508F/C3S). The results indicate that loss of palmitoylation mutation in Lyn(Y508F) does not alter the histology. (Scale bar, 100 μm.)

Table S1. Primers used for cloning or mutagenesis of Src family kinase genes

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Primer name</th>
<th>Primer sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>palm-Src-F</td>
<td>5'-GTCGACTCTAGAAGGACCACGGATCAGTCAACAGGC-3'</td>
</tr>
<tr>
<td>Mouse</td>
<td>palm-Fyn-F</td>
<td>5'-GTCGACTCTAGATGGAATGCTAAAGATGTGCTGCA-3'</td>
</tr>
<tr>
<td>Mouse</td>
<td>palm-lyn-F</td>
<td>5'-CGTGGTACGCCTAAGGAAAGCTGCTGCA-3'</td>
</tr>
<tr>
<td>Mouse</td>
<td>Src(F)-Gene</td>
<td>5'-CATGAATTCTCACAGTCCCTATAGGTCTGCA-3'</td>
</tr>
<tr>
<td>Mouse</td>
<td>Src(R)-Gene</td>
<td>5'-CATGAATTCTCACAGTCCCTATAGGTCTGCA-3'</td>
</tr>
<tr>
<td>Human</td>
<td>Lyn(F)-Gene</td>
<td>5'-CATGAATTCTCACAGTCCCTATAGGTCTGCA-3'</td>
</tr>
<tr>
<td>Mouse</td>
<td>Src(Y529F)-F</td>
<td>5'-CCACGTAGCAGCAGTCCAGGGGGAGACTGCTGCA-3'</td>
</tr>
<tr>
<td>Mouse</td>
<td>Src(Y529F)-R</td>
<td>5'-GTACGTCCTAGAAGGACCACGGATCAGTCAACAGGC-3'</td>
</tr>
<tr>
<td>Mouse</td>
<td>Fyn(Y528F)-R</td>
<td>5'-GCATGAATTCTCACAGTCCCTATAGGTCTGCA-3'</td>
</tr>
<tr>
<td>Mouse</td>
<td>Lyn(Y508F)-R</td>
<td>5'-CATGAATTCTCACAGTCCCTATAGGTCTGCA-3'</td>
</tr>
</tbody>
</table>