An Identity Crisis for *fps/fes*: Oncogene or Tumor Suppressor?

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Abstract

Fps/Fes proteins were among the first members of the protein tyrosine kinase family to be characterized as dominant-acting oncoproteins. Addition of retroviral GAG sequences or other experimentally induced mutations activated the latent transforming potential of Fps/Fes. However, activating mutations in *fps/fes* had not been found in human tumors until recently, when mutational analysis of a panel of colorectal cancers identified four somatic mutations in sequences encoding the Fps/Fes kinase domain. Here, we report biochemical and theoretical structural analysis demonstrating that three of these mutations result in inactivation, not activation, of Fps/Fes, whereas the fourth mutation compromised *in vivo* activity. These results did not concur with a classic dominant-acting oncogenic role for *fps/fes* involving activating somatic mutations but instead raised the possibility that inactivating *fps/fes* mutations might promote tumor progression *in vivo*. Consistent with this, we observed that tumor onset in a mouse model of breast epithelial cancer occurred earlier in mice targeted with either null or kinase-inactivating *fps/fes* mutations. Furthermore, a *fps/fes* transgene restored normal tumor onset kinetics in targeted *fps/fes* null mice. These data suggest a novel and unexpected tumor suppressor role for Fps/Fes in epithelial cells. (Cancer Res 2005; 65(9): 3518-22)

Introduction

Fps/Fes (hereafter called Fps) and Fer are the only two members of a unique family of cytoplasmic protein tyrosine kinases (for recent reviews, see refs. 1, 2). Fps and Fer contain a central Src homology-2 (SH2) domain and a COOH-terminal tyrosine kinase catalytic domain. They are structurally distinguished from other members of cytoplasmic protein tyrosine kinase subfamilies by the presence of NH_2-terminal Fer/CIP4 homology and coiled-coil domains. *fps/fes* was originally identified as an oncogene from avian (*fps*) and feline (*fes*) retroviruses. The viral Fps (v-Fps) proteins encoded by these oncogenes were among the first members of the protein tyrosine kinase family to be characterized as dominant-acting oncoproteins. v-Fps proteins consist of NH_2-terminal GAG sequences fused to either the full-length cellular Fps protein or variants lacking portions of the coiled-coil domains. The viral GAG sequences confer unregulated tyrosine kinase activity that promotes cytokine-independent differentiation of hematopoietic progenitor cells and reduced growth factor requirements in transformed fibroblasts. When transgenically expressed under the control of a heterologous promoter, *v-fps* induced tumors in lymphoid and mesenchymal tissues in mice (3). Deletions and point mutations have also been shown to activate the latent transforming potential of cellular Fps (4). These observations were consistent with well-established correlations between activating mutations in tyrosine kinases and oncogenesis and suggested that activating somatic mutations in *fps* might someday be detected in human cancers. Recently, a mutational analysis of sequences encoding the catalytic domain of 89 tyrosine kinases in a panel of 182 human colorectal cancers revealed four somatic mutations in *fps*, and the authors speculated that these mutations might have activated the Fps kinase and thereby contributed to cancer (5). Here, we provide biochemical evidence that three of these four reported mutants are actually catalytically inactivated, whereas the fourth retained *in vitro* but lacked *in vivo* activity. At first, this biochemical analysis would seem to undermine the pathologic significance of these mutations; however, we also provide genetic evidence that targeted null or kinase-inactivating missense mutations in *fps* correlated with earlier tumor onset in a transgenic mouse model of breast cancer. Together, these observations suggest that inactivating mutations in *fps* could contribute to cancer and that Fps might have an unexpected tumor suppressor role.

Materials and Methods

Transgenic mice. Generation and genotyping of in-bred SVJ/129-CD1 hybrid lines harboring targeted null ($fps^{-/-}$), targeted catalytically inactive ($fps^{KR/KR}$), or transgenic (fps^{Tg}) *fps* alleles in a targeted *fps*-null genetic background ($fps^{-/-}$ fps^{Tg}) have been reported previously (6–8). Generation of in-bred transgenic mice with mouse mammary tumor virus-long terminal repeat–directed, mammary epithelial-specific expression of polyoma virus middle T antigen (PyVmT) has also been described (9). This PyVmT line develops multifocal mammary tumors with 100% penetrance in females. All mice were housed at the Animal Care Facility at Queen's University (Kingston, Ontario, Canada) and procedures were approved by the Queen's University Animal Care Committee in accordance with the regulations set forth by the Canadian Council on Animal Care.

Tumor measurements. Initial tumor onset in abdominal mammary gland was assessed daily by physical palpitation of hemizygous PyVmT progeny in the context of *fps* wild-type (*wt*), $fps^{-/-}$, and $fps^{-/-}$ fps^{Tg} genetic backgrounds. In a second independent series of experiments performed by a different investigator, initial tumor onset was assessed by physical palpitation in hemizygous PyVmT progeny in the context of *wt*, $fps^{KR/KR}$, and $fps^{-/-}$ genetic backgrounds. In this second series of experiments, tumor diameter was continually monitored after the initial onset to generate tumor growth profiles. These profiles were characterized by a dormant phase, which occurred postonset and which preceded emergence of a rapid phase of tumor growth. Estimates of the onset time of this rapid phase of growth were obtained from these profiles.

Biochemical analysis of mutant Fps proteins. PCR mutagenesis was used to generate *Eco*RI cDNA fragments encoding the human Fps SH2 and kinase domains corresponding to K588R (6), M704V, R706Q, V743M, and S759F mutants. Wild-type and mutant *fps* cDNA sequences were

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cloned into the bacterial expression plasmid pGEX-2T (Promega, Madison, WI). For expression in 293T cells, these sequences were substituted into the mammalian expression plasmids encoding full-length Fps with a COOH-terminal *myc* epitope tag (10). All constructs were confirmed by sequencing. Kinase reactions were done in kinase reaction buffer containing 375 μmol/L ATP and 125 μg/mL tubulin (Sigma-Aldrich, Oakville, Ontario, Canada) at 30°C for 20 minutes. Kinase autophosphorylation and phosphorylation of the exogenous substrate tubulin was assessed by Western blotting using a monoclonal anti-phosphotyrosine antibody (Santa Cruz Biotechnology, Santa Cruz, CA). No observable Fps anti-phosphotyrosine signal was apparent in the absence of ATP at the exposure times used to quantify kinase activities. Levels of Fps proteins were determined by immunoblotting with rabbit polyclonal antibody to Fps (for bacterial expression) or with a monoclonal anti-*myc* antibody (for mammalian expression), and tubulin levels were assessed by immunoblotting with a sheep polyclonal anti-tubulin antibody (Cytoskeleton, Inc., Denver, CO).

Structural modeling. A model of the Fps catalytic domain was generated by threading the human Fps sequence onto the solved crystal structure of the inactivated form of chicken Src determined at 2.35 Å (2 protein tyrosine kinases; ref. 11). The resulting models were energy minimized using GROMOS96 (12) and ribbon diagrams were generated using MOLSCRIPT (13).

Results and Discussion

Activating mutations in several tyrosine kinase-encoding genes have been detected in different human malignancies. *In vitro* cell transformation and *in vivo* retroviral infection or transgenic mouse experiments argue strongly that dominant-acting mutations in tyrosine kinases can contribute to human cancer. Although data of this type support the hypothesis that activating mutations in the *fps* proto-oncogene might also contribute to human cancer, until recently, there have been no reports of *fps* mutations in any human tumors. At first, the exciting report by Bardelli et al. (5) seemed to provide the long awaited biological evidence that *fps* could indeed behave like a dominant-acting oncogene in human cancer. Their mutational analysis examined the tyrosine kinome of a panel of 182 colorectal cancers and detected four missense mutations in sequences encoding the kinase domain of Fps (5). Two of these mutations were predicted to cause V743M and S759F substitutions in the COOH-terminal lobe (C-lobe) of the kinase domain (Fig. 1*A-C*), whereas two other mutations predicted M704V and R706Q substitutions in the activation loop (Fig. 1*A* and *D*).

We first assessed these amino acid substitutions by theoretical structural modeling. Energy minimization experiments suggested that three of these mutations had the potential to induce structural perturbations, which might compromise kinase activity. M704 is positioned in the C-lobe of the kinase domain at the beginning of the activation loop near the active site. The M704 side chain points toward the opening of the active site between the NH_2-terminal lobe (N-lobe) and the C-lobe of the kinase domain,

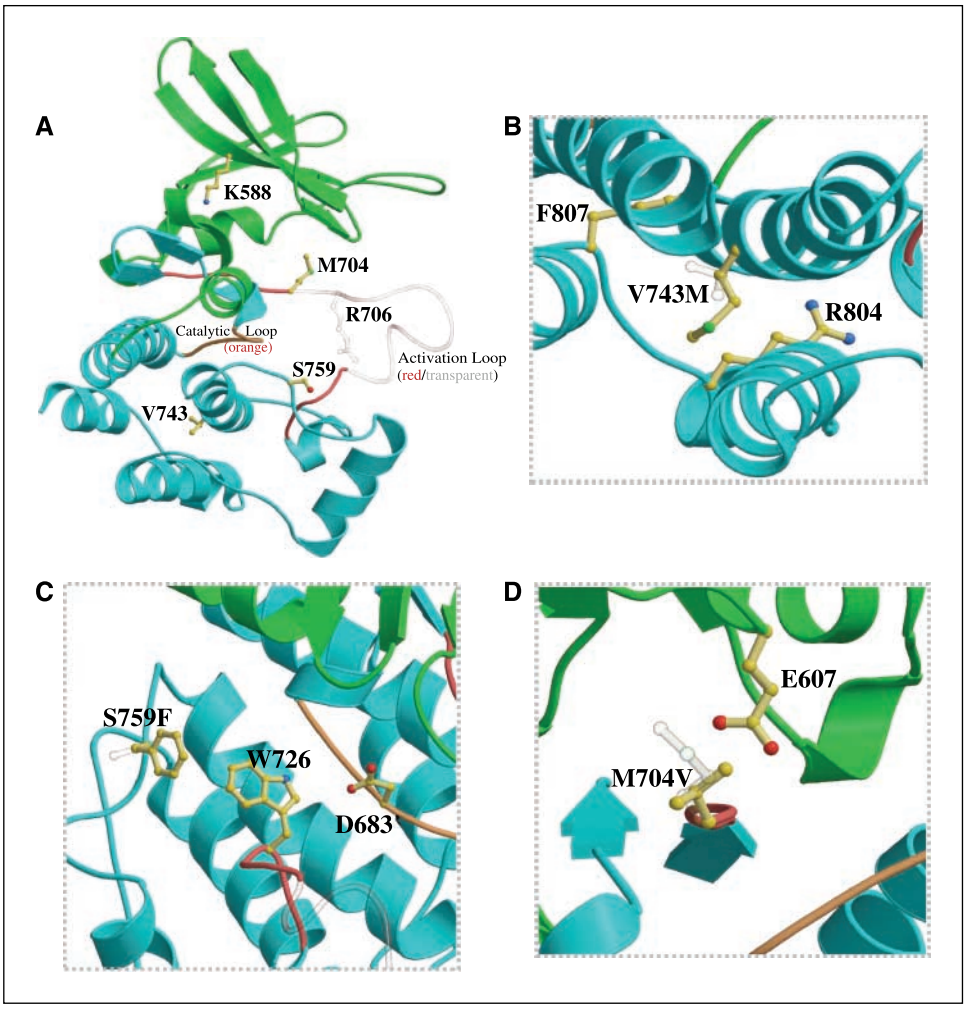

Figure 1. Structural modeling of the Fps kinase domain. *A,* ribbon diagram of the bilobate fold structure of the kinase domain of Fps modeled on a 2.35 Å crystal structure of the kinase domain of chicken Src. The N-lobe and C-lobe of the Fps kinase domain are depicted in green and blue, respectively. The catalytic loop (residues 676-687) is shown in orange and the activation loop (residues 698-728) is shown in red/transparent. The transparent regions corresponding to residues 706 to 722 are hypothetical, as they were undetermined in the Src template. Positions of the K588, M704, R706, S759, and V743 residues are shown. *B-D,* amplified views of the V743M, S759F, and M704V mutations, respectively. Wild-type residues are shown in white and corresponding mutated residues are shown in yellow. Neighboring amino acids are also shown in yellow. N-lobe (*green*), C-lobe (*blue*), activation loop (*red/transparent*), and catalytic loop (*orange*).

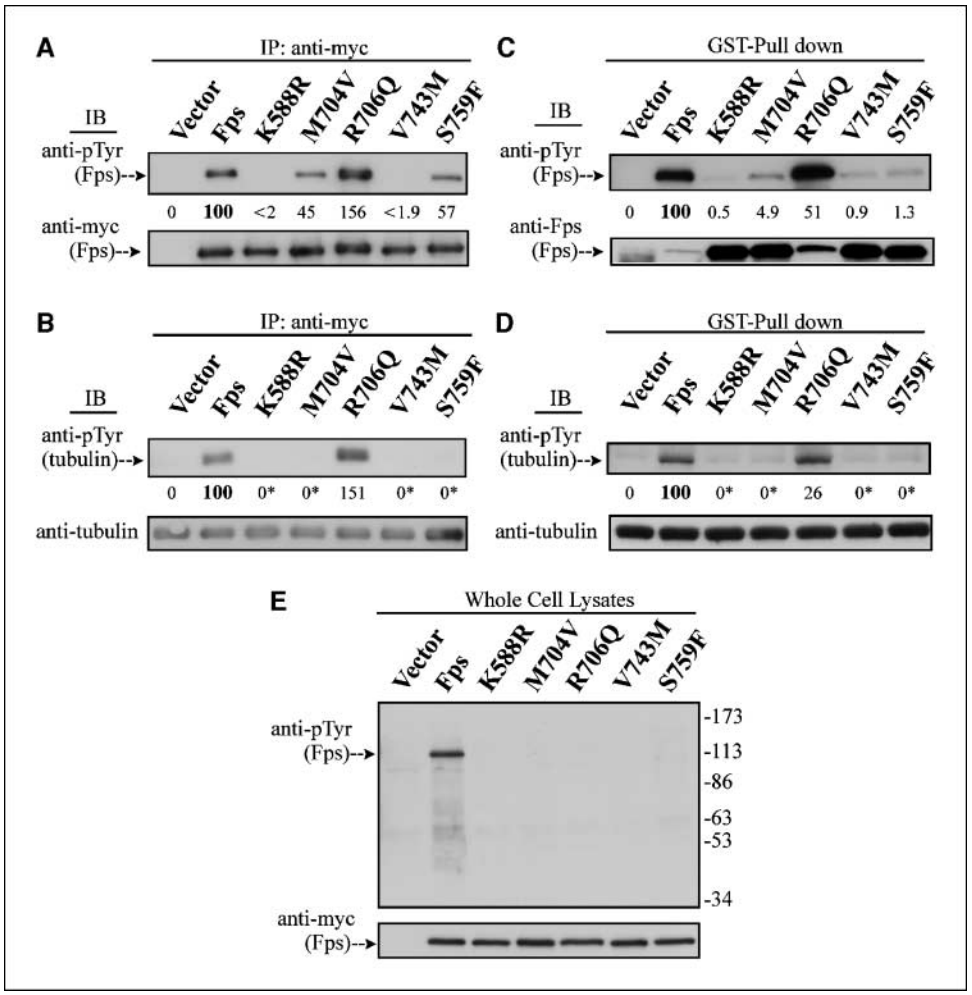

Figure 2. Effect of somatic mutations detected in human colorectal cancer on Fps kinase activity. Full-length Fps proteins with COOH-terminal *myc* epitope tags were transiently expressed in 293T mammalian cells (*A*, *B*, and *E*), or glutathione *S*-transferase (*GST*)-Fps fusion proteins containing the SH2 and kinase domains were expressed in bacteria (*C* and *D*). Fps proteins expressed in mammalian cells were immunoprecipitated with anti-*myc* antibody and glutathione *S*-transferase-Fps proteins expressed in bacteria were isolated on glutathione-agarose beads, and immune complex kinase assays were done using tubulin as an exogenous substrate. *In vitro* kinase autophosphorylation (*A* and *C*) or tubulin phosphorylation (*B* and *D*) activities were quantified as the ratio of pTyr to kinase expression levels and normalized to wild-type Fps. These values are given numerically under the corresponding lanes. *0**, no pTyr signal above background was detected. *E,* anti-pTyr and anti-*myc* blots of whole cell lysates from mammalian cells expressing the different full-length Fps proteins. Longer exposures of the top anti-pTyr blot (data not shown) did not reveal any differences in pTyr-containing proteins other than the signal generated by *in vivo* autophosphorylation of wild-type Fps.

where few interactions are found (Fig. 1*A*). Structural modeling suggested that M704 might play an important role in stabilizing interactions between the ATP-binding cleft and the active site, because it makes several van der Waals interactions with residues in the N-lobe, for example, with E607 (Fig. 1*D*). The shorter side chain of V at this position is predicted to mediate weaker interactions and might therefore destabilize interactions between the ATP-binding cleft and the active site.

R706 is in a region of the activation loop, which was disordered and therefore not determined in the crystal structure template (Fig. 1*A*). We speculate that the apparent flexibility of this loop region might make the R706 position relatively tolerant of the R706Q substitution and it might therefore not have a substantial effect on kinase activity.

V743 is located within a tight hydrophobic pocket in the central core of the C-lobe of the catalytic domain (Fig. 1*A*). Replacement by the larger M residue would not be easily accommodated because it would clash with F807 and R804 (Fig. 1*B*). Consequent crowding would cause repulsion in this hydrophobic pocket that would destabilize the hydrophobic core. This might push apart the two α-helices located immediately below the catalytic loop, and this could in turn disrupt the conformation of the catalytic loop and the active site D683 residue.

S759 is on the surface of the C-lobe of the kinase and is therefore solvent exposed (Fig. 1*A* and *C*). When substituted by the hydrophobic residue F, the aromatic side chain is predicted to fold back toward the protein core to reduce solvent exposure. In so doing, it would interact with W726. Although W726 is ~6 Å away from the catalytic base D683, there is no residue or atom located in between (Fig. 1*C*). Thus, with some conformational flexibility during activation, W726 could influence D683.

To test these predictions, we compared the activity of bacterially expressed glutathione *S*-transferase fusion proteins corresponding to the SH2 and kinase domains of these four mutants with wild-type or a known kinase-inactivating K588R mutant. The position of K588 in the N-lobe is indicated in Fig. 1*A*. This kinase-inactivating K588R substitution has previously been characterized biochemically and introduced into the endogenous *fps* locus in the targeted $fps^{KR/KR}$ mice (6). As predicted by the theoretical modeling, three of the four mutations reported in colon cancer (M704V, V743M, and S759F) greatly compromised the *in vitro* kinase activity of bacterially expressed Fps proteins, both with respect to autophosphorylation (Fig. 2*C*) and phosphorylation of the exogenous substrate, tubulin (Fig. 2*D*). The R706Q mutation reduced *in vitro* activity of the bacterially expressed Fps mutant. This was consistent with our modeling study, which predicted that a conservative R706Q mutation might be tolerated in the flexible activation loop region. Quantified *in vitro* activities of the four mutants, as well as the K588R mutant, relative to wild-type

Fps are shown below the corresponding lanes for autophosphorylation (Fig. 2*C*) and phosphorylation of tubulin (Fig. 2*D*), respectively.

Full-length versions of wild-type and Fps mutants were next expressed in mammalian cells and their *in vitro* activities were assessed in immune complex kinase assays. The M704V, V743M, and S759F mutants were inactive with respect to *in vitro* tubulin phosphorylation (Fig. 2*B*); however, unlike the reduction in activity seen in bacteria, the R706Q mutant displayed a slight (1.5-fold) increase in tubulin phosphorylation relative to wild-type Fps when expressed in mammalian cells. The R706Q mutant was comparably active with respect to *in vitro* autophosphorylation, and the M704V and S759F mutants also displayed some autophosphorylation when expressed in mammalian cells (Fig. 2*A*). Interestingly, anti-phosphotyrosine immunoblotting of whole cell lysates clearly indicated that only the wild-type Fps protein was able to autophosphorylate *in vivo* (Fig. 2*E*). Thus, although some of these mutants retain *in vitro* activity, their *in vivo* activity may be substantially more compromised.

Interestingly, the positions of the M704V and R706Q mutations in Fps correspond closely to the L858R and L861Q mutations in the epidermal growth factor receptor that were shown recently to activate that kinase and render it more sensitive to gefitinib in some lung cancer patients (14). In that case, the authors speculated that structural alterations induced by these nonconservative substitutions might increase the affinity for ATP and the ATP analogue, gefitinib. Although we could not model the R706Q mutant, we speculate that the conservative M704V substitution could destabilize the ATP-binding cleft by compromising interactions between the N-lobe and the C-lobe. Structural studies will be required to determine the relationship between these mutations and conformational changes that might alter the affinity for ATP binding.

These biochemical analyses established that three of four Fps mutations recently identified in colon cancer were inactivating rather than activating, whereas the fourth had slightly increased *in vitro* activity but abolished *in vivo* activity. Although they did not support a hypothesis that activating mutations in *fps/fes* contributed to colon cancer, these observations did raise the interesting new possibility that *fps/fes* might function as a tumor suppressor in epithelial cells. To test this novel hypothesis, we first measured tumor onset in a PyVmT transgenic mouse model of breast cancer in the context of a *fps*-null ($fps^{-/-}$) genetic background. Tumor onset occurred earlier in $fps^{-/-}$ mice relative to the wild-type *fps* genetic background, and this effect could be rescued by a *fps* transgene (fps^{Tg}; Fig. 3, *left*; $P = 0.017$). A second independent study recapitulated the early onset time observed in $fps^{-/-}$ genetic backgrounds (Fig. 3, *right*; $P \leq 10^{-6}$). This second study also showed that mean tumor onset time in a targeted kinase-dead ($fps^{KR/KR}$) *fps* genetic background occurred earlier, although not as early as the onset time observed in $fps^{-/-}$ backgrounds (Fig. 3, *right*; $P \leq 10^{-5}$).

Immediately postonset, tumors remained dormant before entering a rapid growth phase. We observed that the emergence of this rapid growth phase exhibited the same relative pattern of onset as observed for the initial tumor onsets in $fps^{KR/KR}$ and $fps^{-/-}$ backgrounds (*wt*: 79 ± 18 days, $n = 21$; $fps^{-/-}$: 57 ± 6 days, $n = 11$; $P = 0.0004$; $fps^{KR/KR}$: 65 ± 9 days, $n = 24$; $P = 0.002$). However, no differences in the rate of this growth phase were observed (data not shown).

The genetic data presented here strongly support the idea that loss-of-function *fps* genetic backgrounds (null or kinase-inactivating) promote epithelial tumorigenesis. Earlier tumor onset in $fps^{KR/KR}$ and $fps^{-/-}$ backgrounds is consistent with a tumor suppressor function for Fps in epithelial cells and suggests both a phosphorylation-dependent and a protein association–dependent aspect of this Fps function. Our finding that three of the four *fps* mutations detected in human colorectal cancers were kinase-inactivating suggests that the kinase-dependent function of Fps might be a minimal critical requirement for this suppressor

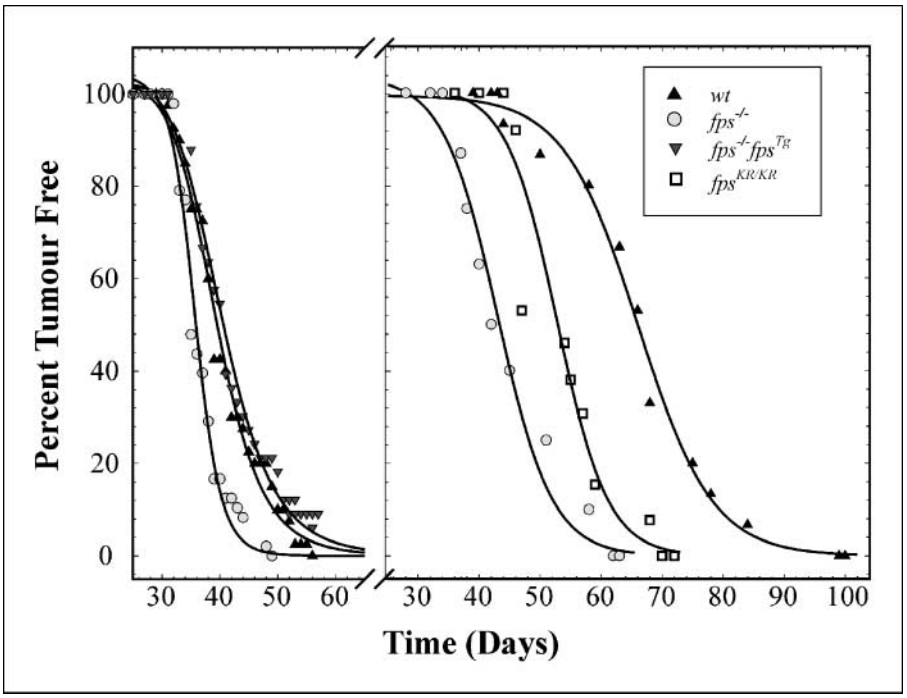

Figure 3. Disruption of *fps/fes* correlates with early tumor onset. Time to tumor onset was assessed by physical palpitation in a PyVmT transgenic mouse model of breast cancer in the indicated *fps* genetic backgrounds. Two independent experiments were done by different investigators (*left* and *right*). Tumors appeared sooner in $fps^{-/-}$ mice than in wild-type (*wt*) mice (*left*: *wt*, $n = 40$; $fps^{-/-}$, $n = 57$; $P = 0.017$). A rescue transgene (fps^{Tg}) restored tumor onset to the *wt* profile in the $fps^{-/-}$ background (*left*: $fps^{-/-}$ fps^{Tg}, $n = 33$; $P = 0.003$). Mice targeted with a kinase-inactivating K588R mutation ($fps^{KR/KR}$) also displayed earlier tumor onset relative to *wt* mice (*right*: *wt*, $n = 24$; $fps^{KR/KR}$, $n = 21$; $P = 5.2 \times 10^{-5}$). Although onset times were earlier in $fps^{KR/KR}$ and $fps^{-/-}$ mice, rates of tumor growth were comparable in all three genotypes.

function. Although we cannot conclude that these kinase-inactivating mutations played a causal or contributing role in these human colon cancers, the mouse model work described here certainly provides strong independent evidence to support that hypothesis. It will be important to determine if tumorigenesis in other oncogene- or carcinogen-based mouse models of epithelial cancers is similarly affected by loss of Fps function.

PyVmT has been proposed to function as a viral analogue of activated growth factor receptors based on its ability to recruit and activate signaling pathways downstream of Shc, phosphatidylinositol 3-kinase, Src, phospholipase Cγ, and protein phosphatase 2A (15). The observed early tumor onset in the PyVmT model of breast cancer therefore implicates Fps in negatively regulating PyVmT signaling pathways. In this respect, increased tyrosine phosphorylation in *v-fps*-transformed fibroblasts has been observed in several growth factor–inducible signaling proteins, including RasGAP, Shc, and phosphatidylinositol 3-kinase (reviewed in ref. 2). Hence, diminished pathway activity due to hypophosphorylated states of the latter signaling proteins may affect PyVmT-mediated tumorigenesis in loss-of-function *fps* genetic backgrounds. Potential tumor suppressor roles for the tyrosine kinases Syk (16), Csk (17), and EphB2 (18) have been described. However, to our knowledge, this is the first example of a tyrosine kinase with known oncogenic properties that might also act as a tumor suppressor. In fact, the opposite has been shown for Src, which was required for breast tumorigenesis in the same PyVmT-based breast tumor model used here (19).

In preliminary experiments, we failed to observe an effect of wild-type Fps or kinase-dead Fps on the transforming ability of PyVmT in NIH3T3 cells. However, because Fps is expressed in epithelial cells but not in fibroblasts, it will be important to repeat these experiments on epithelial cells from the $fps^{-/-}$ mice. If transduction with wild-type Fps reduces PyVmT-induced transformation of these cells, it would further support the hypothesis that Fps might act as a tumor suppressor in breast epithelial cells.

The loss of adhesion junctions as a result of E-cadherin dysfunction in epithelial cells is thought to contribute to tumorigenesis (20). It has been shown recently that the closely related Fer kinase plays a role in maintaining adherens junctions by regulating the tyrosine phosphorylation status of β-catenin (21). It will be interesting to see if tumorigenesis is similarly affected in mice lacking Fer and if Fps plays a role in regulating adherens junctions in epithelial cells.

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References

1. Smithgall TE, Rogers JA, Peters KL, et al. The c-Fes family of protein-tyrosine kinases. Crit Rev Oncog 1998;9:43–62.
2. Greer PA. Closing in on the biological functions of Fps/Fes and Fer. Nat Rev Mol Cell Biol 2002;3:278–89.
3. Yee SP, Mock D, Greer P, et al. Lymphoid and mesenchymal tumors in transgenic mice expressing the v-fps protein-tyrosine kinase. Mol Cell Biol 1989;9:5491–9.
4. Cheng HY, Schiavone AP, Smithgall TE. A point mutation in the N-terminal coiled-coil domain releases c-Fes tyrosine kinase activity and survival signaling in myeloid leukemia cells. Mol Cell Biol 2001;21:6170–80.
5. Bardelli A, Parsons DW, Silliman N, et al. Mutational analysis of the tyrosine kinome in colorectal cancers. Science 2003;300:949.
6. Senis Y, Zirngibl R, McVeigh J, et al. Targeted disruption of the murine fps/fes proto-oncogene reveals that Fps/Fes kinase activity is dispensable for hematopoiesis. Mol Cell Biol 1999;19:7436–46.
7. Greer P, Maltby V, Rossant J, Bernstein A, Pawson T. Myeloid expression of the human c-fps/fes proto-oncogene in transgenic mice. Mol Cell Biol 1990;10:2521–7.
8. Zirngibl RA, Senis Y, Greer PA. Enhanced endotoxin-sensitivity in Fps/Fes-null mice with minimal defects in hematopoietic homeostasis. Mol Cell Biol 2002;22:2472–86.
9. Guy CT, Cardiff RD, Muller WJ. Induction of mammary tumors by expression of polyomavirus middle T oncogene: a transgenic mouse model for metastatic disease. Mol Cell Biol 1992;12:954–61.
10. Craig AW, Zirngibl R, Greer P. Disruption of coiled-coil domains in Fer protein-tyrosine kinase abolishes trimerization but not kinase activation. J Biol Chem 1999;274:19934–42.
11. Williams JC, Weijland A, Gonfloni S, et al. The 2.35 A crystal structure of the inactivated form of chicken Src: a dynamic molecule with multiple regulatory interactions. J Mol Biol 1997;274:757–75.
12. Scott WRP, Hunenberger PH, Tironi IG, et al. The GROMOS biomolecular simulation program package. J Phys Chem A 1999;103:3596–607.
13. Kraulis P. MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures. J Appl Crystallogr 1991;24:946–50.
14. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129–39.
15. Dilworth SM. Polyoma virus middle T antigen and its role in identifying cancer-related molecules. Nat Rev Cancer 2002;2:951–6.
16. Moroni M, Soldatenkov V, Zhang L, et al. Progressive loss of Syk and abnormal proliferation in breast cancer cells. Cancer Res 2004;64:7346–54.
17. Read RD, Bach EA, Cagan RL. Drosophila C-terminal Src kinase negatively regulates organ growth and cell proliferation through inhibition of the Src, Jun N-terminal kinase, and STAT pathways. Mol Cell Biol 2004;24:6676–89.
18. Huusko P, Ponciano-Jackson D, Wolf M, et al. Nonsense-mediated decay microarray analysis identifies mutations of EPHB2 in human prostate cancer. Nat Genet 2004;36:979–83.
19. Guy CT, Muthuswamy SK, Cardiff RD, Soriano P, Muller WJ. Activation of the c-Src tyrosine kinase is required for the induction of mammary tumors in transgenic mice. Genes Dev 1994;8:23–32.
20. Wheelock MJ, Soler AP, Knudsen KA. Cadherin junctions in mammary tumors. J Mammary Gland Biol Neoplasia 2001;6:275–85.
21. Xu G, Craig AW, Greer P, et al. Continuous association of cadherin with β-catenin requires the non-receptor tyrosine-kinase Fer. J Cell Sci 2004;117:3207–19.

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