

Chapter VIII

Non-receptor Protein Tyrosine Kinase Fes as a Candidate for Anticancer Molecular Targeting Therapy

Shigeru Kanda^{1,3}, Yasuyoshi Miyata² and Hiroshi Kanetake²*

¹Department of Molecular Microbiology and Immunology,
Division of Endothelial Cell Biology

²Department of Urology, Nagasaki University Graduate School of Biomedical Sciences,
1-7-1 Sakamoto, Nagasaki 852-8501, Japan

³Department of Experimental and Clinical Laboratory Medicine, National Hospital
Organization, Nagasaki Hospital, 41-6 Sakuragi-machi, Nagasaki 851-0251, Japan

Abstract

Fes (also known as Fps), together with Fer, form the nonreceptor protein tyrosine kinase subfamily. The expression of Fes in normal tissues has been shown to be limited to monocytic hematopoietic cells, endothelial cells, and some neuronal and epithelial cells. Studies using mice expressing activated mutant Fes or cultured endothelial cells indicated that Fes might be involved in pathological angiogenesis, suggesting it could be a potential target for anti-angiogenic therapy. Fes was initially identified as a protooncogene product. However, recent studies have indicated that Fes may act also as a tumor suppressor protein in some carcinomas, such as colon cancer, based on the identification of inactivating mutated Fes in colon cancer tissues and downregulated expression of Fes in some cancer cells. Thus, during treatment of these cancers, inhibition of Fes activity may stimulate progression of the pathological process. In this short communication, we will review the recent reports on the roles of Fes in tumor progression and discuss the problems that must be solved before targeting of this kinase in any anti-cancer therapy.

* E-mail address: skanda-jua@umin.net. Phone: +81 95 823 2261. Fax: +81 95 828 2616. Address all correspondence to: Shigeru Kanda, M.D., Ph.D., National Hospital Organization, Nagasaki Hospital, 41-6 Sakuragi-machi, Nagasaki 851-0251, Japan

Introduction

Fes (also known as Fps) and Fer define a unique subfamily of non-receptor protein-tyrosine kinases encoded by a protooncogene, *c-fes/fps* [1, 2]. Transforming oncogenes, *v-fps* (from Fujinami and PRC-type chicken sarcoma virus) and *v-fes* (from feline sarcoma virus) encode proteins consisting of N-terminal viral *gag* sequence fused to *c-fes*- or *c-fps*-derived kinase sequence. The *gag*-fusion forms exhibit constitutive activation of tyrosine kinase that is essential for their transforming potential. Unlike the ubiquitously distributed Fer, Fes is only expressed on myeloid hematopoietic cells, vascular endothelial cells, some neuronal and epithelial cells [3]. Structurally, Fes consists of a long N-terminal unique region followed by an SH2 domain and a protein-tyrosine kinase domain with two autophosphorylation sites (Y713 and Y811) [1, 4]. Y713 is a key regulatory residue for Fes kinase activity [5]. The unique N-terminal region contains two coiled-coil homology domains (CCDs), which are involved in homophilic intermolecular oligomerization, followed by autophosphorylation and subsequent activation of tyrosine kinase [6]. Studies using mutant Fes proteins suggested that the first CCD negatively regulates kinase activity while the second CCD has a positive regulatory role [7]. Regulation of the protein-tyrosine kinase activity by coiled-coil domains is a unique defining feature of the Fes/Fer kinase family.

Mice with targeted deletion of *fes* gene or mutant mice expressing kinase-inactive Fes are viable and fertile, and showed no significant defect during development [8, 9]. Mice expressing kinase-inactive Fes showed markedly reduced tyrosine phosphorylation of Stat3 and Stat5A in bone marrow-derived macrophages in response to granulocyte-macrophage colony-stimulating factor (GM-CSF) but not to interleukin (IL)-3 or IL-6 [8]. This suggests a distinct nonredundant role for Fps/Fes in signaling from the GM-CSF receptor that does not extend to the closely related IL-3 receptor. Mice lacking the expression of Fes had reduced numbers of bone marrow myeloid progenitors and circulating mature myeloid cells, and these cells were more sensitive to lipopolysaccharide than those from wild type mice [9]. These results suggest that the bulk of Fes function *in vivo* could be dispensable for other protein tyrosine kinases. The critical point is that mice positive for the expression of activated mutant Fes or v-Fps exhibited increased vascularity or tumorigenicity, indicating that Fes may play important roles in tumor development and progression [10, 11].

Signal Transduction Pathways Upstream or Downstream of Fes (Tables 1 and 2)

Fes is activated by a number of external stimuli. In cells of hematopoietic origin, IL-3, IL-4, IL-6, GM-CSF, and erythropoietin activate Fes and Fes is also activated downstream of c-kit and IgE receptor FcεRI [12-18]. Furthermore, fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor-A (VEGF-A), angiopoietins (Ang), stromal cell-derived factor-1α (SDF-1α), and sonic hedgehog (Shh) activate Fes in endothelial cells [19-26]. Nerve growth factor (NGF) also activates Fes in PC12 cells [27]. These results indicate that growth factor receptor tyrosine kinases and activated cytokine receptors or G-protein-coupled receptors are involved at least in part as upstream activators of Fes. Activation of Fes requires oligomerization. The common beta subunit of the IL-3 receptor is shared by the receptors for IL-5 and GM-CSF. GM-CSF induces physical association between Fes and the common beta

subunit of the IL-3 receptor [12]. Receptors for IL-6, oncostatin M, and leukemia inhibitory factor, transduce signal through a common β -chain called gp130. Fes associates with gp130 independently of ligand stimulation [13]. IL-4 induces the association of Fes with the IL-4

Table 1. Upstream activators of Fes.

Ligand	Possible receptors and/or components	Cell type	References
IL-3	Common β -subunit of IL-3R	Hematopoietic cells	[12]
GM-CSF	Common β -subunit of IL-3R	Hematopoietic cells	[12]
IL-6	gp130	Hematopoietic cells	[13]
IL-4	α chain of IL-4R	Hematopoietic cells	[14]
EPO	EPOR	Hematopoietic cells	[15]
	c-kit	Hematopoietic cells	[16,17]
IgE	Fc ϵ RI	Hematopoietic cells	[18]
FGF-2	FGFR-1	Endothelial cells	[19,20]
VEGF-A	VEGFR-2	Endothelial cells	[21,22]
Ang1, 2	Tie 2	Endothelial cells	[23,24]
SDF-1 α	CXCR4	Endothelial cells	[25]
Shh	Smoothened	Endothelial cells	[26]
PEDF	unknown	Endothelial cells	[35]
NGF	NGFR	PC-12 cells	[27]
No	Src	COS-7 cells	[34]
No	Bcr-Abl	Hematopoietic and 293T cells	[60]
No	KAP-1	293T cells	[40]

IL; interleukin, GM-CSF; granulocyte-macrophage colony stimulating factor, EPO; erythropoietin, EPOR; EPO receptor, IgE; immunoglobulin E, FGF; fibroblast growth factor, FGFR; FGF receptor, VEGF; vascular endothelial growth factor, VEGFR; VEGF receptor, Ang; angiopoietin, PDGF; platelet-derived growth factor, PDGFR; PDGF receptor, SDF-1 α ; stromal cell-derived factor-1 α , Shh; sonic hedgehog, PEDF; pigment epithelium-derived factor, NGF; nerve growth factor, KAP-1; Kruppel-associated box-associated protein-1.

Table 2. Downstream signaling molecules of Fes.

Signaling molecule	Function	References
Bcr	Binding to Fes and recruitment of Grb2-Sos complex	[29]
	Activation of Rac1 and Cdc42, leading to neurite outgrowth of PC-12 cells	[31]
PI3-kinase	Differentiation of ECs treated by SDF-1 α , Shh, and VEGF-A	[22, 25, 26]
	Migration of ECs treated by Ang2 and VEGF-A	[22, 23]
	Proliferation of ECs treated by Ang1	[24]
	Neurite outgrowth of PC12 cells treated by NGF	[27]
Fyn	Inhibition of differentiation of PEDF-treated ECs by inactivation	[35]
Src	Focal adhesion disassembly and migration of ECs treated by FGF-2	[20]
STAT1 and 3	Tyrosine phosphorylation and activation	[15, 37]
HSH2	Binding to Fes followed by tyrosine phosphorylation	[39]

EC; endothelial cells, SDF-1 α ; stromal cell-derived factor-1 α , Shh; sonic hedgehog, VEGF; vascular endothelial growth factor, Ang; angiopoietin, NGF; nerve growth factor, PEDF; pigment epithelium-derived factor, FGF; fibroblast growth factor, STAT; signal transducers and activators of transcription, HSH2; hematopoietic SH2 protein.

receptor alpha chain [14]. These cytokine receptor subunits aggregate by ligand binding, which is subsequently followed by autophosphorylation and activation of non-receptor protein tyrosine kinases, such as JAK [28]. Therefore, the binding of Fes to aggregated receptor subunits may help the oligomerization of Fes, followed by its activation. However, neither direct association of Fes with receptor tyrosine kinases, such as FGF receptor-1, Tie 2, VEGF receptor-2, and NGF receptors, nor binding of Fes to particular adaptor proteins downstream of these receptor tyrosine kinases to promote Fes oligomerization have been demonstrated to date.

A panel of signaling molecules act downstream of activated Fes. The breakpoint-cluster region protein (Bcr) is composed of N-terminal serine/threonine kinase domain, a central region homologous to the Dbl guanine nucleotide exchange factor, and a C-terminal GTPase activating protein domain for Rho family small GTPases. Fes associates with Bcr between the N-terminal unique region and SH2 domain of Fes and N-terminal kinase domain of Bcr [29]. Fes-tyrosine phosphorylated Bcr associates with Grb2-Sos complex, an activator of Ras [29]. This observation implies the potential role of Fes in Ras/mitogen-activated protein kinase (MAPK) signaling. Indeed, constitutively activated MAPK/extracellular signal-regulated kinase kinase (MEK) and its target ERK are essential for the proliferation of v-Fes-overexpressing murine macrophages [30]. Other targets of Fes/Bcr pathway are Rho family GTPases, Rac1 and Cdc42. Fes-induced neurite extension in PC12 cells is inhibited by the expression of dominant negative Rac1 or Cdc42, and a fragment of Bcr containing Fes binding and tyrosine phosphorylation sites [31]. Therefore, Fes-induced neurite extension seems to require Bcr-driven Rac1 and Cdc42 activities. On the other hand, Ras, Rac, and Cdc42 and their downstream molecules, ERK and c-Jun N-terminal kinase, have been reported to be involved in v-Fps-induced fibroblast transformation [32]. These results suggest that Fes-regulated Bcr and its downstream signaling diverges either proliferation or differentiation in a cell context-dependent manner.

Another important downstream signaling molecule is phosphoinositide 3-kinase (PI3-kinase). One of the mechanisms involved in the activation of class I PI3-kinase is the binding to tyrosine phosphorylated proteins [33]. In mouse T-cells, Fes is tyrosine phosphorylated upon IL-4-stimulation, followed by association with PI3-kinase [14]. In endothelial cells, Ang1 and 2 induce autophosphorylation of Fes and activation of PI3-kinase [23, 24]. Ang-activated PI3-kinase activity was co-immunoprecipitated with wild type Fes, but not with kinase-inactive Fes [23]. In the yeast two hybrid system, wild type, but not kinase-inactive Fes associated with p85 regulatory subunit of PI3-kinase [23], as has been shown in IL-4-treated mouse T-cells [14]. Ang1- and 2-induced activation of PI3-kinase is dependent on Fes activity, because expression of kinase-inactive Fes (dominant negative for endogenous Fes) abolished Ang-induced PI3-kinase activation [23, 24]. Similar results were obtained in endothelial cells treated with SDF-1 α and Shh [25, 26], suggesting that Fes is an activator of PI3-kinase in endothelial cells. PI3-kinase activity was involved in Abg2-directed chemotaxis [23] and morphological differentiation of endothelial cells promoted by SDF-1 α and Shh [25, 26]. In VEGF-A-treated endothelial cells, activated PI3-kinase was associated with not only activated Fes, but also activated VEGF receptor-2, Src, and tyrosine phosphorylated insulin receptor substrate 1 [21]. Therefore, expression of kinase-inactive Fes does not exert a dominant negative effect on PI3-kinase-sensitive chemotaxis and capillary morphogenesis induced by VEGF-A in endothelial cells. However, our recent study have shown that

downregulation of Fes by small interfering RNA (siRNA) significantly inhibited VEGF-A-promoted chemotaxis and capillary morphogenesis [22], suggesting that Fes may act as not only a protein tyrosine kinase, but also a scaffold protein for proper activation of PI3-kinase in VEGF-A-treated endothelial cells. In PC12 cells, PI3-kinase was activated and was required for neurite extension induced by NGF [27].

Interaction between Fes and Src family kinases is cell-context dependent. Co-expression with wild-type Src and Fyn resulted in potent tyrosine phosphorylation of kinase-inactive Fes in Sf-9 cells [19]. In this system, whether tyrosine phosphorylation of Fes results in its activation remains elusive. However, coexpression of Fes and active Src in COS-7 cells resulted in Fes activation, leading to colocalization of Fes with microtubules [34]. Thus, Src acts as an activator of Fes in transfected COS-7 cells. Fyn is inactivated by tyrosine phosphorylation of its C-terminal tail by Fes. This inactivation occurred within focal adhesions of endothelial cells treated with pigment epithelium-derived factor, which is linked to the inhibition of FGF-2-promoted capillary morphogenesis of endothelial cells [35]. In FGF-2-treated endothelial cells, activation of Fes enhanced FAK-dependent activation of Src within focal adhesions, leading to disassembly of focal adhesion and subsequent migration toward FGF-2 [20].

Signal transducers and activators of transcription (STATs) are SH2 domain containing transcription factors. They are tyrosine phosphorylated followed by their dimerization upon stimulation by a variety of cytokines/growth factors and subsequently enter into nuclei for transcriptional activation [36]. STAT3 is tyrosine phosphorylated and activated by Fes in human 293T cells or insect Sf-9 cells co-transfected with STAT3 and wild type Fes, but not with kinase-inactive Fes [37]. Unlike STAT3, activation of STAT5 was not observed in these systems [37]. Fes was involved in EPO-induced activation of STAT1 and STAT3 in UT-7/EPO cells and 293T cells [15]. Thus, STAT1 and STAT3 act as downstream effectors of Fes.

The adapter protein hematopoietic SH2 protein (HSH2) is involved in the survival and differentiation of B-cells [38]. It has been shown that Fes and activated Cdc42-associated tyrosine kinase bind to and tyrosine phosphorylate HSH2 [39]. Fes would thus be a possible regulator of B-cell survival and differentiation.

The transcriptional co-repressor Kruppel-associated box-associated protein (KAP)-1 was found to interact with Fes second coiled-coil domain [40]. KAP-1 forms a complex with heterochromatin proteins and histone deacetylases to repress transcriptions [41-43]. Co-expression of Fes and KAP-1 in 293T cells induced Fes autophosphorylation and subsequent tyrosine phosphorylation of KAP-1. Thus, KAP-1 acts as both an upstream and downstream signaling molecule of Fes [40].

Expression of Fes in Human Malignant Cells and Neoplastic Tissues

Although Fes is expressed in only a few types of normal cells, tumor cells from various origins have been found to express Fes gene and/or protein. Since early studies demonstrated that Fes was expressed in hematopoietic cells of myeloid origin [44], expression of Fes was preferentially observed in myelogenous leukemia cells rather than lymphoid leukemia cells [45, 46]. However, the expression has been also detected in some B-cell lymphomas and cell lines derived from patients with Hodgkin's disease [47, 48]. In solid tumors, Fes protein is

expressed in lung cancers (51.2% of adenocarcinoma, 26.3% of squamous cell carcinoma, 35.7% of large cell carcinoma, and 15.4% of small cell carcinoma) [49]. Copy number gain of *fes* gene has been observed in diffuse gastric cancers [50], oral squamous cell carcinomas [51], and glioblastomas [52]. *Fes* transcript is increased in ovarian adenocarcinomas, mixed-Mullerian tumors [53], and renal cell carcinomas [54]. In contrast, expression of *Fes* gene is reported to be downregulated in melanomas and anaplastic thyroid carcinomas when compared with normal melanocytes or thyroid tissue, respectively [55, 56].

It is not clear at this stage whether the expression of *Fes* gene and protein correlates with tumor progression and prognosis because of the lack of large clinicopathological studies. Furthermore, whether the expression of *Fes* benefits the proliferation, survival, and invasion of tumor cells needs further analysis.

Is *Fes* a Tumor Suppressor Protein?

Fusion proteins, Bcr-Abl (p210 and p185), produced by chromosomal translocation, are associated with leukemogenesis (p210 in chronic myelogenous leukemia, CML and p185 in acute lymphocytic leukemia) [57, 58]. Fusion to the N-terminal Bcr sequence induces constitutive activation of Abl protein tyrosine kinase. Introduction of *Fes* into the CML cell line K-562 induced growth arrest and terminal differentiation [59], suggesting that *Fes* may inhibit the progression of CML through interaction with Bcr-Abl. Coexpression of *Fes* with Bcr-Abl in 293T cells promoted reciprocal transphosphorylation, resulting in the activation of *Fes* [60]. This result suggests that Bcr-Abl may act as an upstream activator of *Fes*. Interestingly, Bcr-Abl-induced cytokine-independent outgrowth of myeloid leukemia cells, DAGM cells, was markedly inhibited by the presence of wild type *Fes*, but not by kinase-inactive *Fes* [60]. Thus, it seems likely that *Fes* exerts antiproliferative effect on leukemia cells expressing Bcr-Abl and that inhibition of *Fes* activity may favor the progression of Bcr-Abl-driven leukemia.

Analysis of 147 colorectal cancer tissues by Bardelli and coworkers [61] found 4 somatic mutations of the kinase domain of *Fes*, including M704V, R706Q, V743M, and S759F. These mutations were not found in Korean or Japanese colorectal cancer patients [62, 63]. Functional studies by two independent groups have shown that M704V, V743M, and S759F, or M704V, R706Q, and V743M mutations downregulated the kinase activity of *Fes* [64, 65]. Both groups expressed the mutant *Fes* in 293T cells. It is not clear why either R706Q or S759F mutations did not commonly exhibit reduced *Fes* kinase activity. Sangrar et al. [64] also showed that mice targeted with either null or kinase-inactive *fes* developed early-onset breast cancer in transgenic mice with mammary epithelial-specific expression of polyoma middle T antigen. Furthermore, Delfino and colleagues [65] demonstrated that introduction of wild type *Fes* into colorectal cancer cells, HT-29 and HCT 116 cells, inhibited anchorage-independent growth of these cells in soft agar. These results suggest that *Fes* may have tumor suppressor functions in epithelial malignancies. However, the downstream signaling molecules that are involved in these tumor suppressive functions have not been identified yet.

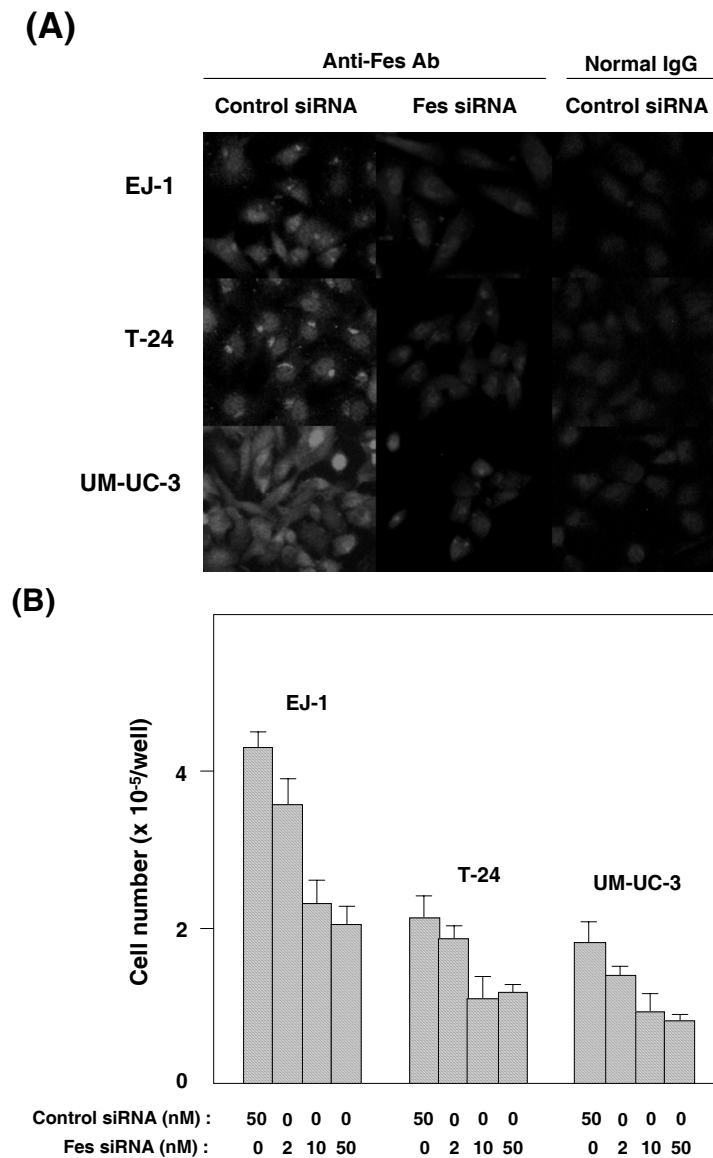


Figure 1. (A) Treatment of bladder cancer cells (EJ-1, T-24, and UM-UC-3 cells) with Fes siRNA (50 μ M) downregulates the expression of endogenous Fes. EJ-1 and T-24 cells were obtained from Japanese Cancer Research Resources Bank and UM-UC-3 cells were kindly provided by Dr. Hiro-omi Kanayama at the Department of Urology, The University of Tokushima Graduate School Institute of Health Bioscience. Cells were seeded onto coverslips and then treated with either negative control siRNA or Fes siRNA (Qiagen, Ref. [22]). Three days later, cells were fixed and Fes protein was visualized with anti-Fes (H-65) antibody (Santa Cruz Biotechnologies) by indirect immunofluorescent staining. Endogenous Fes distributed mainly in the nuclei and perinuclear area in EJ-1 and T-24 cells, whereas Fes was observed throughout the cytoplasm in UM-UC-3 cells. (B) Treatment with Fes siRNA dose-dependently inhibits the proliferation of bladder cancer cells. Cells were seeded into wells of 24-well plates and then treated with siRNA at indicated concentrations. Three days later, cells were detached by trypsin and their number was counted. Data are expressed as means \pm SD for triplicate wells.

To our knowledge, there are no reports describing Fes expression in bladder carcinoma cells. We examined the expression and the effect of Fes downregulation by siRNA in three human bladder carcinoma cell lines. As shown in Fig. 1, treatment with siRNA for Fes downregulated the expression of Fes protein in these cells. Interestingly, downregulation of Fes inhibited the proliferation of three bladder carcinoma cell lines. Furthermore, downregulation of Fes expression suppresses its kinase activity and scaffold function of Fes protein. These results raise the possibility that targeting Fes by a specific siRNA may inhibit angiogenesis as well as proliferation of human bladder cancer cells in clinics. Although the absence of Fes protein accelerated breast carcinogenesis in mutant mice [64], downregulation of Fes decreased the proliferation of bladder cancer cells. The reason for the discrepancy between the results of mutant mice and ours is unknown at present. One possibility is that breast carcinogenesis involves initiation and progression steps to transform normal cells into cancer cells, whereas cancer cells do not require further progression steps. Thus, cancer cells may use Fes for proliferation, and expression of Fes protein may affect the transformation of normal cells during development.

Conclusion

Fes is involved in a variety of angiogenic cellular responses by endothelial cells. Approaches based on Fes targeting may be a potentially useful therapeutic strategy in cancer patients through inhibition of tumor-related angiogenesis. Before this strategy could be ascertained, it is important to examine whether inhibition or downregulation of Fes inhibits angiogenesis *in vivo*. Also, Fes may act as a tumor suppressor in certain malignancies, and thus inhibition of Fes kinase activity in any treatment of human cancers may favor the progression of the disease. Hence, it is important to examine the role of Fes in proliferation, survival and motility of a wide variety of tumor cells.

Because of the lack of small molecular weight synthetic kinase inhibitors for Fes [4], downregulation of Fes (*e.g.*, treatment with specific siRNA) or introduction of kinase-inactive Fes into tumor cells may be helpful [66, 67]. Clinically applicable strategy for *in vivo* gene delivery of Fes siRNA to tumor tissue can be achieved by transcatheter intra-arterial administration of liposomes or vectors containing siRNA via feeding arteries of tumors. This approach can be used for the treatment of advanced renal cell carcinomas, primary and secondary hepatic tumors, and bladder cancers.

Progress in proteome analyses and microarray studies may reveal a relationship between Fes expression and progression of human cancers. If overexpression of Fes is associated with high tumor stage and poor prognosis in certain cancers, downregulation of Fes may be effective in halting cancer progression. Correlation analysis of clinicopathological features and Fes expression is therefore important to conjecture whether Fes plays a role in tumor progression and suppression.

Finally, it is also important to identify the signaling pathways involved in Fes-mediated tumor suppressive function. Many signaling molecules have diverse functions, such as induction of differentiation, growth arrest and stimulation of cell survival. In many cases, the mechanisms how signaling molecules decide the cell fate (*e.g.*, proliferation or differentiation) in response to external stimuli are not well understood. However, if the effector molecule that mediates Fes-induced tumor suppression is identified, simultaneous

inhibition of this molecule and Fes kinase activity could be beneficial in the control of advanced cancers.

References

- [1] Smithgall, T. E., Rogers, J. A., Peters, K. L., Li, J., Briggs, S. D., Lionberger, J. M., Cheng, H., Shibata, A., Scholtz, B., Schreiner, S., Dunham, N. (1998) The c-Fes family of protein-tyrosine kinases. *Crit Rev Oncog.* **9**, 43-62.
- [2] Greer, P. (2002) Closing in on the biological functions of Fps/Fes and Fer. *Nat Rev Mol Cell Biol.* **3**, 278-289.
- [3] Haigh, J., McVeigh, J., Greer, P. (1996) The fps/fes tyrosine kinase is expressed in myeloid, vascular endothelial, epithelial, and neuronal cells and is localized in the trans-Golgi network. *Cell Growth Differ.* **7**, 931-944.
- [4] Kanda, S., Miyata, Y., Kanetake, H., Smithgall, T. E. (2007) Non-receptor protein-tyrosine kinases as molecular targets for anti-angiogenic therapy. *Int J Mol Med.* **20**, 113-121.
- [5] Rogers, J. A., Read, R. D., Li, J., Peters, K. L., Smithgall, T. E. (1996) Autophosphorylation of the Fes tyrosine kinase. Evidence for an intermolecular mechanism involving two kinase domain tyrosine residues. *J Biol Chem.* **271**, 17519-17525.
- [6] Read, R. D., Lionberger, J. M., Smithgall, T. E. (1997) Oligomerization of the Fes tyrosine kinase. Evidence for a coiled-coil domain in the unique N-terminal region. *J Biol Chem.* **272**, 18498-18503.
- [7] Cheng, H. Y., Schiavone, A. P., Smithgall, T. E. (2001) 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.* **21**, 6170-6180, 2001.
- [8] Senis, Y., Zirngibl, R., McVeigh, J., Haman, A., Hoang, T., Greer, P. A. (1999) Targeted disruption of the murine fps/fes proto-oncogene reveals that Fps/Fes kinase activity is dispensable for hematopoiesis. *Mol Cell Biol.* **19**, 7436-7446.
- [9] Zirngibl, R. A., Senis, Y., Greer, P. A. (2002) Enhanced endotoxin sensitivity in fps/fes-null mice with minimal defects in hematopoietic homeostasis. *Mol Cell Biol.* **22**, 2472-2486.
- [10] Greer, P., Haigh, J., Mbamalu, G., Khoo, W., Bernstein, A., Pawson, T. (1994) The Fps/Fes protein-tyrosine kinase promotes angiogenesis in transgenic mice. *Mol Cell Biol.* **14**, 6755-6763.
- [11] Yee, S. P., Mock, D., Greer, P., Maltby, V., Rossant, J., Bernstein, A., Pawson, T. (1989) Lymphoid and mesenchymal tumors in transgenic mice expressing the v-fps protein-tyrosine kinase. *Mol Cell Biol.* **9**, 5491-5499.
- [12] Hanazono, Y., Chiba, S., Sasaki, K., Mano, H., Miyajima, A., Arai, K., Yazaki, Y., Hirai, H. (1993) c-fps/fes protein-tyrosine kinase is implicated in a signaling pathway triggered by granulocyte-macrophage colony-stimulating factor and interleukin-3. *EMBO J.* **12**, 1641-1616.
- [13] Matsuda, T., Fukada, T., Takahashi-Tezuka, M., Okuyama, Y., Fujitani, Y., Hanazono, Y., Hirai, H., Hirano, T. (1995) Activation of Fes tyrosine kinase by gp130, an

- interleukin-6 family cytokine signal transducer, and their association. *J Biol Chem.* **270**, 11037-11039.
- [14] Izuhara, K., Feldman, R. A., Greer, P., Harada, N. (1996) Interleukin-4 induces association of the c-fes proto-oncogene product with phosphatidylinositol-3 kinase. *Blood.* **88**, 3910-3918.
- [15] Kirito, K., Nakajima, K., Watanabe, T., Uchida, M., Tanaka, M., Ozawa, K., Komatsu, N. (2002) Identification of the human erythropoietin receptor region required for Stat1 and Stat3 activation. *Blood.* **99**, 102-110.
- [16] Petti, F., Thelemann, A., Kahler, J., McCormack, S., Castaldo, L., Hunt, T., Nuwaysir, L., Zeiske, L., Haack, H., Sullivan, L., Garton, A., Haley, J. D. (2005) Temporal quantitation of mutant Kit tyrosine kinase signaling attenuated by a novel thiophene kinase inhibitor OSI-930. *Mol Cancer Ther.* **4**, 1186-1197.
- [17] Voisset, E., Lopez, S., Dubreuil, P., De Sepulveda, P. (2007) The tyrosine kinase FES is an essential effector of KITD816V proliferation signal. *Blood.* **110**, 2593-2599.
- [18] Udell, C. M., Samayawardhena, L. A., Kawakami, Y., Kawakami, T., Craig, A. W. (2006) Fer and Fps/Fes participate in a Lyn-dependent pathway from FcepsilonRI to platelet-endothelial cell adhesion molecule 1 to limit mast cell activation. *J Biol Chem.* **281**, 20949-20957.
- [19] Kanda, S., Lerner, E. C., Tsuda, S., Shono, T., Kanetake, H., Smithgall, T. E. (2000) The nonreceptor protein-tyrosine kinase c-Fes is involved in fibroblast growth factor-2-induced chemotaxis of murine brain capillary endothelial cells. *J Biol Chem.* **275**, 10105-10111.
- [20] Kanda, S., Miyata Y., Kanetake, H., Smithgall, T. E. (2006) Fibroblast growth factor-2 induces the activation of Src through Fes, which regulates focal adhesion disassembly. *Exp Cell Res.* **312**, 3015-3022.
- [21] Kanda, S., Mochizuki, Y., Miyata, Y., Kanetake, H. (2003) The role of c-Fes in vascular endothelial growth factor-A-mediated signaling by endothelial cells. *Biochem Biophys Res Commun.* **306**, 1056-1063, 2003.
- [22] Kanda, S., Kanetake, H., Miyata, Y. (2007) Downregulation of Fes inhibits VEGF-A-induced chemotaxis and capillary-like morphogenesis by cultured endothelial cells. *J Cell Mol Med.* **11**, 495-501.
- [23] Mochizuki, Y., Nakamura, T., Kanetake, H., Kanda, S. (2002) Angiopoietin 2 stimulates migration and tube-like structure formation of murine brain capillary endothelial cells through c-Fes and c-Fyn. *J Cell Sci.* **115**, 175-183.
- [24] Kanda, S., Miyata, Y., Mochizuki, Y., Kanetake, H. (2005) Angiopoietin 1 is mitogenic for cultured endothelial cells. *Cancer Res.* **65**, 6820-6827.
- [25] Kanda, S., Mochizuki, Y., Kanetake, H. (2003) Stromal cell-derived factor-1 α induces tube-like structure formation of endothelial cells through phosphoinositide 3-kinase. *J Biol Chem.* **278**, 257-262.
- [26] Kanda, S., Mochizuki, Y., Suematsu, T., Miyata, Y., Nomata, K., Kanetake, H. (2003) Sonic hedgehog induces capillary morphogenesis by endothelial cells through phosphoinositide 3-kinase. *J Biol Chem.* **278**, 8244-8249.
- [27] Shibata, A., Laurent, C. E., Smithgall, T. E. (2003) The c-Fes protein-tyrosine kinase accelerates NGF-induced differentiation of PC12 cells through a PI3K-dependent mechanism. *Cell Signal.* **15**, 279-288.

- [28] Ihle, J. N., Kerr, I. M. (1995) Jaks and Stats in signaling by the cytokine receptor superfamily. *Trends Genet.* **11**, 69-74.
- [29] Maru, Y., Peters, K. L., Afar, D. E., Shibuya, M., Witte, O. N., Smithgall, T. E. (1995) Tyrosine phosphorylation of BCR by FPS/FES protein-tyrosine kinases induces association of BCR with GRB-2/SOS. *Mol Cell Biol.* **15**, 835-842.
- [30] Rovida, E., Marra, F., Baccarini, M., Dello Sbarba, P. (2000) Constitutive activation of the MAPK pathway mediates v-fes-induced mitogenesis in murine macrophages. *Blood.* **95**, 3959-63.
- [31] Laurent, C. E., Smithgall, T. E. (2004) The c-Fes tyrosine kinase cooperates with the breakpoint cluster region protein (Bcr) to induce neurite extension in a Rac- and Cdc42-dependent manner. *Exp Cell Res.* **299**, 188-198.
- [32] Li, J., Smithgall, T. E. (1998) Fibroblast transformation by Fps/Fes tyrosine kinases requires Ras, Rac, and Cdc42 and induces extracellular signal-regulated and c-Jun N-terminal kinase activation. *J Biol Chem.* **273**, 13828-13834.
- [33] Fruman, D. A., Meyers, R. E., Cantley, L. C. (1998). Phosphoinositide kinases. *Annu Rev Biochem.* **67**, 481-507.
- [34] Laurent, C. E., Delfino, F. J., Cheng, H. Y., Smithgall, T. E. (2004) The human c-Fes tyrosine kinase binds tubulin and microtubules through separate domains and promotes microtubule assembly. *Mol Cell Biol.* **24**, 9351-9358.
- [35] Kanda, S., Mochizuki, Y., Nakamura, T., Miyata, Y., Kanetake, H. (2005) Pigment epithelium-derived factor inhibits fibroblast growth factor-2-induced capillary morphogenesis of endothelial cells through c-Fyn. *J Cell Sci.* **118**, 961-970.
- [36] Finbloom, D. S., Larner, A. C. (1995) Regulation of the Jak/STAT signalling pathway. *Cell Signal.* **7**, 739-745.
- [37] Nelson, K. L., Rogers, J. A., Bowman, T. L., Jove, R., Smithgall, T. E. (1998) Activation of STAT3 by the c-Fes protein-tyrosine kinase. *J Biol Chem.* **273**, 7072-7077.
- [38] Herrin, B. R., Justement, L. B. (2006) Expression of the adaptor protein hematopoietic Src homology 2 is up-regulated in response to stimuli that promote survival and differentiation of B cells. *J Immunol.* **176**, 4163-4172.
- [39] Oda, T., Muramatsu, M. A., Isogai, T., Masuho, Y., Asano, S., Yamashita, T. (2001) HSH2: a novel SH2 domain-containing adapter protein involved in tyrosine kinase signaling in hematopoietic cells. *Biochem Biophys Res Commun.* **288**, 1078-1086.
- [40] Delfino, F. J., Shaffer, J. M., Smithgall, T. E. (2006) The KRAB-associated co-repressor KAP-1 is a coiled-coil binding partner, substrate and activator of the c-Fes protein tyrosine kinase. *Biochem J.* **399**, 141-150.
- [41] Lechner, M. S., Begg, G. E., Speicher, D. W., Rauscher, F. J. 3rd. (2000) Molecular determinants for targeting heterochromatin protein 1-mediated gene silencing: direct chromoshadow domain-KAP-1 corepressor interaction is essential. *Mol Cell Biol.* **20**, 6449-6465.
- [42] Matsuda, E., Agata, Y., Sugai, M., Katakai, T., Gonda, H., Shimizu, A. (2001) Targeting of Kruppel-associated box-containing zinc finger proteins to centromeric heterochromatin. Implication for the gene silencing mechanisms. *J Biol Chem.* **276**, 14222-14229.
- [43] Schultz, D. C., Friedman, J. R., Rauscher, F. J. 3rd. (2001) Targeting histone deacetylase complexes via KRAB-zinc finger proteins: the PHD and bromodomains of KAP-1 form

- a cooperative unit that recruits a novel isoform of the Mi-2alpha subunit of NuRD. *Genes Dev.* **15**, 428-443.
- [44] Samarut, J., Mathey-Prevot, B., Hanafusa, H. (1985) Preferential expression of the *c-fps* protein in chicken macrophages and granulocytic cells. *Mol Cell Biol.* **5**, 1067-1072.
- [45] MacDonald, I., Levy, J., Pawson, T. (1985) Expression of the mammalian c-fes protein in hematopoietic cells and identification of a distinct fes-related protein. *Mol Cell Biol.* **5**, 2543-2551.
- [46] Mavilio, F., Sposi, N. M., Petrini, M., Bottero, L., Marinucci, M., De Rossi, G., Amadori, S., Mandelli, F., Peschle, C. (1986) Expression of cellular oncogenes in primary cells from human acute leukemias. *Proc Natl Acad Sci U S A.* **83**, 4394-4398.
- [47] Lee, J., Talpaz, M., Walters, R., Gutterman, J. U., Blick, M. (1988) Aberrant expression of the K-ras proto-oncogene in B-cell malignancies. *Hematol Pathol.* **2**, 229-237.
- [48] Jucker, M., Schaadt, M., Diehl, V., Poppema, S., Jones, D., Tesch, H. (1990) Heterogeneous expression of proto-oncogenes in Hodgkin's disease derived cell lines. *Hematol Oncol.* **8**, 191-204.
- [49] Nishio, H., Nakamura, S., Horai, T., Ikegami, H., Matsuda, M. (1992) Clinical and histopathologic evaluation of the expression of Ha-ras and fes oncogene products in lung cancer. *Cancer.* **69**, 1130-1136.
- [50] Peng, D. F., Sugihara, H., Mukaiho, K., Tsubosa, Y., Hattori, T. (2003) Alterations of chromosomal copy number during progression of diffuse-type gastric carcinomas: metaphase- and array-based comparative genomic hybridization analyses of multiple samples from individual tumours. *J Pathol.* **201**, 439-450.
- [51] Liu, C. J., Lin, S. C., Chen, Y. J., Chang, K. M., Chang, K. W. (2006) Array-comparative genomic hybridization to detect genome-wide changes in microdissected primary and metastatic oral squamous cell carcinomas. *Mol Carcinog.* **45**, 721-731.
- [52] Nakahara, Y., Shiraishi, T., Okamoto, H., Mineta, T., Oishi, T., Sasaki, K., Tabuchi, K. (2004) Detrended fluctuation analysis of genome-wide copy number profiles of glioblastomas using array-based comparative genomic hybridization. *Neuro Oncol.* **6**, 281-289.
- [53] Kacinski, B. M., Carter, D., Kohorn, E. I., Mittal, K., Bloodgood, R. S., Donahue, J., Kramer, C. A., Fischer, D., Edwards, R., Chambers, S. K., et al. (1989) Oncogene expression in vivo by ovarian adenocarcinomas and mixed-mullerian tumors. *Yale J Biol Med.* **62**, 379-392.
- [54] Karthaus, H. F., Schalken, J. A., Feitz, W. F., Debruyne, F. M., de Haan, P. T., Bloemers, H. P., Van de Ven, W. J. (1986) Expression of the human fes cellular oncogene in renal cell tumors. *Urol Res.* **14**, 123-127.
- [55] Onda, M., Emi, M., Yoshida, A., Miyamoto, S., Akaishi, J., Asaka, S., Mizutani, K., Shimizu, K., Nagahama, M., Ito, K., Tanaka, T., Tsunoda, T. (2004) Comprehensive gene expression profiling of anaplastic thyroid cancers with cDNA microarray of 25 344 genes. *Endocr Relat Cancer.* **11**, 843-854.
- [56] Easty, D. J., Herlyn, M., Bennett, D. C. (1995) Abnormal protein tyrosine kinase gene expression during melanoma progression and metastasis. *Int J Cancer.* **60**, 129-136.
- [57] Ben-Neriah, Y., Daley, G. Q., Mes-Masson, A. M., Witte, O. N., Baltimore, D. (1986) The chronic myelogenous leukemia-specific p210 protein is the product of the *bcr/abl* hybrid gene. *Science.* **233**, 212-214.

- [58] Hermans, A., Heisterkamp, N., von Lindern, M., van Baal, S., Meijer, D., van der Plas, D., Wiedemann, L. M., Groffen, J., Bootsma, D., Grosveld, G. (1987) Unique fusion of *bcr* and *c-abl* genes in Philadelphia chromosome-positive acute lymphoblastic leukemia. *Cell*. **51**, 33-40.
- [59] Yu, G., Smithgall, T. E., Glazer, R. I. (1989) K562 leukemia cells transfected with the human *c-fes* gene acquire the ability to undergo myeloid differentiation. *J Biol Chem*. **264**, 10276-10281.
- [60] Lionberger, J. M., Smithgall, T. E. (2000) The c-Fes protein-tyrosine kinase suppresses cytokine-independent outgrowth of myeloid leukemia cells induced by Bcr-Abl. *Cancer Res*. **60**, 1097-1103.
- [61] Bardelli, A., Parsons, D. W., Silliman, N., Ptak, J., Szabo, S., Saha, S., Markowitz, S., Willson, J. K., Parmigiani, G., Kinzler, K. W., Vogelstein, B., Velculescu, V. E. (2003) Mutational analysis of the tyrosine kinase in colorectal cancers. *Science*. **300**, 949.
- [62] Kim, H. S., Kim, S. Y., Nam, S. W., Park, W. S., Lee, J. Y., Yoo, N. J., Lee, S. H. (2006) Absence of FES exon 16 and 17 mutation in the colorectal carcinomas in Korean patients. *Dig Liver Dis*. **38**, 213-214.
- [63] Shao, R. X., Kato, N., Lin, L. J., Murayama, R., Moriyama, M., Ikenoue, T., Watabe, H., Otsuka, M., Guleng, B., Ohta, M., Tanaka, Y., Kondo, S., Dharel, N., Chang, J. H., Yoshida, H., Kawabe, T., Omata, M. (2007) Absence of tyrosine kinase mutations in Japanese colorectal cancer patients. *Oncogene*. **26**, 2133-2135.
- [64] Sangrar, W., Zirgnibl, R. A., Gao, Y., Muller, W. J., Jia, Z., Greer, P. A. (2005) An identity crisis for *fps/fes*: oncogene or tumor suppressor? *Cancer Res*. **65**, 3518-3522.
- [65] Delfino, F. J., Stevenson, H., Smithgall, T. E. (2006) A growth-suppressive function for the c-fes protein-tyrosine kinase in colorectal cancer. *J Biol Chem*. **281**, 8829-8835.
- [66] Putral, L. N., Gu, W., McMillan, N. A. (2006) RNA interference for the treatment of cancer. *Drug News Perspect*. **19**, 317-324.
- [67] Kim, D. H., Rossi, J. J. (2007) Strategies for silencing human disease using RNA interference. *Nat Rev Genet*. **8**, 173-184.

