1. ABSTRACT

The fes gene was first discovered as a protein-tyrosine kinase-encoding retroviral oncogene. The ability of v-fes to transform cells in vitro and initiate cancer in vivo has been established by cell culture, engraftment and transgenic mouse studies. The corresponding cellular c-fes proto-oncogene encodes a cytoplasmic FES protein-tyrosine kinase with restrained catalytic activity relative to its retrovirally encoded homologs. These observations have stimulated a search for mutations or inappropriate expression of c-fes in human cancers and research aimed at understanding the functions of the FES kinase and its potential involvement in cancer and other diseases. Paradoxically, although first identified as an oncogene, genetic evidence has also implicated c-fes as a potential tumor suppressor. This review will describe observations from basic and translational research which shapes our current understanding of the physiological, cellular and molecular functions of the FES protein-tyrosine kinase and its potential roles in tumorigenesis. We also propose a model to reconcile the conflicting oncogenic and tumor suppressor roles of c-fes in tumorigenesis.

2. INTRODUCTION AND HISTORICAL PERSPECTIVES

FES (also known as FPS in the case of the orthologous avian protein) is the founding member of the F-BAR domain-containing subgroup of cytoplasmic protein-tyrosine kinases (PTKs) (previously reviewed in (1; 2; 3). FES and the FES related FER PTK are the only two members of this subgroup, and are encoded by paralogous human fes and fer genes located at chromosome positions 15q26.1 and 5q21, respectively. This review will focus on FES and will draw on work done over the past three decades to elucidate the normal biological functions of this PTK and its potential involvement in cancer. We begin with a brief historical perspective that dates back to the original discovery of the retroviral v-fes/fps oncogenes.

The cellular transforming and in vivo tumorigenic potential of fes/fps was first suggested by its discovery as an oncogene encoded by feline and avian tumor-associated retrovirus. These include Gardner-Arnstein and Snyder-Theilen strains of feline sarcoma virus (v-fes); or the chicken PRCII avian sarcoma and Fujinami poultry
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Figure 1. FES and related F-BAR family members. Protein domains of representative members of the F-BAR family include the protein-tyrosine kinase, SRC-homology 2 (SH2), SRC-homology 3 (SH3), RHO GTPase activating protein (RHOGAP), coiled-coil (CC), retroviral GAG and F-BAR domains. The illustrated retroviral encoded GAG-FPS protein is from Fujinami poultry sarcoma virus (FPS), and contains all the domains included in the cellular avian FPS and mammalian FES proteins, as well as the paralogous FER protein. CDC42 interacting protein 4 (CIP4) and PACSIN/syndapin represent members of the SH3-domain-containing F-BAR adaptor group, while srGAP is a member of the RHO-GAP family. Putative CC-like domains are included in several proteins; and in the case of FER this region has recently been suggested to be a phosphatidic acid binding FX domain (116).

sarcoma viruses (v-fps) (4; 5; 6; 7; 8). The viral v-fes/fps alleles were shown to encode chimeric proteins consisting of N-terminal retrovirally-derived GAG sequences fused with most or all of the corresponding cellular FES/FPS proteins (Figure 1). The dominant-acting in vitro cell transforming properties of v-fes/fps alleles and the in vivo tumorigenic potential of v-fps/fes transformed cells were demonstrated in cell culture and engrafment studies, respectively (9; 10). Transgenic mice studies also showed that retroviral GAG-FPS could drive the formation of mesenchymal and lymphoid tumors (11; 12). Informed by the seminal work of Harold Varmus, Michael Bishop and colleagues, which revealed the cellular origins of retroviral oncogenes (13), it was soon determined that v-fes/fps alleles were homologous to cellular c-fes/fps proto-oncogenes (14; 15) and the corresponding human and chicken orthologs were identified (16; 17).

In addition to being one of the first described PTKs, FES was one of the first proteins to be dissected using molecular biology-based structure-function methods. With the help of Michael Smith, who pioneered DNA oligonucleotide chemical synthesis, James Stone, Ivan Sadowski and Tony Pawson studied the effects of engineered dipeptide insertions on GAG-FPS, the retroviral transforming protein encoded by Fujinami sarcoma virus. Specific insertion points were found to compromise kinase activity and cell transformation properties. Interestingly, some insertion mutations outside the kinase domain displayed intriguing temperature sensitivity and host range cell transformation restrictions (18). The phenotypes of these mutants led to the hypothesis that a distinct protein subdomain consisting of sequences immediately N-terminal to the kinase domain plays regulatory roles which might involve both intramolecular (in cis) interactions and in trans interactions with cellular proteins. The homology of these sequences with SRC and some other cytoplasmic PTKs led to the name SRC homology 2 (SH2) domain (19; 20), where SH1 represents the kinase domain itself and SH3 was recognized as a third region of sequence homology that is conserved in ABL and SRC-family PTKs, but not in FPS/FES. It was soon discovered that SH2 domains were conserved in proteins other than tyrosine kinases, starting with phospholipase Cγ1 (21) and RAS GTPase activating protein (RASGAP/p120GAP/RASA1) (22; 23); and that this domain mediates interactions with tyrosine phosphorylated peptides (24; 25; 26; 27; 28; 29). The ability of the now recognized 120 SH2 domains encoded by the human genome to bind phosphotyrosine-containing peptides and mediate interactions between key signaling proteins has since been extensively studied (30; 31; 32) and forms a cornerstone of our understanding of how protein-protein interactions regulate signal transduction (reviewed in (33)). From an historical perspective, it is remarkable to consider that this all started with the intriguing phenotypes of a couple dipeptide insertion mutants of viral GAG-FPS (18; 19; 20; 27; 34). SH2 binding partners and kinase substrates of FES itself are still relatively poorly understood. However, proteomic and biochemical studies have implicated a number of interesting candidates, including several with potential relevance to cancer (31; 32; 35) (reviewed in (2)).

The recently solved crystal structure of the isolated kinase and SH2 domains of FES has revealed a unique in cis regulatory interaction whereby the SH2 domain may promote phosphorylation of specific substrates by mediating their interactions with the catalytic site and simultaneously stabilizing an active configuration of the N-terminal lobe of the kinase domain (36). These structural
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insights have provided a potential mechanistic basis for the regulatory function of the FES SH2 domain which was postulated twenty years earlier (19). However, it is important to note that we still do not know the identity of the hypothesized in trans interacting cellular protein (s) which might explain the host range cellular transformation restriction phenotype associated with SH2 mutants in GAG-FPS. One would expect these to be both SH2-binding partners and substrates of the FES kinase. The identification of these hypothetical proteins could provide important mechanistic insights into the dominant-acting cell intrinsic transforming properties of FES.

3. FES GETS ADOPTED BY THE F-BAR FAMILY

In addition to the C-terminal tyrosine kinase domain (37) and central SH2 domain (19), FES also contains an N-terminal membrane binding/bending BIN/Amphiphysin/RSV (BAR) domain (38; 39) (reviewed in (2)) (Figure 1). The BAR domain of FES and FER includes an N-terminal α-helix that shares close homolog with a group of adaptor proteins, for which CDC42 interacting protein 4 (CIP4) and yeast CDC15p are prototypic members (38). This FER/CIP4 homology (FCH) motif distinguishes F-BAR domains from the closely related N-BAR or I-BAR domains of proteins like endophilin A1 or IRSp53, respectively (reviewed in (40)). Structural studies have revealed that BAR domains consist of a triple helical bundle that forms banana-shaped dimers. These dimers present clusters of positively charged side chains on a membrane-binding surface that interact with negatively charged phosphate head groups of specific membrane-associated phospholipids. Membrane binding may induce either positive or negative curvature, depending upon whether the phospholipid-binding surfaces are on the concave or convex surface of the BAR dimers, or their ability to insert a short hydrophobic peptide into the membrane.

BAR domains are implicated in membrane/cytoskeletal dynamics including filapodia extension and endocytosis. The F-BAR-containing adaptors CIP4 and FBPI7 have been shown to promote endocytosis and membrane tubulation (41; 42). While their F-BAR proteins have intrinsic membrane binding and bending properties, their SH3 domains recruit effectors including dynamin (a membrane “pinchase”) and N-WASP (which recruits the actin polymerizing complex Arp2/3). These effectors play key roles in membrane-actin complex nucleating events, possibly by initiating membrane curvature at sites of accumulation of specific phospholipids (45). Endocytosis of ligand-receptor complexes and their subsequent trafficking within the cell plays an important regulatory role in signaling and cellular responses (46). These processes represent important potential mechanistic functions for F-BAR-containing proteins, including FES.

FES was first implicated in receptor endocytosis when macrophages from fes knockout mice where observed to have defective ligand-induced internalization of the lipopolysaccharide (LPS)-TLR4 receptor complex, which correlated with enhanced and prolonged activation of NF-κB and increased production of the inflammatory cytokine TNFα (47). This in vitro macrophage phenotype correlated with increased in vivo sensitivity of fes knockout mice to LPS challenge (48; 49). These observations suggest that FES contributes to the regulation of receptor internalization, trafficking and signaling output.

Interestingly, members of the F-BAR adaptor family contain several highly conserved tyrosine residues which may represent sites of regulatory phosphorylation by PTKs. Of particular interest are conserved tyrosine residues located immediately N-terminal to the SH3 domain in the F-BAR adapters PSTPIP1, PACSIN/Syndapin-1 and -3 which have been shown to be phosphorylated (50; 51; 52). It will be important to determine if phosphorylation at these sites regulates interactions of effectors such as dynamin and N-WASP with the SH3 domains, or the ability of the F-BAR domains to bind to and induce membrane curvature. It will also be interesting to determine if FES/FER kinases play direct or indirect roles in phosphorylation of these F-BAR adaptors.

The only other class of proteins reported to contain F-BAR domains are a small subset of RHO GAPs (53), including the Slit-Robo GAPs that are involved in axon pathfinding; however, recent work indicates that the srgap2 protein actually functions more like an I-BAR, inducing filopodia-like extensions that facilitate cell-cell interactions (54). RHO-family proteins including RAC, RHO and CDC42 are well known to play important roles in regulating membrane-cytoskeletal dynamics (55; 56). Several of the F-BAR adaptors, including CIP4, have been shown to bind to and be regulated by RHO family members (38). These observations suggest that F-BAR-containing adaptors, kinases and RHO GAPs may work in concert with RHO family members to regulate membrane-cytoskeletal remodeling and other cellular behaviors, including cell transformation.

Dominant-negative variants of RAS, RAC and CDC42 were found to inhibit the ability of viral or myristoylated FES to promote the growth of Rat2 fibroblasts in soft agar and this correlated with inhibition of FES-induced JNK activity (57). FES has also been implicated in RAC-dependent actin dynamics associated with neurite outgrowth from PC12 cells (58), and FES and FER have been shown to regulate semaphorin-induced axon collapse in dorsal root ganglion cells (59). PACSIN/Syndapin-1 has also been shown to interact with the RAS-RAC GTP exchange factor, SOS (60). Thus, F-BAR domains are structural components of both positive and negative regulators of the small GTPases, while the F-BAR proteins are themselves potentially regulated by RHO family proteins. Interactions between RHO GTPases and the F-BAR-containing adaptors, RHO GAPs and FES/FER kinases represents an important future research direction.
that may reveal insights into how these PTKs contribute to normal cellular functions and to cancer and other diseases.

4. INSIGHTS FROM TRANSGENIC AND GENE TARGETED MOUSE MODELS

Early investigations of c-fes suggested that its expression might be confined to cells of the myeloid lineage (61; 62). This led to expectations that activating mutations or tissue-specific over-expression might contribute to hematological malignancies, in particular to myeloid leukemias. Although FES expression was described in a number of human myeloid leukemic cell lines, no activating mutations have been reported to date. Furthermore, the fes gene has not yet been implicated in translocations, rearrangements or gene amplifications in myeloid cancers or other malignancies. Antisense-based knockdown and over-expression studies with myeloid progenitor-like cell lines showed that FES promotes survival and differentiation, but inhibits mitogenesis (63; 64; 65; 66; 67). These observations did not support the hypothesis that FES could promote myeloid malignancy through cell intrinsic dominant acting oncogenic mechanisms. In contrast, they provided the first evidence that FES might be capable of playing a cell intrinsic tumor-suppressor role.

After observing that ectopic over-expression of retroviral GAG-FPS in transgenic mice resulted in lymphoid and mesenchymal tumors (11), follow-up transgenic mice studies were performed using the complete human c-fes gene to achieve tissue-specific over-expression of wild type FES (68), or an activated mutant fes allele that encoded an N-terminally myristoylated FES protein (69). These transgenic mice tissue-specifically over-expressed human FES with the expected high levels observed in myeloid tissues. Mice over-expressing wild type FES displayed no apparent phenotypes, showing that greater than 10-fold over-expression was well tolerated and did not result in disruption of myelopoesis or any other evidence of malignancies (68). However, similarly engineered transgenic mice that tissue-specifically over-expressed kinase-activated N-terminally myristoylated FES died in utero or perinatally, showing signs of hemorrhaging (69). One stable line of mice with a single copy of the myristoylated FES transgene was established which displayed slightly elevated levels of myeloid cells and some hemostasis defects, but no myeloid leukemia or other hematological malignancies were observed (69; 70; 71; 72). The most striking phenotype observed in these myristoylated FES transgenic mice was hypervascularity that progressed to benign hemangiomas. This surprising phenotype led to the discovery that FES is normally expressed at high levels in endothelial lineages; furthermore, it suggested that endothelial cells are intrinsically more sensitive to expression of activated FES than are myeloid cells (69). This transgenic line of mice was also used to establish a yolk sac derived vascular endothelial cell line which is capable of acting as a feeder layer to support the growth of lymphoid cells (73; 74; 75). FES can also affect the responsiveness of endothelial cells to a number of angiogenic growth factors including PDGF, FGF, VEGF, and angiopoietins (71; 76; 77; 78; 79). In one of these studies, myristoylated FES partially rescued the developmental vasculogenesis defect of VEGFR2 null embryonic stem cells (78).

Mice have since been engineered with targeted null or kinase-inactivating mutations in the fes locus, and these were found to develop normally with only subtle defects in hematopoiesis and essentially normal vasculogenesis and angiogenesis (49; 80). However, a separate group who independently generated fes knockout mice did report more substantial defects in hematopoiesis, including abnormal myeloid proliferation (81). The differences in the phenotypes observed by these two groups have not been reconciled, but mouse strain background differences and the specific molecular approaches used to target the fes locus could be contributing factors (49).

Among the most intriguing phenotypes observed in targeted fes null mice was the hyper-responsiveness to lipopolysaccharide (LPS) challenge (49). This has since been mechanistically linked to a defect in LPS-induced endocytosis of the TLR4 receptor complex on the surface of cultured macrophages, enhanced and prolonged activation of NF-κB, excessive TNFα production (47) and enhanced tissue recruitment of leukocytes in response to localized LPS challenge (48). Considering these observations and the epidemiologic links between inflammation and epithelial cancers of the gastrointestinal system, breast, prostate and kidney (reviewed in (82)), as well as other observations described below, it has become apparent that FES might contribute to cancer through both tumor cell intrinsic functions and roles in stromal cell types, including myeloid and endothelial lineages.

5. EVIDENCE FOR TUMOR SUPPRESSOR FUNCTIONS OF FES

In 2003, missense mutations in fes were reported in human colon cancer (83). At first, it appeared these might represent the long awaited occurrence of activating oncogenic mutations in fes contributing to human cancer through tumor cell intrinsic mechanisms. However, subsequent biochemical analysis of these mutations showed they were not activating; indeed, all four of the observed mutations proved to be kinase-inactivating (84). Using the MMTV-polyoma virus middle T oncogene transgenic mouse model of mammary tumorigenesis, it was found shown that tumor onset occurred earlier in mice targeted with fes mutations compared with wild type mice; furthermore, this earlier tumor onset phenotype in targeted fes-null mice was reversed by interbreeding with a human fes rescue transgene (84). These observations provided the first compelling genetic evidence that FES could play a tumor suppressor role in epithelial tumorigenesis. However, the molecular, cellular and physiological bases of this apparent tumor suppressor effect were not elucidated in this study.

To our knowledge, mutations in fes or dysregulated FES expression have not been reported in human breast cancer. FES expression was recently shown
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to be significantly induced in mouse mammary epithelial cells during lactation, and this correlated with a dramatically enhanced FES in vivo phosphorylation status (85). FES was associated with E-cadherin at the adherens junctions and in cytosolic vesicles in lactating epithelial cells; and based on nursing pup weights it was concluded that milk production was reduced in fes-null lactating mice. Primary mouse mammary epithelial cell cultures also showed induced FES expression when exposed to the differentiation promoting agents insulin, prolactin and dexamethasone (85). These observations support a cell intrinsic role for FES in driving cell differentiation, and together with the earlier tumor onset seen in FES-deficient MMTV-polyoma virus middle T transgenic mice they are consistent with a potential tumor cell intrinsic tumor suppressor role. However, it should again be emphasized that these studies lacked mechanistic insights into what that tumor suppressor function might be (84).

Some reports using cultured human carcinoma cell lines have also suggested cell intrinsic tumor suppressor roles for FES and have shed some light on potential molecular roles. In colorectal cancer cells, expression of wild type FES inhibited their anchorage-independent growth, a typical characteristics of transformed cells (86). More recently, inhibition of DNA methylation in human colon cancer cell lines was shown to induce expression of FES in vitro. Furthermore, while the fes promoter was observed to be hypermethylated in cultured colon cancer cell lines, it was shown to be hypomethylated in normal colon epithelium; hypomethylation correlated with detectable FES protein expression in vivo (87). These colon cancer studies suggested a potential cell intrinsic tumor suppressor role for FES in epithelial cancer which could involve the promotion of survival, differentiation and mitotic arrest, as was previously suggested in studies of myeloid cells (67).

In contrast to the above mentioned examples of potential tumor suppressor functions in human cancer, there have also been a few studies supporting a potential tumor cell intrinsic oncogenic function for FES in human cancer. In renal carcinoma cells, in which the expression of FES at a protein level has been reported earlier (88), down-regulation of FES protein by small interfering RNA inhibited cell growth in monolayer culture (89). However, introduction of neither wild type nor kinase-inactive FES in these cells significantly affected their growth in monolayer or in nude mice. Down-regulation of FES by siRNA was associated with decreased e-Akt1 phosphorylation, nuclear translocation of NF-κB, and cyclin D1 expression. The molecular mechanisms underlying these discrepancies and apparently conflicting tumor cell intrinsic functions of FES remain elusive and will require further studies.

As described earlier, fes-null mice displayed a hyperinflammatory response to LPS which was associated with prolonged activation of NF-κB in macrophages. NF-κB has been implicated by many groups to be a key molecular player in inflammatory signaling in epithelial cancers (reviewed in (82)). Its activity has been shown to potentiate tumor initiation in colon cancer by acting at the

level of epithelial cells where it plays a pro-survival role, as well as in myeloid cells where it plays a tumor-promoting pro-inflammatory role (90). Indeed, inflammation has been linked to cancer of the colon as well as other tissues, including the breast (82). In light of these observations, it will be interesting to see if the earlier tumor onset observed in fes-null MMTV-polyomavirus middle T transgenic mice is associated with a tumor-initiating effect of pro-inflammatory FES-deficient tissue macrophages. Accordingly, one might hypothesize that FES plays a tumor suppressor role at this level of carcinogenesis by attenuating the NF-κB pathway in tissue macrophages, just as it appears to do in the context of LPS stimulation.

6. STROMAL ROLES FOR FES IN REGULATING TUMORIGENESIS

In addition to tumor cell intrinsic roles for FES, either as an oncogene or tumor suppressor, FES expression in myeloid and endothelial cells raises the possibility of stromal roles in carcinogenesis. Orthotopic tumor cell engraftment experiments have recently been used to separately examine tumor cell intrinsic and stromal roles of FES in breast cancer (91). When FES expression was ectopically manipulated in a highly metastatic engraftable mouse mammary carcinoma cell line, there was no apparent effect on tumor growth at the orthotopic injection site or metastasis to the lungs. That manipulation included over-expression of wild type FES, kinase-dead FES or activated (myristoylated) FES in the engrafted cancer cells. However, when the role of FES in the tumor niche was explored by comparing tumorigenesis after tumor cell engraftment into wild type or fes knockout mice, significant reductions in tumor growth rates and metastasis were observed in the fes knockout mice. This correlated with reductions in tumor angiogenesis, tumor-associated macrophages and circulating tumor cells. Furthermore, fes knockout macrophages did not promote the in vitro invasive properties of co-cultured tumor cells to the same extend as fes wild type macrophages did, and fes knockout macrophages were also deficient in their ability become more invasive in the presence of co-cultured tumor cells. These observations have provided a compelling argument for important tumor promoting (oncogenic) roles for FES in stromal cells within the tumor niche (91).

Tumor-associated angiogenesis is considered one of the hallmarks of cancer and is believed to play a rate limiting role in tumor growth and metastasis (92). Reduced tumorigenesis in engrafted fes knockout mice may therefore involve defective responsiveness of FES-deficient endothelial cells to tumor-produced paracrine acting angiogenic factors, including VEGF, PDGF, bFGF and angiopoietin. Other FES-expressing cells of the tumor niche which might interact in a paracrine fashion with tumor cells and the endothelium to promote tumor growth and metastasis would include platelets (72; 93), mast cells (39; 94; 95; 96) granulocytic cell types (63; 65; 67; 97; 98) and macrophages (80). Of these cell types, macrophages are particularly intriguing because of their high level of FES expression and accumulating evidence linking tumor associated macrophages to tumorigenesis (99; 100; 101;
Figure 2. FES influences on macrophages in tumorigenesis. Potential molecular roles for FES in signaling pathways regulating monocyte/macrophage lineage differentiation, polarization and functions. Solid lines indicate roles that are supported by published studies, while dotted lines indicate speculative roles. FES involvement in signaling from IL-3, GM-CSF and CSF-1 may support survival and differentiation. FES appears to attenuate NF-κB activation downstream of LPS during classical activation of M1-like macrophages (M1-MΦ). The pro-inflammatory functions of M1-MΦ, including ROS production, may initially contribute to initiation of tumorigenesis (indicated in green). In contrast, expression of MHC, TNFα and other pro-inflammatory cytokines may endow M1-MΦ with anti-tumorigenic functions at later stages in tumorigenesis (indicated in red). Positive or negative effects on signalling or tumorigenesis are indicated with green or red lines, respectively. Arg-1, arginase 1; bFGF, basic fibroblast growth factor; COX-2, cyclooxygenase-2; CSF-1 colony stimulating factor 1; EGF, epidermal growth factor; GM-CSF, granulocyte monocyte colony stimulating factor; HGF, hepatocyte growth factor; IFNγ, interferon gamma; IL, interleukin; LPS, lipopolysaccharide; macrophage, MΦ; MMP, matrix metalloproteinase; MHC, major histocompatibility complex; NF-κB, nuclear factor kappa B; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; TF, tissue factor; TGFβ, transforming growth factor beta; TNFα, tumor necrosis factor alpha; VEGF, vascular endothelial cell growth factor; VEGFR-1, vascular endothelial cell growth factor-1.

The observed reduction in circulating tumor cells in tumor cell engrafted mice is particularly significant in light of a recent report correlating clinical metastasis and tumor cell interactions with macrophages and endothelial cells in breast cancer (103). The cell co-culture experiments which revealed a defect in the ability of FES knockout macrophages to promote in vitro tumor cell invasion into collagen I gels and to respond to co-cultured tumor cells with enhanced invasion argues that FES plays roles in mediating paracrine interactions between macrophages and tumor cells which could be important in metastasis (91). Possible roles for FES in macrophages to explore include regulating the secretion of growth factors or proteases such as EGF and MMPs that may promote tumor cell migration and invasion, or responsiveness to factors produced by the tumor cells, including CSF-1, which could in turn influence macrophage migration and invasion. Other studies might investigate FES involvement in signaling pathways contributing to monocyte differentiation and polarization of tumor-associated macrophages into specialized phenotypes (Figure 2).
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Oxygen species (ROS), and they also induce MHC expression. These characteristics endow macrophages with anti-microbial and anti-tumorigenic properties (reviewed in (106)). However, ROS can also serve as a mutagen which could play a role in tumor initiation. Thus, tissue macrophages could play either pro- or anti-tumorigenic functions, depending upon their specific phenotype and the stage in tumorigenesis at which they are engaged.

Alternative activation of macrophages by IL-4- and IL-13-mediated STAT-6 activation leads to a wound healing M2 phenotype. Through production of TGF-β, EGF, MMPs and VEGF, these M2 macrophages may promote tumorigenesis through effects on angiogenesis and metastasis. M2 macrophages also inhibit M1 macrophages by secreting the anti-inflammatory mediator IL-10 (reviewed in (107)). The relationship between M1 and M2 macrophages and hypoxic tumor-associated macrophages is unclear (Figure 2), but they may tend to acquire a M2 phenotype. Fewer phagocytic F4/80phae macrophages were observed in the tumor-associated stroma of fes knockout mice, suggesting that FES might potentiate the formation of M2 macrophages (91). It will be important to determine if FES regulates the responses of macrophages to TLR receptor ligands and IFNγ or IL-4 and IL-13, which drive M1 or M2 polarization, respectively (106; 108). Accordingly, FES-deficient macrophages might be relatively more likely to polarize toward an M1 phenotype in response to TLR receptor ligands or IFNγ, and relatively refractory to IL-4 or IL-13 induced M2 polarization. FES has been reported to interact with the IL-4 receptor in B cells and potentiate recruitment of PI3K to IRS2 (96; 109; 110). There have been no reports yet linking FES to IL-4 or IL-13 signaling in macrophages, but this would certainly be important to investigate. It also seems plausible that FES-deficient tumor-associated macrophages will be hypersensitive to M1 polarization in response to IFNγ and TLR ligands. This speculation is supported by previous studies showing that fes knockout mice display hyperinflammatory responses to LPS (49). This was further characterized in vivo by increased leukocyte recruitment to locally inflamed tissues (48); as well as a systemic increase of TNFα and decrease of IL-10 (47). Furthermore, cultured fes knockout macrophages displayed prolonged LPS-induced activation of NF-κB, increased TNFα production and reduced internalization of the TLR4 receptor complex (47). It will be important to determine if FES-deficiency promotes an M1 polarization at the expense of M2 macrophages. In that case, FES inhibition might not only interfere with tumor-promoting functions of M2 polarized macrophages, but it might also promote the M1-based anti-tumor functions.

7. A MODEL OF THE CONFLICTING ROLES FOR FES IN TUMORIGENESIS

In the course of studying the c-fes proto-oncogene and its potential involvement in tumorigenesis it has become apparent that we need to distinguish between tumor cell intrinsic and stromal cell roles. In the former case, it is clear from observations with retrovirally encoded fes alleles that hyperactive FES can indeed drive cell intrinsic transformation in vitro and tumorigenesis in vivo. So theoretically, activating mutations in fes may contribute to human cancer through cell intrinsic mechanisms; however, at this point, there have been no examples of this described in the literature. The endogenous expression pattern of FES includes hematopoietic cells of the myeloiderythroid lineages, as well as endothelial, epithelial and neuronal lineages. Where FES function in these cell types has been studied, it has been linked to promotion of survival and differentiation. These cell intrinsic functions could in theory contribute to either oncogenic or tumor suppressor functions in human cancer. While there are currently no reports supporting oncogenic roles in human cancer, there have been some interesting correlations of inactivating missense mutations and promoter methylation-based transcriptional silencing of c-fes in human colon cancer that have raised the intriguing possibility of a cell intrinsic tumor suppressor role. In summary, at the level of tumor cell intrinsic roles in human cancer, the jury is still out as to whether FES might be involved in either pro- or anti-tumorigenic functions; there is circumstantial evidence for both possibilities.

More recently we have given more consideration to the possibility that FES might contribute to tumorigenesis through roles in stromal cells of the tumor niche, particularly endothelial cells and macrophages (91). The vascular hyperplasia observed in transgenic mice expressing myristoylated FES was perhaps the first clue suggesting a potential stromal role for FES in promoting cancer (69). The important role played by tumor-associated angiogenesis in cancer growth and metastasis is well recognized and this has spurred tremendous activity in the development of anti-angiogenic cancer treatments (92). Significant therapeutic benefits have been achieved in clinical trials of antibodies against VEGF and kinase inhibitors, several of which have been approved by the FDA; however, low response rates and modest delays in disease progression have been observed (111; 112; 113). Recent animal model studies have even suggested that anti-angiogenic treatment may promote metastasis [114; 115]. There is a clear need for biomarkers which can predict which patients will respond to antiangiogenic treatment and a better understanding of how tumor angiogenesis is regulated and how tumorigenesis is affected by its inhibition.

We now appreciate that FES plays roles in promoting mitogenic, survival and differentiation signaling in cells of the endothelial lineage. These observations favor placement of FES in the “oncogene corner” through a role in promoting tumor-associated angiogenesis. This idea is supported by slower tumor growth, reduced metastasis and angiogenesis in fes knockout mice compared to fes wild type mice in engraftment studies designed to isolate the stromal role of FES (91). However, we should not forget that fes knockout mice carrying the MMTV-polymavirus middle T transgene developed mammary tumors earlier than control wild type mice (84). So FES-deficiency in the vascular endothelial lineage apparently did not significantly impede tumor development; in
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contrast, FES behaved as a tumor suppressor in this particular model system (84).

This brings us back to macrophages, the cell types where highest levels of FES expression were originally observed. Through consideration of roles for FES in different phenotypes of macrophages we can now offer a tentative model that may in part reconcile the apparently conflicting roles of FES as initiator or inhibitor of tumor progression. Inflammation has been shown to correlate with cancer at a number of anatomical sites, and roles for innate immune cells including macrophages have been proposed which involve production of ROS and other potential mutagens. Thus, inflammatory M1-like macrophages may promote tumor initiation or early progression (Figure 2). The observed hyperactivation of the NF-κB pathway in fes knockout macrophages suggests that FES plays a role in restraining the activity of classically activated M1 inflammatory macrophages {Parsons, 2006 #525}. Thus, increased inflammation and ROS levels in the mammary tissues of fes knockout mice might have contributed to earlier tumor initiation in the MMTV-polyomavirus middle T transgenic model {Sangrar, 2005 #420}. So in the context of tumors where inflammation might play an important role in initiation, FES could be acting as a tumor suppressor by retraining the production of ROS and other mutagenic mediators by M1-like macrophages. However, at later stages in tumorigenesis, tumor-associated macrophages tend to more M2-like, which are associated with increased angiogenesis and metastasis through paracrine interactions with both tumor cells and vascular endothelial cells. The reduced numbers of tumor-associated macrophages observed in more developed tumors in fes knockout engrafted mice suggest that FES might promote the polarization of macrophages toward this M2 phenotype {Zhang, #5249}. Thus, FES could provide an oncogenic role at later stages in tumorigenesis through its roles in M2 macrophages.

Thus, the apparent contradictory involvement of macrophages in tumorigenesis may reflect distinct types of macrophages and their engagement at different stages in carcinogenesis. In this model, inflammatory (M1-like) macrophages may contribute to tumor initiation or early progression events and FES might restrain those functions (thus acting as a tumor suppressor); while wound healing (M2-like) macrophages might promote later tumor progression events including angiogenesis and metastasis and FES might promote these functions (thus serving a tumor promoting role). At early or late stages in tumorigenesis, M1-like macrophages may also play anti-tumorigenic roles through presentation of tumor antigens and activation of cytotoxic T cells. It will be important to determine what role FES might play in this process.

8. SUMMARY AND PERSPECTIVE

In summary, the current literature suggests FES may have a very complex involvement in cancer, including oncogenic and tumor suppressor effects that are intrinsic to cancer cells; as well as pro- and anti-tumorigenic effects acting through a variety of stromal cells, including distinct subtypes of macrophages and endothelial cells. A more complete understanding of these multiple roles will be essential in the context of developing therapeutic targeting strategies for the treatment of human cancers.

Although we have restricted the focus of this review to FES, we must also consider that the paralogous FER kinase is ubiquitously expressed and may therefore contribute to the regulation of similar functions as FES in cells where their expression is overlapping. Unpublished findings have implicated FER in playing both tumor cell intrinsic and stromal cell roles in promoting tumorigenesis. This will have to be carefully considered when exploring therapeutic strategies aimed at inhibiting these highly homologous kinases.

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**Send correspondence to:** Peter A. Greer, Queen’s Cancer Research Institute, Botterell Hall, Room A309, Queen’s University, Kingston, Ontario, K7L 3N6, Canada, Tel: 613-533-2813, Fax: 613-533-6830, E-mail: greerp@queensu.ca

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