The human c-fes locus encodes a non-receptor protein-tyrosine kinase implicated in myeloid, vascular endothelial, and neuronal cell differentiation. A recent analysis of the tyrosine kinase in colorectal cancer identified c-fes as one of only seven genes with consistent kinase domain mutations. Although four mutations were identified (M704V, R706Q, V743M, S759F), the consequences of these mutations on Fes kinase activity were not explored. To address this issue, Fes mutants with these substitutions were co-expressed with STAT3 in human 293T cells. Surprisingly, the M704V, R706Q, and V743M mutations substantially reduced Fes autophosphorylation and STAT3 Tyr-705 phosphorylation compared with wild-type Fes, whereas S759F had little effect. These mutations had a similar impact on Fes kinase activity in a yeast expression system, suggesting that they inhibit Fes by affecting kinase domain structure. We have also demonstrated for the first time that endogenous Fes is strongly expressed at the base of colonic crypts where it co-localizes with epithelial cells positive for the progenitor cell marker Musashi-1. In contrast to normal colonic epithelium, Fes expression was reduced or absent in colon tumor sections from most individuals. Fes protein levels were also low or absent in a panel of human colorectal cancer cell lines, including HT-29 and HCT 116 cells. Introduction of Fes into these lines with a recombinant retrovirus suppressed their growth in soft agar. Together, our findings strongly implicate the c-Fes protein-tyrosine kinase as a tumor suppressor rather than a dominant oncogene in colorectal cancer.

c-fes, and the closely related gene fcr, encode distinct non-receptor protein-tyrosine kinases characterized by a unique amino-terminal region critical for kinase regulation and downstream signaling, a central SH2 domain, and a carboxyl-terminal kinase domain (1, 2). Fes lacks an SH3 domain, a negative regulatory tail, and other features that contribute to negative regulation of c-Src, c-Abl, and other cytoplasmic tyrosine kinases. Fes activation in response to growth factors and cytokines has been linked to diverse cellular events including survival, proliferation, differentiation, and reorganization of the cytoskeleton (2—4).

MATERIALS AND METHODS

Plasmids and Retroviruses—Coding sequences for wild-type human Fes, a kinase-active coiled-coil domain mutant (L145P), and a kinase-inactive mutant (KE) were fused to the enhanced yellow-shifted variant of green fluorescent protein (YFP) in the plasmid vector pEYFP-C1 (BD Bioscience-Clontech) as described elsewhere (4). In this report, YFP is referred to as green fluorescent protein (GFP) for simplicity. Codons for the colon cancer-associated mutations M704V, R706Q, V743M, and
S759F were incorporated into wild-type or L145P Fes cDNA clones via standard PCR-based methods. Yeast expression vectors for the wild-type and L145P forms of Fes have been described elsewhere (13). The M704V, R706Q, V743M, and S759F single mutants as well as the L145P, M704V, L145P, R706Q, L145P, V743M, and L145P, S759F double mutants were subcloned from the corresponding YFP-Fes fusion plasmids into the yeast expression vector pESC-Ura (Stratagene). Retrovirus for the expression of GFP alone or wild-type Fes fused to GFP have been described previously (14). To generate the active Fes-Fps chimera, the coding region for residues 610–822 of c-Fes were replaced with the homologous region of v-Fps (15). The Fes-Fps coding region was then amplified by PCR, subcloned into pSRαGFP (14), and used for retrovirus production as described (6, 14). STAT3 was expressed in 293T cells using the mammalian expression vector pCDNA3.1(−) (Invitrogen) as described elsewhere (16).

**Cell Culture, Transfection, and Retroviral Transduction**—All cell lines were grown at 37 °C in a 5% CO2 humidified incubator. 293T, TF-1, and K-562 cell culture has been described elsewhere (6, 14). Caco-2 cells were grown in Dulbecco’s modified Eagle’s medium (Invitrogen), HT-29 and HCT 116 cells were cultured in McCos modified 5A medium (ATCC or Invitrogen), and SNU-1040, DLD-1, and Colo 320 cells were cultured in RPMI 1640 medium (Invitrogen). All culture media were supplemented with 10% fetal bovine serum (Atlanta Biological) and Antibiotic-Antimicotic (Invitrogen). 293T cell transient transfection was performed using standard calcium phosphate techniques, and retroviral transduction of HT-29 and HCT 116 cells was performed as described previously (17).

**Immunoprecipitation, Immunoblotting, and Antibodies**—Transiently transfected 293T cells as well as stably transduced HT-29 and HCT 116 cells were lysed in radioimmune precipitation assay buffer, whereas all other cell lines were lysed in Fes lysis buffer as described previously (6). Immunoprecipitation and immunoblotting assays were performed as described elsewhere (4). Fes immunoprecipitation utilized a rabbit polyclonal antiserum raised against the Fes amino-terminal and SH2 domains (18). Fes was immunoblotted with goat polyclonal antiserum raised against the Fes amino-terminal region of Fes (Fes N-19; Santa Cruz) and YFP-Fes was detected with a mouse monoclonal antibody raised against GFP (GFP B-2; Santa Cruz). The active phosphorylated form of Fes was detected with either a mouse monoclonal anti-phosphotyrosine antibody (PY99; Santa Cruz) or a rabbit polyclonal antiserum produced against a peptide corresponding to the phosphorylated activation loop of Fes (pY713) (13). STAT3 was immunoblotted with a rabbit polyclonal antibody against the carboxyl terminus of STAT3 (STAT3 C-20; Santa Cruz), and pY705 STAT3 was detected with a mouse monoclonal antibody (Upstate). Immunoblot analysis of actin was performed with a mouse monoclonal antibody (Chemicon International).

**Immunohistochemistry**—Formalin-fixed and paraffin-embedded adult human colon tissue microarrays (Clonemics Bioscience, CytoMyx, and Asterand) were deparaffinized in xylene, rehydrated through a graded alcohol series, and permeabilized for 20 min in 0.5% Tween 20. Endogenous peroxidase activity was quenched in 3% H2O2 for 20 min, and antigen retrieval was performed by microwave oven incubation (high power, 12 min) in IHC select citrate buffer (Chemicon). Slides were cooled in citrate buffer for 20 min and blocked for 1 h with normal serum. Tissue sections were then incubated with rabbit polyclonal antibodies raised against Musashi-1 (Chemicon) or with goat polyclonal antibodies generated against either Fes amino-terminal (Fes N-19) or carboxyl-terminal (Fes C-19; Santa Cruz) peptides. Antibody blocking experiments were performed by preincubation of the Fes carboxyl-termin}

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**RESULTS**

**Colon Cancer-associated Mutations Block Fes Kinase Activity**—Fig. 1A shows a model of the Fes kinase domain in its down-regulated form and illustrates the positions of the four residues mutated in colon cancer (Met-704, Arg-706, Val-743, and Ser-759). Both Met-704 and Arg-706 localize to the activation loop (shown in red) and are in close proximity to the major site of tyrosine autophosphorylation (Tyr-713) (17). Tyrosine 713 plays a critical role in the regulation of Fes kinase and biological activities (17, 19), and Met-704 and Arg-706 are positioned to directly impact autophosphorylation and kinase domain function. The other two sites of colon cancer-associated mutations, Ser-759 and Val-743, localize to the carboxyl-terminal lobe of the kinase domain away from the active site but may affect kinase activity through an allosteric mechanism.

To determine the impact of the colon cancer-associated mutations on Fes kinase activity, individual mutants were created and expressed in human 293T cells. Kinase activity was assessed via immunoblot analysis with a general anti-phosphotyrosine antibody (pTyr) as well as a phosphospecific antibody against the Fes activation loop (pY713). As shown in Fig. 1B, wild-type (WT) Fes reacted strongly with both antibodies, indicative of kinase activation resulting from the high level of Fes expression in this system. Surprisingly, the M704V, R706Q, and V743M mutations all substantially reduced Fes autophosphorylation, whereas S759F caused a partial reduction. To address the effects of these mutations on substrate phosphorylation in vivo, wild-type and mutant forms of Fes were co-expressed with STAT3, a previously identified Fes sub-strate (16). As demonstrated in Fig. 1C, co-expression of wild-type Fes (WT) resulted in robust STAT3 Tyr-705 phosphorylation, consistent with previous results (16). The M704V, R706Q, and V743M mutations substantially reduced STAT3 phosphorylation by Fes, whereas the
S759F mutant had little impact, consistent with the effects of these mutations on Fes autophosphorylation. The observation that three of four colon cancer-associated mutations inhibit Fes kinase activity argues against a contributory role for Fes in colon cancer.

Colon Cancer-associated Mutations Inhibit Fes Kinase Activity Independently of Mammalian Cell Factors—To determine whether the suppressive effects of the colon cancer-associated mutations are intrinsically to the structure of Fes or require interaction with other proteins in mammalian cells, the WT and mutant Fes proteins were expressed in S. cerevisiae. Yeast expression provides an excellent model system to explore the functional consequences of these mutations on Fes structure and activity. Prior studies have shown that wild-type Fes naturally adopts an inactive conformation in yeast that can be activated with a point mutation (L145P) in the amino-terminal coiled-coil regulatory region (13). The yeast system therefore allowed us to assess the effects of the colon cancer-associated mutations in both the down-regulated and active forms of Fes without interference from endogenous tyrosine kinases. Moreover, ectopic expression of active Fes (as well as other non-receptor tyrosine kinases) inhibits yeast cell growth, providing a convenient in vivo biological assay for Fes activity (13, 20, 21).

S. cerevisiae cultures transformed with Fes expression plasmids were started in liquid culture, and aliquots were spotted on agar plates containing either galactose (+Gal) or glucose (+Glu). Transformed yeast were also grown in liquid culture for 6 h in the presence of galactose, and lysates were analyzed for Fes expression and tyrosine phosphorylation as described in the legend to Fig. 1, except that a Fes-specific antibody was used in place of the GFP antibody to determine Fes protein levels. Data are representative of two independent experiments.

Expression of Fes proteins carrying the individual colorectal cancer-associated mutations had little impact on yeast growth. Fes autophosphorylation, or yeast protein phosphotyrosine content, clearly demonstrating that these mutations do not activate wild-type Fes (Fig. 2). In contrast, the M704V, R706Q, and V743M mutations all strongly suppressed Fes-L145P autophosphorylation, caused a substantial reduction in tyrosine phosphorylation of yeast cell proteins, and reversed the growth inhibitory effect. This reduction in tyrosine phosphorylation of Fes substrate proteins in yeast agrees with the STAT3 result in Fig. 1C and provides further support for the idea that these mutations directly affect Fes kinase function. As in 293T cells, the S759F mutation did not markedly alter Fes-L145P kinase or biological activity in yeast. Demonstration that colon cancer-associated mutations affect Fes kinase activity in the same way in yeast and human cells suggests that these mutations affect kinase structure independently of cellular regulatory factors.

Fes Expression in Human Colonic Epithelium—Although high Fes mRNA levels have been observed in the developing gut (22), Fes protein expression in the adult has not been reported for this tissue type. To determine whether Fes is expressed in the adult colon, sections of normal colon tissue from 58 colon cancer patients were probed with antibodies against the carboxyl-terminal region of human c-Fes. Nearly 86% of these patients exhibited a staining pattern similar to that represented in Fig. 3A, in which Fes expression was restricted to the cytoplasm of a distinct subpopulation of intestinal crypt epithelial cells. The remaining sections exhibited either diffuse Fes staining in all cells or no detectable expression (data not shown). Interestingly, longitudinal

![FIGURE 1](Image)

**FIGURE 1. Mutations associated with colorectal cancer reduce Fes kinase activity.** A, model of the Fes kinase domain based on the predicted Fer structure (45) illustrates the proximity of residues Met-704, Arg-706, Val-743, and Ser-759 to the activation loop (shown in red) and autophosphorylation site (Tyr-713). B, 293T cells were transiently transfected with plasmids expressing GFP fused to kinase-inactive Fes (KE), wild-type Fes (WT), or the colon cancer-associated Fes mutants (M704V, R706Q, V743M, S759F). Clarified cell lysates were immunoblotted with anti-phosphotyrosine antibodies (pY713), or antibodies against GFP for detection of Fes protein. C, 293T cells were transiently transfected as described for panel B except that Fes expression plasmids were co-transfected with a STAT3 expression vector. Clarified lysates were immunoblotted with antibodies against STAT3 phosphotyrosine 705 (pY705 STAT3), STAT3 protein, or GFP for detection of Fes protein. Data are representative of two independent experiments.

![FIGURE 2](Image)

**FIGURE 2. Mutations associated with colorectal cancer reduce Fes activity in yeast.** Transformed yeast expressing no gene (Control), wild-type Fes (WT), an active Fes mutant (L145P), or Fes colon cancer-associated mutants either alone (M704V, R706Q, V743M, S759F) or in combination with the activating L145P mutation (LP) were grown on agar plates containing either galactose (+Gal) or glucose (+Glu). Transformed yeast were also grown in liquid culture for 6 h in the presence of galactose, and lysates were analyzed for Fes expression and tyrosine phosphorylation as described in the legend to Fig. 1, except that a Fes-specific antibody was used in place of the GFP antibody to determine Fes protein levels. Data are representative of two independent experiments.
sections of colonic crypts (Fig. 3, B and C) exhibited cells with strong Fes staining that localized to the lower portion of the crypts. Normal colon tissue stained with the Fes carboxyl-terminal antibody is shown at higher magnification in Fig. 3D and more clearly shows robust Fes expression in the cytoplasm of individual crypt cells. The basal nuclei of these cells are evident from their blue counterstaining with hematoxylin. A serial section from this same sample was stained with an antibody raised against the amino-terminal region of Fes and shows a very similar pattern of Fes immunoreactivity (Fig. 3E). Preincubation of the Fes carboxyl-terminal antibody with the corresponding peptide antigen completely eliminated immunostaining to this tissue section, providing evidence for antibody specificity (Fig. 3F). As an additional control for antibody specificity, the Fes-negative colorectal cancer cell line HT-29 was transduced with retroviruses carrying either GFP alone (Fig. 3G) or GFP fused to Fes (Fig. 3, H and I). Immunostaining with the Fes carboxyl-terminal antibody demonstrated no immunoreactivity in GFP-expressing cells consistent with the lack of Fes protein in HT-29 cells observed via immunoblotting (see Fig. 5B). In contrast, robust Fes staining was observed in HT-29 cells expressing the GFP-Fes fusion protein (Fig. 3H), further validating Fes antibody specificity. Together, these data provide the first evidence for Fes protein kinase expression in the crypt cells of normal human colonic epithelium.

**Fes Localizes to Musashi-1-expressing Cells**—Intestinal epithelial cells are believed to arise from a small population of stem cells located near the base of colonic crypts (23–25). Localization of Fes to distinct epithelial cells near the base of the colonic crypts (Fig. 3, B and C) suggested that Fes expression might be restricted to these colonic epithelial progenitors. Unfortunately, lack of clear morphological criteria and definitive molecular markers has hindered identification of intestinal stem cells. However, Musashi-1, an RNA-binding protein required for asymmetric cell division during Drosophila neural development, was recently characterized as a putative stem cell marker in human colonic epithelium where it exhibited an expression pattern similar to that of Fes described here (26–28). To investigate whether Fes is expressed in Musashi-1-positive cells, serial human colon tissue sections were stained with Fes or Musashi-1 antibodies. As demonstrated in Fig. 4, serial sections from three separate patients exhibited Musashi-1 and Fes staining in the same cells, suggesting that Fes expression may be restricted to the stem cells of the gut epithelium.

**Fes Expression Is Reduced or Absent in Colorectal Cancer**—To determine whether Fes expression is altered in colorectal cancer, human colon tumor tissue and matched normal control sections from 49 patients were immunostained with the Fes carboxyl-terminal antibody. As shown in Table 1, Fes expression was observed in all 49 normal tissues examined but was reduced in tumor tissue from 67% (33 of 49) of these patients. Moreover, Fes expression was undetectable in 16 tumor tissues examined. In contrast to normal colonic epithelium, Fes expression was not observed in tumor sections from these same individuals. The reduction or loss of Fes expression in nearly 70% of the tumor samples is consistent with a tumor suppressor function for Fes in colonic epithelium.
Fes was strongly expressed in TF-1 cells but was not detected in K-562 cells, in agreement with prior studies (7, 29, 30). Consistent with the loss of Fes expression in human colon tumor samples, Fes protein was undetectable in all colorectal cancer cell lines with the exception of Caco-2 cells. Here, Fes expression was observed although at a level significantly lower than that seen in the TF-1 myeloid leukemia cell line. These data further suggest that the loss of Fes protein expression, like the inactivating mutations described above, may contribute to colorectal cell transformation.

Fes Suppresses Anchorage-independent Growth of Colorectal Cancer Cell Lines—To test directly whether Fes modulates colorectal cancer cell growth, the Fes-negative HT-29 and HCT 116 cell lines were transduced with recombinant retroviruses carrying either GFP alone or fused to wild-type or kinase-active forms of Fes. The active form of Fes used in these experiments (Fes-Fps) is a chimera in which residues 610–822 of the c-Fes kinase domain have been replaced with the corresponding region of an active viral homologue (v-Fps) (15). Each of the cell lines was then assayed for anchorage-independent growth in a soft agar colony-forming assay. As shown in Fig. 6, wild-type Fes expression reduced soft agar colony formation to 60% of the levels observed with the GFP control in HT-29 cells and to 54% of control levels in HCT 116 cells. Expression of the active Fes-Fps mutant further reduced colony numbers to 31% of control levels in HT-29 cells but did not further suppress the growth of the HCT 116 cell line. This difference in Fes-Fps biological activity may reflect genetic differences between these cell lines. Assessment of Fes phosphotyrosine content via immunoblot analysis demonstrated robust activity for Fes-Fps in both cell lines, whereas wild-type Fes activity was low (HCT 116) or fully repressed (HT-29). In contrast to Fes, expression of an active form of the Src family kinase Hck had very little impact on HT-29 colony formation (data not shown), supporting the idea that the Fes tyrosine kinase has a unique role in growth suppression. Together, these data show that Fes suppresses anchorage-independent growth of colorectal cancer cell lines.

### Table 1

**Fes expression in normal colon versus tumor**

Colon tissue sections from 49 colorectal cancer patients were immunostained with an antibody raised against a c-Fes carboxyl-terminal peptide as described under “Materials and Methods” and the legend to Figure 3. Relative Fes staining was scored using the following scale: Strong, robust Fes expression in five or more cells; Moderate, robust Fes expression in 1–4 cells; Weak, low levels of diffuse staining with an absence of robust Fes-positive cells; Undetectable, no expression detected. Patient age, sex, and TNM tumor staging are also included. A detailed explanation of the TNM tumor staging system (T, extent of primary tumor; N, metastasis to lymph nodes; M, distal metastasis) can be found at the American Cancer Society web site (www.cancer.org). NA, not available.

| Patient | Age, sex | TNM staging | Fes staining |
|---------|----------|-------------|-------------|
| 1       | 63, F    | T3 N0       | Strong      |
| 2       | 83, F    | T3 Nx      | Moderate    |
| 3       | 74, M    | T3 N1      | Strong      |
| 4       | 68, M    | T3 N0      | Moderate    |
| 5       | 70, F    | T3 N2      | Strong      |
| 6       | 74, M    | T3 N2      | Weak        |
| 7       | 89, F    | T3 N0      | Strong      |
| 8       | 72, F    | T3 N1      | Strong      |
| 9       | 86, F    | T2 N1      | Strong      |
| 10      | 74, M    | T3 N0      | Strong      |
| 11      | 46, F    | T4 N0 M1   | Undetectable|
| 12      | 60, F    | T3 N0      | Strong      |
| 13      | NA       | T4 N0 Mx   | Strong      |
| 14      | NA       | TX N1 Mx   | Strong      |
| 15      | NA       | T3 N0 Mx   | Strong      |
| 16      | NA       | T4 N2 Mx   | Strong      |
| 17      | 48, M    | NA         | Strong      |
| 18      | 60, F    | T3 N1 Mx   | Strong      |
| 19      | 66, F    | T3 N0 M0   | Strong      |
| 20      | 59, M    | T1 N0 M0   | Strong      |
| 21      | 86, F    | T3 N1 Mx   | Strong      |
| 22      | 64, F    | T3 N0 M0   | Strong      |
| 23      | 64, M    | T3 N0 M0   | Strong      |
| 24      | 72, M    | NA         | Undetectable|
| 25      | 51, M    | NA         | Strong      |
| 26      | 60, F    | NA         | Strong      |
| 27      | 77, M    | NA         | Strong      |
| 28      | 62, F    | T3 N0 M0   | Strong      |
| 29      | 52, F    | T2 N1 M0   | Strong      |
| 30      | 73, F    | T4 N1 Mx   | Strong      |
| 31      | 51, F    | T4 N0 M0   | Strong      |
| 32      | 63, M    | T3 N0 M0   | Strong      |
| 33      | 60, F    | T4 N0 M0   | Strong      |
| 34      | 63, M    | NA         | Strong      |
| 35      | 62, M    | NA         | Strong      |
| 36      | 45, M    | T3 N0 M0   | Strong      |
| 37      | 69, M    | T3 N1 Mx   | Strong      |
| 38      | 64, F    | NA         | Strong      |
| 39      | 77, M    | T3 N0 M0   | Strong      |
| 40      | 55, M    | T3 N2 M0   | Strong      |
| 41      | 60, M    | T3 N2 M0   | Strong      |
| 42      | 60, M    | T2 N1 M0   | Strong      |
| 43      | 57, M    | T2 N1 M0   | Strong      |
| 44      | 79, M    | T3 N1 M0   | Strong      |
| 45      | 40, F    | T4 N1 M1   | Strong      |
| 46      | 74, M    | NA         | Strong      |
| 47      | 55, F    | NA         | Strong      |
| 48      | 50, M    | T4 N2 M0   | Strong      |
| 49      | 72, M    | T3 N1 M0   | Strong      |
Fes Tyrosine Kinase Suppresses Colon Cancer Cell Growth

FIGURE 5. Fes expression is reduced or absent in colorectal cancer cells. A, adult human colon sections from normal (a and b) and tumor (c and d) tissue were probed with antibodies raised against the Fes carboxy-terminal region as described in Fig. 3. B, Fes was immunoprecipitated from the indicated cell lines with antiserum generated against the Fes amino-terminal and SH2 regions followed by immunoblotting with an antibody against the Fes amino-terminal domain (upper panel). Clarified lysates were also immunoblotted with actin-specific antibodies as a control for input protein levels (lower panel). Data are representative of two independent experiments.

FIGURE 6. Fes expression suppresses anchorage-independent growth of colorectal cancer cells. HT-29 and HCT 116 cells expressing GFP alone or fused to wild-type (WT) or active forms of Fes (Fes-Fps) were grown in soft agar. Representative images of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide-stained plates are shown at the top. Three separate plates were stained for each cell population, and the average colony number was expressed relative to the GFP control cells. The bar graphs show the mean values and S.E. for HT-29 (n = 4) and HCT 116 (n = 2) experiments. Cell lysates were immunoblotted for Fes expression (Fes) and autophosphorylation (pTyr; bottom panels).

shown that c-fes mutations previously associated with colon cancer (8) dramatically inhibit rather than stimulate Fes kinase activity. Fes protein levels were consistently reduced or absent in primary human colon tumors compared with matched normal tissue. Moreover, in colon cancer cell lines where Fes expression was undetectable (HT-29 and HCT 116), re-establishing Fes expression by gene transfer was sufficient to suppress anchorage-independent growth, providing direct evidence that Fes acts as a growth suppressor in this cell type.

Our study is the first to report Fes protein expression in epithelial cells of the adult human colon, suggesting an important physiological role in normal colon function. Fes has previously been shown to promote differentiation of hematopoietic, endothelial, and neuronal cells (3, 5, 14, 31), suggesting that it may affect the differentiation program of colonic epithelium as well. Surprisingly, Fes was expressed in a distinct subpopulation of colonic epithelial cells localized near the base of some crypts (Fig. 3). Colonic epithelial stem cells have also been proposed to reside in limited numbers at the base of colonic crypts (24, 25), suggesting that Fes might be expressed in these cells. In support of a role for Fes in colon progenitor cells, Fes localized to cells expressing the putative colon stem cell marker Musashi-1 (Fig. 4). However, further studies are necessary to identify the precise physiological role of Fes in normal colon.

Data presented here suggest that both the presence of the c-Fes protein as well as its tyrosine kinase activity may contribute to its putative tumor suppressor function. Three of four mutations in the Fes kinase domain associated with colon cancer substantially inhibited Fes kinase activity, whereas a fourth had little effect (Figs. 1 and 2). Although Fes protein was expressed in normal colon tissue, it was reduced or absent in tumor tissue from 67% of these same individuals (Fig. 5 and Table 1). It will be of great interest to determine whether colon tumors staining positive for Fes exhibit inactivating mutations in the c-fes locus. In HT-29 cells, which fail to express endogenous Fes, re-introduction of wild-type Fes induced partial suppression of anchorage-independent growth, whereas introduction of a kinase-active mutant suppressed growth further (Fig. 6). Taken together, these data suggest that although kinase-inactivating mutations may promote colorectal cancer progression, a further selective advantage may be conferred following loss of Fes expression.

The mechanism by which Fes inhibits colorectal cell growth is currently unclear. Mutations in components of the Wnt signaling network that activate β-catenin are very common in colorectal cancer (10, 12, 23, 32), suggesting that Fes may exert growth suppression via inhibition of β-catenin function. In support of this idea, co-immunoprecipitation experiments showed strong association of active Fes with endogenous β-catenin in HCT 116 cells (data not shown), one of the colon cancer cell lines that responded to Fes expression with reduced colony-forming activity (Fig. 6). Moreover, the Fes homologue, Fer, has been shown to modulate β-catenin function at adherens junctions, resulting in enhanced cell adhesion (33). A similar role for Fes would fit with the suppression of anchorage-independent growth of colon tumor cells reported here.

Protein-tyrosine kinases are generally regarded as oncogenic, and activating mutations, enhanced expression, and constitutive kinase activity have been reported for a wide variety of cancers, including colon tumors (34 – 37). For example, protein-tyrosine kinases of the Src family are constitutively active in many colon cancer cell lines where they disrupt cadherin-catenin complexes and promote anchorage-independent growth (37 – 40). Along these lines, a tumor suppressor function has been suggested for Csk, the tyrosine kinase responsible for negative regulation of c-Src and other members of the Src kinase family (41).
Although a tumor suppressor role for a non-receptor protein tyrosine kinase such as Fes is very unusual, a similar loss of Syk expression has been associated with development of breast cancer (42). However, Fes has previously been shown to inhibit growth and induce terminal differentiation of chronic myelogenous leukemia cells, suggesting a more general role as a tumor suppressor (6, 7). Indeed, mice with either null or kinase-inactivating mutations have very recently been shown to develop breast cancer with a shorter latency, and this effect can be rescued with a c-fes transgene (43). The Fes kinase and/or the downstream mediators of its tumor suppressor function may therefore represent unexplored targets for drug discovery efforts aimed at reducing cancer progression. Conversely, an important consideration related to kinase-directed drug discovery (44) is that candidate inhibitors remain free from nonspecific effects on Fes tyrosine kinase activity, as inhibition of this kinase pathway may favor tumor development.

Acknowledgments—We thank Peter Greer, Queens University, Ontario for the generous gift of the Fes antiserum and Protein Data Bank coordinates for the fes transgene (43). The Fes kinase and/or the downstream mediators of its tumor suppressor function may therefore represent unexplored targets for drug discovery efforts aimed at reducing cancer progression. Conversely, an important consideration related to kinase-directed drug discovery (44) is that candidate inhibitors remain free from nonspecific effects on Fes tyrosine kinase activity, as inhibition of this kinase pathway may favor tumor development.

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