Cancer and Metastasis Reviews **9**: 191–202, 1990.

Autocrine regulation of cell growth and transformation by basic fibroblast growth factor

Avner Yayon* and Michael Klagsbrun
Departments of Surgery and Biological Chemistry, Children's Hospital and Harvard Medical School, Boston MA 02115, USA (Current adress: Department of Chemical Immunology, Weizman Institute of Science, Rehovot 76100, Israel)*

Key words: FGF, autocrine transformation, oncogenes

Abstract

Basic FGF (bFGF) and acidic FGF (aFGF) are multipotential factors that stimulate and support proliferation, migration and differentiation. Both bFGF and aFGF are non-secreted growth factors consistent with the lack of a signal peptide. However, bFGF and aFGF are deposited in extracellular matrix (ECM) suggesting that an alternative mechanism for FGF release exists. Four oncogenes, *int*-2, *hst*/K-*fgf*, FGF-5 and FGF-6 have been isolated that are highly homologous to aFGF and bFGF. Unlike bFGF and aFGF, they possess signal peptides and are secreted. These oncogenes transform cells and induce tumors, ostensibly via an autocrine mechanism. The involvement of bFGF and aFGF in autocrine transformation has been clarified by studies using FGF cDNA transfection. NIH-3T3 cells transfected with native bFGF cDNA and expressing 20 to 100 times as much bFGF as parental 3T3 cells acquire an enhanced proliferation rate and higher saturation density. NIH cells transfected with a construct in which bFGF cDNA is altered by addition of a signal peptide, undergo autocrine transformation and exhibit morphological and biochemical alterations characteristic of highly transformed cells. Injection of cells expressing native bFGF even at levels 100 times greater than parental 3T3 cells fails to induce tumors or lung metastasis in syngeneic mice. Signal peptide bFGF-transected cells on the other hand, acquire a high tumorigenic and metastatic potential with tumor incidence and numbers comparable to those induced by *ras* transformed cells. Acquisition of a signal peptide converts bFGF into a transforming protein analogous to FGF-related oncogenes which naturally have signal peptide sequences.

Introduction

Growth and differentiation of mammalian cells is under the control of various cellular growth factors and their corresponding receptors. Uncontrolled, autonomous cell growth and tumorigenicity have been suggested to result from a constitutive interaction of cellular growth factors and their receptors [1]. An autocrine mechanism of transformation has been demonstrated for a variety of polypeptide growth factors including v-*sis*, CSF-1 and IL-3 [2–4]. Autocrine transformation via an external stimulatory loop occurs when a secreted growth factor binds to cell surface receptors to generate a growth signal (Fig. 1A). Internal autocrine transformation results from the co-migration and continuous interaction of growth factor and growth factor receptors along the secretory pathway of the cell (Fig. 1B). Experimental evidence exists for the involvement of both external [4] and internal [5] autocrine loops in neoplastic cell proliferation. Autocrine transformation has been investigated most extensively for the v-*sis* oncogene which encodes for a PDGF-B homologue [2, 6]. v-*sis* is believed to

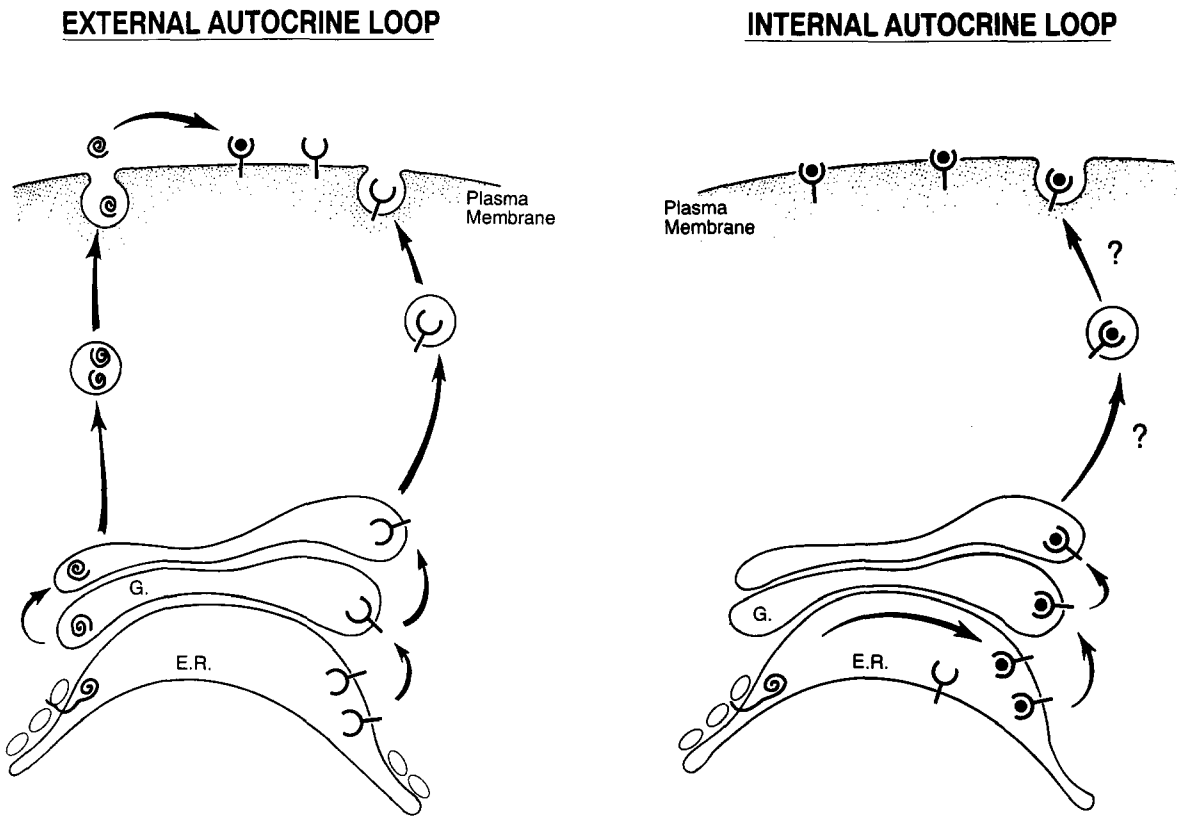

Fig. 1. External (A) and internal (B) autocrine loops. Schematic diagram of 2 types of autocrine loops involving growth factor and its receptor. G – golgi apparatus; E.R. – endoplasmic reticuluum.

transform cells by activation of an intracellular immature form of the PDGF receptor [5]. Evidence for internal autocrine transformation by v-*sis* includes the lack of growth inhibition by neutralizing antibodies to PDGF [7] and the prominent down-regulation of surface receptors for PDGF [8].

Basic fibroblast growth factor (bFGF) and acidic fibroblast growth factor (aFGF) are potent growth and angiogenic factors abundant in normal and malignantly transformed cells [9–11]. Four oncogenes, *int*-2, *hst*/K-*fgf*, FGF-5 and FGF-6, have been isolated that are highly homologous to aFGF and bFGF [12–16]. High expression of bFGF in cells transfected with bFGF cDNA leads to phenotypic transformation *in vitro* [17–20]. However, other reports suggest that bFGF-transfected lines are neither transformed nor tumorigenic [21–24]. Therefore, unlike FGF related-oncogenes, it is not clear whether aFGF and bFGF have a transforming potential. This article will focus on the role of bFGF in the growth of normal and transformed cells with particular emphasis on the relationship of bFGF expression, cellular localization and autocrine transformation.

The diverse biological activities of basic and acidic FGF

Basic FGF and aFGF are multipotential factors that stimulate and support a variety of normal cell functions such as proliferation, migration and differentiation. They stimulate the proliferation of a large number of cells of mesodermal and neuroectodermal origin including fibroblasts, vascular endothelial and smooth muscle cells, chondrocytes, myoblasts, glial and rat neuronal precursor cells [9–11]. bFGF and aFGF are chemotactic factors for

a number of cell types including endothelial cells, fibroblasts and astroglial cells [25–27]. In the presence of bFGF, endothelial cells in culture retain their contact-inhibited monolayer appearance seen *in vivo* [28], chondrocytes produce collagen type II [29], sheep preadipocyte fibroblasts differentiate into adipocytes [30], and neurite outgrowth is induced in PC12 cells and hippocampal neurons [31–33]. Both bFGF and aFGF are angiogenic factors [9, 34, 35] as they support the complex angiogenic process involving degradation of capillary basement membrane, migration, proliferation and endothelial tube formation. Another FGF-like protein found in adult tissue is the keratinocyte growth factor (KGF). KGF is a mitogen present in stromal cells derived from epithelial tissue [36]. KGF is unique among the FGF family in that it is a specific mitogen for epithelial cells.

FGF-related oncogenes

Several oncogenes have been identified to be members of the FGF family (Table 1). These oncogenes share with bFGF the 3 exon/2 intron structure and the conservation of 2 cysteine residues. They all encode for proteins that are 40% to 50% homologous to bFGF and aFGF, however with addition of a potential signal peptide sequence at the amino terminus. Apart from encoding for secreted FGF-like molecules these oncogenes also differ from bFGF and aFGF in that they are rarely found in adult tissues. In contrast, they are expressed during embryogenesis, neo-natal development and in many tumors.

The first FGF homologue to be described was *int*-2 [12]. *int*-2 is a cellular gene that is induced to become transcriptionally active after integration (int) of mouse mammary tumor virus (MMTV) into the mouse genome [37]. *int*-2 encodes for a protein of about 240 amino acids with a short stretch of non-charged amino acids that may function as an atypical signal sequence. Evidence for possible secretion of *int*-2 was suggested by its localization in the Golgi apparatus and endoplasmic reticulum of overexpressing COS cells [38]. *int*-2 is not detectable in normal mammary glands nor in other adult tissues. *int*-2 is expressed at several different stages in embryogenesis and in mouse embryonal cells [39–41] and is amplified in a number of tumors such as breast cancer and squamous cell carcinoma of the head and neck [42, 43]. *hst*/K-*fgf* are identical oncogenes. *hst* was originally identified in DNA isolated from a human stomach tumor (hst) and from a non-cancerous portion of human mucosa that had the ability to transform NIH 3T3 cells after transfection [13, 44]. The gene has an open reading frame that encodes a protein of

Table 1. The FGF family of homologous polypeptides

	Common name	MW	Signal peptide	Found in	Target cells
FGF 1	acidic FGF (HBGF-1)	18000	–	adult (neural)	fibroblasts endothelial cells neuronal cells
FGF 2	basic FGF (HBGF-2)	18000	–	adult	fibroblasts endothelial cells neuronal cells
FGF 3	*int*-2	27000	+	mouse embryo tumors	ND
FGF 4	*hst*/K-*fgf*	23000	+	mouse embryo tumors	fibroblasts endothelial cells
FGF 5	FGF 5	29000	+	mouse embryo tumors	fibroblasts
FGF 6	FGF.6	ND	+	tumors	ND
FGF 7	KGF	28000	+	skin	keratinocytes

N.D. – no data available

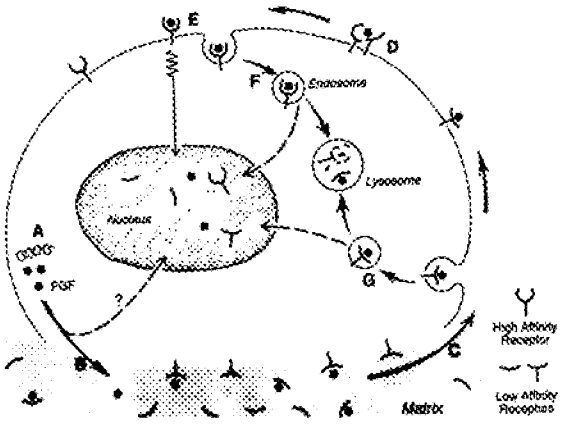

Fig. 2. Multiple cellular pathways for bFGF action. Schematic diagram of possible bFGF pathways in a cell producing bFGF and the high and low affinity FGF receptors. bFGF is synthesized and retained as a cytoplasmic protein (A); Polar secretion of bFGF into extracellular matrix (B); Matrix-bound bFGF is released by matrix-degrading enzymes or heparin-like molecules (C) and interacts with cell surface low and high affinity FGF receptors; Direct transfer of bFGF from low to high affinity FGF receptors (D); bFGF binds to high affinity receptors generating a mitogenic signal (E) and is rapidly internalized and degraded in lysosomes (F); Intact bFGF is translocated into the nucleus by either high (F) or low (G) affinity receptor mediated endocytosis. It is not yet known wether direct translocation of bFGF to the nucleus occurs.

206 amino acids. *hst* has a long non-homologous N-terminal extension region of about 55 amino acids relative to bFGF with a potential hydrophobic signal peptide sequence. K-*fgf*, an oncogene derived from transfection of Kaposi's sarcoma DNA into NIH 3T3 cells, appears to be identical to *hst* [14, 45]. The mature K-FGF protein is a glycosylated 23 kDa protein containing 175–176 amino acids that is secreted into the conditioned medium [46]. K-FGF is a mitogen for fibroblasts and endothelial cells. *hst*/K-*fgf* and *int*-2 are both located on chromosome 11 band q13, 40 to 50 kB apart and are co-amplified in human melanoma [42, 47]. Another FGF-related oncogene, FGF-5, was originally isolated by transfection of human bladder tumor DNA into NIH 3T3 cells [15]. FGF-5 encodes a 267 amino acid protein with a long hydrophobic amino acid terminus. FGF-5 has been found to be expressed in mouse embryos [48]. A fourth FGF like oncogene, FGF-6, was isolated from a mouse cosmid library by screening with the *hst* gene [16]. The cloned gene transforms NIH 3T3 cells and has 70% identity to the *hst* oncoprotein.

That FGF-like oncogenes encode for proteins that are secreted and expressed in tumors and in the developing embryo suggests that these oncogenes may be involved in malignant autocrine transformation. The possession of a signal peptide sequence therefore appears to be an important factor in determining the transforming potential of members of the FGF family.

Multiple cellular pathways for basic FGF

To understand the mode of FGF action, it is necessary to consider pathways for both exogenous and endogenous FGF. A schematic overview of possible intracellular and pericellular pathways for bFGF-mediated mitogenic activity is shown in Fig. 2. The biological activity of exogenous bFGF is mediated through specific high affinity saturable cell surface receptors [24, 49–55]. A low affinity, large capacity class of binding sites for bFGF has also been identified [56–58]. Unlike most other polypeptide growth factors, basic FGF is a non-glycosylated, non secreted, cell associated protein. However, despite the lack of signal sequence there is considerable evidence for the release and deposition of bFGF into the extracellular matrix (ECM) of normal cells *in vitro* [59–62] and basement membranes *in vivo* [63–65].

The mechanism of bFGF release is still unknown. However, there appears to be a class of secretory proteins that do not possess hydrophobic signal sequences and are released by a secretory pathway distinct from the classical endoplasmic reticulum and Golgi apparatus route [66]. A number of possibilities have been proposed for bFGF release, none of which have been proven yet. One proposed mechanism for FGF translocation into ECM is release via cell death, cell lysis or micro-injury. However it is improbable that such an uncontrolled growth factor release would lead to polar deposition and the specific pattern of distribution observed in basement membranes *in vivo*.

Another proposed mechanism is polar secretion and comigration of bFGF with surface and matrix

heparan sulfate (HS) containing proteoglycans (Fig. 2). Basic FGF in the ECM is bound to HS-proteoglycans which constitute a low affinity, large capacity class of binding sites for bFGF. Matrix-bound bFGF is continuously mobilized to interact with cell surface high affinity FGF receptors [56, 57, 67]. Matrix-bound bFGF could be locally released by either matrix and heparan sulfate degrading enzymes [59, 63, 68, 69] or direct competition by secreted heparin-like molecules [63, 68]. Another possible mechanism could be direct transfer of bFGF from low to high affinity membrane FGF-receptors (Fig. 2).

The mitogenic activity of bFGF administered exogenously is receptor-mediated. Most cells possess FGF receptors which are phosphorylated and internalized upon binding of FGF [57, 70–73] (Fig. 2). Radio-labeled bFGF cross linking studies suggest that FGF receptors are composed of two major glycoproteins of 130 and 150 kDa [24, 54]. Recently a 130 kD high affinity receptor for FGF has been purified and cloned [74–77]. The FGF receptor was found to be identical to the *flg* gene product and to contain an intracellular tyrosine kinase domain.

Upon binding to high affinity receptors and generation of the mitogenic signal, bFGF is rapidly internalized [57, 73] (Fig. 2). Lysosomal degradation of the receptor-ligand complex may account for the down-regulation of surface receptors and the self-limiting response of the cell. However the finding that intact bFGF is translocated into the nuclei of target cells [78, 79] requires that an alternative non-lysosomal transport pathway exists. Moreover, unlike growth factors such as EGF or PDGF, which are totally degraded, internalized non-nuclear bFGF is only partially degraded into discrete large fragments of 8–16 kDa which persist for up to 24 hrs [57]. One alternative pathway could involve high affinity receptor-mediated endocytosis with direct translocation of the FGF-receptor complex to the nucleus as suggested for other polypeptide growth factors [80]. Low affinity receptor-mediated endocytosis of a bFGF-HS complex is another attractive alternative for bFGF internalization and nuclear translocation (Fig. 2). It has been demonstrated that cell surface and matrix heparan sulfates are rapidly endocytosed [81], and that direct nonlysosomal transport of heparan sulfate from the cell surface to the nucleus occurs [82]. Heparin and heparan sulfates have been shown to protect bFGF from proteolytic degradation even under acidic conditions such as those found in mammalian lysosomes [83–85]. Endocytosis of a HS-bFGF complex could therefore account for the fact that a full-length bFGF is found in the nucleus and that cytoplasmic bFGF is only partially degraded into large fragments upon internalization.

A potential role for bFGF in the nucleus

It is generally believed that polypeptide growth factors generate their biological activities through interactions with cell surface-specific receptors. The interaction activates an intracellular signal transduction cascade which eventually results in cell division. There is little doubt that high affinity cell surface receptors provide the specificity and sensitivity of cells to polypeptide growth factors. However, there is accumulating evidence that various growth factors also have nuclear sites of action. Nuclear binding sites for insulin, epidermal growth factor, nerve growth factor and PDGF have been demonstrated, and their accumulation in the nucleus has been found to be rapid and specific [80]. bFGF administered to G_1-arrested aortic endothelial cells has been localized to the nucleolus and shown to stimulate ribosomal gene transcription [78]. Furthermore, it has been shown that nuclear and nucleolar localization of bFGF in adult bovine aortic endothelial cells are related to the state of proliferation [79]. Exogenously administered bFGF enters the nucleus and nucleolus of growing cells at the G_1-S transition. bFGF is not detected in the nucleus of quiescent confluent cells despite a continuous cellular uptake of bFGF throughout the cell cycle. It is of interest to note that direct nuclear translocation of endogenous bFGF was not observed suggesting that bFGF has to get to the cell surface first in order to be transported to the nucleus. Nuclear bFGF may control basal cell growth by regulation of gene transcription via a direct interaction with DNA or through activation of nuclear protein kinases.

Autocrine transformation by bFGF

Basic FGF is synthesized by many normal cells and adult tissues. Despite the abundance of bFGF in the pericellular space *in vitro* and *in vivo,* cells with available, functional FGF receptors (e.g. endothelial cells) do not appear to be transformed. NIH-3T3 cells transfected with native bFGF cDNA and expressing 20 to 100 times as much bFGF as parental 3T3 cells and 10 times as much as endothelial cells acquire an enhanced proliferation rate (Fig. 3) and a higher saturation density. However they are density-arrested *in vitro* and non-tumorigenic in syngeneic mice (Table 2) [21, 24, 86]. Cells transfected with native acidic FGF cDNA show similar properties *in vitro* and *in vivo* [87]. Extremely high over-expression of bFGF has been reported to result in cell transformation [19, 20]. 3T3 cell lines that over-express bFGF at a very high level, 300–1000 fold greater than endothelial cells are morphologically altered, and their FGF receptors are down-regulated. The conditioned media of these cells contain high levels of biologically active bFGF. Thus, it is possible that cells over-expressing of bFGF are transformed in culture as a result of continuous external stimulation by released bFGF. Tumors produced in nude mice by cells over-expressing either acidic or basic FGF are of low frequency, small, slow-growing and spontaneously regressing [20, 86, 87] and might arise from a local paracrine stimulation of host cells.

It is possible that external autocrine stimulation by bFGF released from cells over-expressing bFGF can confer growth advantage *in vitro* but may prove to be inefficient *in vivo* since soluble, non-matrix bound bFGF is very labile and rapidly inactivated [83–85]. The instability of secreted bFGF may account for the observation that melanocytes transfected with native bFGF acquire properties *in vitro* similar to those of metastatic melanoma cells but are not tumorigenic *in vivo* [88]. Moreover in a reconstituted skin environment, bFGF transformants revert to a normal melanotic phenotype with restricted growth indicating that constitutive production of bFGF by itself is insufficient to make melanocytes tumorigenic.

NIH cells transfected with a construct, in which bFGF cDNA is altered by addition of a signal sequence, undergo autocrine transformation and exhibit morphological and biochemical alterations characteristic of highly transformed cells [21, 24]. The signal peptide bFGF (spbFGF)-transformed cells have an accelerated proliferation rate, are not density arrested and are capable of anchorage-independent growth. spbFGF cells possess few functional FGF receptors at the cell surface supporting the idea that these cells are transformed by constitutive interaction with, and down-regulation of, the FGF receptor [24]. FGF receptors in NIH 3T3 cells transformed by the FGF-related oncogene, K-*fgf,* are also down-regulated [19, 24] suggesting that autocrine transformation by K-*fgf* is mediated via the FGF receptor. FGF receptors are not down-regulated in *ras*-transformed cells even though these cells produce substantial amounts of bFGF [89], suggesting that the bFGF produced by *ras*-transformed cells is not involved in autocrine transformation.

We suggest that spbFGF transforms cells via an internal autocrine loop, since these cells do not secrete biologically active bFGF despite the presence of a signal peptide, and their proliferation rate is not affected by neutralizing antibodies to bFGF. Such an 'internal' interaction may include all subcellular sites including the plasma membrane where bound bFGF cannot be dissociated from the high affinity receptor by a large excess of native bFGF or other measures known to release FGF from the receptor.

Suramin, a drug reported to interfere with growth factor receptor interactions [8, 90, 91], rapidly reverts the transformed phenotype, inhibits accelerated and anchorage-independent growth and induces restoration of cross-linkable FGF receptors in the plasma membrane of spbFGF-transformed cells [24]. Suramin would have to be taken up by spbFGF-transformed cells in order to disrupt an internal autocrine loop, and suramin uptake into endosomes has been demonstrated in v-*sis* transformed cells [8]. However, suramin is a highly charged, membrane-impermeable molecule [92], and it cannot be excluded that in spbFGF-transformed cells, occupied FGF-receptor complexes reside in the plasma membrane in a state which is

inaccessible to exogenous bFGF and neutralizing antibodies but which is accessible to suramin. Suramin might also work indirectly, via a secondary yet unidentified mediator, to interrupt bFGF receptor interactions.

A basic question still remains as to the molecular mechanism by which a signal peptide-bearing bFGF or FGF related-oncoprotein results in transformation. It could be that a structural, post-translational modification of FGF now being processed through the endoplasmic reticulum and Golgi apparatus may lead to an atypical interaction with the receptor. The localization of bFGF-receptor interaction is very likely to play an important role in spbFGF-induced autocrine transformation. While native bFGF can interact with the FGF receptor only at the cell surface, a signal peptide-bFGF might be able to bind the receptor inside the cell anywhere along the secretory pathway. Such an intracellular interaction may activate different modes of signal transduction by exposing novel substrates to the tyrosine kinase activity of the FGF receptor.

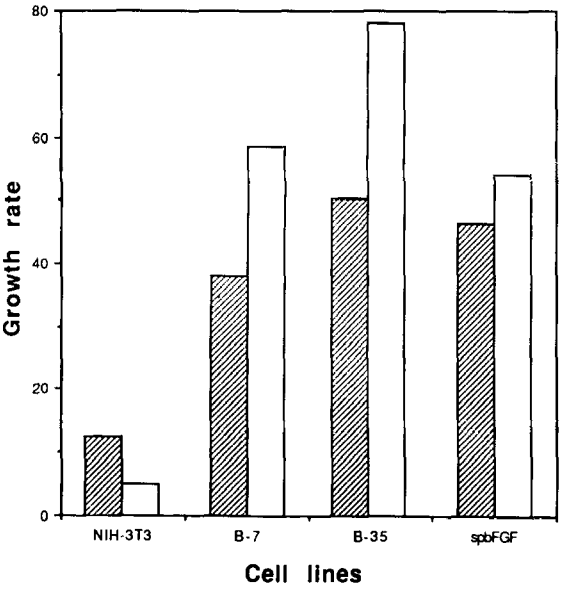

Fig. 3. The relation of basal growth rates of cloned bFGF-transfected NIH 3T3 cells and the amount of bFGF synthesized by these cells. Hatched bars – growth rate, open bars – levels of bFGF as determined by immunoprecipitation. 1) NIH 3T3 cells; 2) B-7 cells – NIH 3T3 cells expressing native bFGF; 3) B-35 – NIH 3T3 cells expressing higher levels of native bFGF; 4) spbFGF – NIH 3T3 cells expressing signal peptide bFGF. Cells were plated in 24 well plates at 10^4 cells/well and counted after 72 hours in culture. The amount of bFGF synthesized was determined by laser densitometry of bands generated in a quantitative immunoprecipitation of bFGF.

The malignant potential of signal peptide bFGF

Cancer cells are characterized by their autonomous unrestrained growth together with their ability to invade and colonize sites and tissues inaccessible to normal cells. Activation of a tumorigenic and metastatic phenotype *in vivo* is a more critical and appropriate index for evaluating the transforming potential of an oncogene than is transformation in culture. When injected into syngeneic mice, spbFGF-transformed cells form large and rapidly growing tumors. The differential transforming and malignant potential of native and signal peptide

Table 2. Tumorigenic and metastatic potential of NIH 3T3 cells expressing native bFGF and signal peptide bFGF

Cell lines	bFGF activity units/10^4 cells	Tumors frequency	%	Metastases frequency	%
NIH 3T3	0.05	0/12	0	0/12	0
bFGF (B7)	0.80	0/9	0	0/12	0
bFGF (B35)	4.60	0/9	0	0/6	0
spbFGF	0.80	21/24	88	23/28	82
NIH EJ 3T3	0.45	11/12	92	17/22	77

NIH 3T3 cells expressing moderate levels of bFGF (B7 cells), high levels of bFGF (B35 cells) and signal peptide bFGF (spbFGF cells), as well as *ras*-transformed cells (NIH EJ 3T3) were injected into syngeneic NIH/NSF mice. The number of mice with apparent tumors was measured 4 weeks after a subcutaneous injection of 5×10^5 cells. The number of mice with apparent lung metastases were estimated by explorative thoracotomy performed 4 weeks after tail vein injection of 3×10^5 cells.

bFGF has been analyzed in an experimental metastasis model *in vivo*. Injection of cells expressing native bFGF even at levels 100 times greater than parental 3T3 cells failed to induce any detectable macroscopic or microscopic lung metastases (Table 2). spbFGF cells on the other hand, demonstrated a high metastatic potential with tumor incidence and numbers comparable to those induced by *ras*-transformed cells. spbFGF cells formed large and rapidly progressive and aggressive lung tumors apparent within 2 to 3 weeks after the injection of the transformed cells. The metastatic and tumorigenic potential of spbFGF-transformed cells may be related to their increased ability to degrade matrix and to their possession of altered fibronectin receptors, i.e., integrins (Yayon and Klagsbrun, in preparation).

Conclusions

Basic FGF does not appear to be a transforming protein. Transfection and over-expression of bFGF in cells alters morphology and increases growth rates but does not induce tumorigenicity or promote metastatic potential *in vivo*. These results are consistent with numerous observations showing that a transformed phenotype *in vitro* is not necessarily indicative of a neoplastic phenotype *in vivo*. For example, malignant and non-tumorigenic human prostate carcinoma cell lines show similar morphology and growth rates *in vitro* [93]. In a model system for cancer suppression genes, transfer of a normal chromosome 11 into Wilm's tumor cells supresses their tumorigenicity, but the cells are unaltered in culture [94].

The lack of a signal peptide ostensibly prevents bFGF from being involved in autocrine stimulatory loops. However if bFGF is altered by the addition of a signal sequence, cells transfected with this construct exhibit morphological and biochemical alterations characteristic of highly transformed cells and acquire a highly tumorigenic and metastatic potential as well. Possession of a signal sequence may be a prerequisite for a growth factor gene to become a transforming oncogene. Acquisition of a signal peptide converts bFGF into a transforming protein analogous to FGF-related oncogenes which naturally have signal peptide sequences and are transforming. We speculate that the hydrophobic signal sequences allow transforming growth factors to gain access to otherwise inaccessible compartments and that inappropriate subcellular localization plays a major role in bFGF-induced cellular transformation.

Key unanswered questions

There has been major progress in recent years in understanding the biochemical and biological characteristics of the FGF family. However, our knowledge is still very limited as to how bFGF expression is regulated at the molecular level and what role bFGF and related oncogenes play in physiological and pathological processes *in vivo*. A number of specific key questions need to be addressed. For example:

1. Where is bFGF localized in cells and is the subcellular localization of bFGF different in normal and transformed cells?
2. What is the significance of differential localization to the mechanism of autocrine transformation in general?
3. What are the mechanisms for transport of bFGF into the extracellular matrix?
4. What is the biological role of extracellular matrix bFGF?
5. What are the signal transduction pathways associated with bFGF mitogenesis on one hand and differentiation on the other hand?
6. How is the activity of bFGF regulated in tissues which have abundant amounts of bFGF and FGF receptors but which are neither growing nor undergoing active angiogenesis.

These and many other questions need to be addressed so that ultimately the role of bFGF in growth control and transformation can be understood.

Acknowledgements

This work was supported by National Institutes of

Health Grants CA7392 and CA45548. We thank Patricia Farber for editing the manuscript.

References

1. Sporn MB, Roberts AB: Autocrine growth factors and cancer. Nature 313: 745–747, 1985
2. Waterfield MD, Scrace GT, Whittle N, Stroobant P, Johnsson A, Wasteston A, Westermark B, Heldin CH, Huang JS, Deuel TF: Platelet-derived growth factor is structurally related to the putative transforming protein p28 of simian sarcoma virus. Nature 304: 35–39, 1983
3. Stocking C, Loliger C, Kawai M, Suciu S, Gough N, Osterag W: Identification of genes involved in growth autonomy of hematopoietic cells by analysis of factor-independent mutants. Cell 53: 869–879, 1988
4. Browder TM, Abrams JS, Wong PMC, Nienhuis AW: Mechanism of autocrine stimulation in hematopoietic cells producing interleukin 3 after retro virus-mediated gene transfer. Mol Cell Biol 9: 204–213, 1989
5. Keating MT, Williams LT: Autocrine stimulation of intracellular PDGF receptors in v-sis transformed cells. Science 239: 914–916, 1988
6. Doolittle RF, Hunkapiller MW, Hood LE, Devare SG, Robbins KC, Aaronson SA, Antoniades NH: Simian sarcoma virus oncogene, v-sis, is derived from the gene (or genes) encoding a platelet-derived growth factor. Science 221: 275–277, 1983
7. Huang JS, Huang SS, Deuel TF: Transforming protein of simian sarcoma virus stimulates autocrine growth of SSV-transformed cells through PDGF cell-surface receptors. Cell 39: 79–87, 1984
8. Huang SS, Huang JS: Rapid turnover of the platelet-derived growth factor receptor in sis-transformed cells and reversal by suramin. J Biol Chem 263: 12608–12618, 1988
9. Folkman J, Klagsbrun M: Angiogenic factors. Science 235: 442–447, 1987
10. Gospodarowicz D: Fibroblast growth factor. In: Pimental E, Perucho M (eds) Critical Reviews in Oncogenesis. CRC Press, Boca Raton, FL., 1989, pp 1–26
11. Klagsbrun M: The fibroblast growth factor family: structural and biological properties. Progress in Growth Factor Research 1: 207–235, 1989
12. Dickson C, Peters G: Potential oncogene product related to growth factors. Nature 326: 833, 1987
13. Sakamoto H, Mori M, Taira M, Yoshida T, Matsukawa S, Shimizu K, Sekiguchi M, Terada M, Sugimura T: Transforming gene from human stomach cancers and a noncancerous portion of stomach mucosa. Proc Natl Acad Sci USA 83: 3997–4001, 1986
14. Delli-Bovi PD, Basilico C: Isolation of a rearranged human transforming gene following transfection of Kaposi sarcoma cDNA. Proc Natl Acad Sci USA 84: 5660–5664, 1987
15. Zhan X, Bates B, Hu X, Goldfarb M: The human FGF-5 oncogene encodes a novel protein related to fibroblast growth factors. Mol Cell Biol 8: 3487–3495, 1988
16. Marics I, Adelaide J, Raybaud F, Mattei M-G, Coulier F, Planche J, De Lapeyriere O, Birnbaum D: Characterization of the *HST*-related FGF-6 gene, a new member of the fibroblast growth factor gene family. Oncogene 4: 335–340, 1988
17. Neufeld G, Mitchell R, Ponte P, Gospodarowicz D: Expression of human basic fibroblast growth factor cDNA in baby hamster kidney-derived cells results in autonomous cell growth. J Cell Biol 106: 1385–1394, 1988
18. Sasada R, Kurokawa T, Iwane M, Igarashi K: Transformation of mouse BALB/c 3T3 cells with human basic fibroblast growth factor cDNA. Mol Cell Biol 8: 588–594, 1988
19. Moscatelli D, Quarto N: Transformation of NIH 3T3 cells with basic fibroblast growth factor or the hst/K-fgf oncogene causes down-regulation of the fibroblast growth factor receptor: reversal of morphological transformation and restoration of receptor number by suramin. J Cell Biol 109: 2519–2527, 1989
20. Quarto ND, Talarico A, Sommer R, Florkiewicz HD, Basilico C, Rifkin DB: Transformation by basic fibroblast growth factor requires high levels of expression: comparison with transformation by *hst*/K-fgf. Oncogene Res 1989
21. Rogelj S, Weinberg RA, Fanning P, Klagsbrun M: Basic fibroblast growth factor fused to a signal peptide transforms cells. Nature 331: 173–175, 1988
22. Blam SB, Mitchell R, Tischer E, Rubin JS, Silva M, Silver S, Fiddes JC, Abraham JA. Aaronson SA: Addition of growth hormone secretion signal to basic fibroblast growth factor results in cell transformation and secretion of aberrant forms of the protein. Oncogene 3: 129–136, 1988
23. Rogelj S, Klagsbrun M: Oncogenic transformation by basic fibroblast growth factor. In: Krey LC, Gulyas BJ, McCracken JA (eds) Autocrine and Paracrine mechanisms in reproductive endocrinology. Plenum Publishing Corporation, 1989, pp 19–28
24. Yayon A, Klagsbrun M: Autocrine transformation by signal peptide basic fibroblast growth factor; reversal by suramin. Proc Natl Acad Sci USA 87: 5346–5350, 1990
25. Connolly DT, Stoddard BL, Harakas NK, Feder J: Human fibroblast-derived growth factor is a mitogen and chemoattractant for endothelial cells. Biochem Biophys Res Commun 144: 705–712, 1987
26. Sato Y, Rifkin D: Autocrine activities of basic fibroblast growth factor: regulation of endothelial cell movement, plasminogen activator synthesis, and DNA synthesis. J Cell Biol 107: 1199–1205, 1988
27. Senior RM, Huang SS, Griffin GL, Huang JS: Brain derived growth factor is a chemoattractant for fibroblasts and astroglial cells. Biochem Biophys Res Commun 14: 67–72, 1986
28. Gospodarowicz D, Moran J, Braun D, Birdwell CR: Clonal growth of bovine vascular endothelial cells in culture: fibroblast growth factor as a survival agent. Proc Natl Acad Sci USA 73: 4120–4124, 1986

29. Kato Y, Gospodarowicz D: Sulfated proteoglycan synthesis by confluent cultures of rabbit costal chondrocytes grown in the presence and absence of fibroblast growth factor. J Cell Biol 100: 477–485, 1985
30. Broad TE, Ham RG: Growth and adipose differentiation of sheep preadipocyte fibroblasts in serum free medium. Eur J Biochem 135: 33–39, 1983
31. Togari A, Dickens G, Kuzuya H, Guroff G: The effect of fibroblast growth factor on PC12 cells. J Neurosci 5: 307–316, 1985
32. Walicke P, Cowan M, Ueno N, Baird A, Guillemin R: Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension. Proc Natl Acad Sci USA 83: 3012–3016, 1986
33. Neufeld G, Gospodarowicz D, Dodge L, Fujii DK: Heparin modulation of the neurotropic effects of acidic and basic fibroblast growth factors and nerve growth factors on PC-12. J Cell Physiol 131: 131–140, 1987
34. Klagsbrun M: Angiogenesis factors. In: Ryan U (eds) Endothelial Cells, Vol 1. CRC Press, Boca Raton, 1988, pp 37–50
35. Folkman J, Klagsbrun M: A family of angiogenic peptides. Nature 329: 671–672, 1987
36. Rubin JS, Hiroyuki O, Finch PW, Taylor WG, Rudikoff S, Aaronson SA: Purification and characterization of a newly identified growth factor specific for epithelial cells. Proc Natl Acad Sci USA 86: 802–806, 1989
37. Dickson C, Smith R, Brookes S, Peters G: Tumorigenesis by mouse mammary tumor virus: proviral activation of a cellular gene in the common integration region *int*-2. Cell 37: 529–536, 1984
38. Dickson C, Dixon M, Deed R, Smith R, Brookes S, Acland P, Peters G: The *int*-2 gene; a protein implicated in normal fetal development and virally induced mammary tumorigenesis. J Cell Biochem Supp 13b: 78, 1989
39. Jakobovits A, Shackleford GM, Varmus HE, Martin GR: Two proto-oncogenes implicated in mammary carcinogenesis, Int-1 and *Int*-2, are independently regulated during mouse development. Proc Natl Acad Sci USA 83: 7806–7810, 1986
40. Mansour SL, Martin GR: Four classes of mRNA are expressed from the mouse *int*-2 gene, a member of the FGF gene family. EMBO J 7: 2035–2041, 1988
41. Smith R, Peters G, Dickson C: Multiple RNAs expressed from the *int*-2 gene in mouse embryonal carcinoma cell lines encode a protein with homology to fibroblast growth factors. EMBO J 7: 1013–1022, 1988
42. Adelaide J, Mattei M-G, Marics I, Raybaud F, Planche J, DeLapeyriere O, Birnbaum D: Chromosomal localization of the *hst* oncogene and its co-amplification with the *int*-2 oncogene in a human melanoma. Oncogene 2: 413–416, 1988
43. Zhou DJ, Casey G, Cline M: Amplification of human *int*-2 in breast cancers and squamous carcinomas. Oncogene 2: 279–282, 1988
44. Yoshida T, Miyagawa K, Odagiri H, Sakamoto H, Little P, Terada M, Sugimura T: Genomic sequence of *hst*, a transforming gene encoding a protein homologous to fibroblast growth factors and the *int*-2-encoded protein. Proc Natl Acad Sci USA 84: 7305–7309, 1987
45. Delli-Bovi P, Curatola AM, Kern FG, Greco A, Ittmann M, Basilico C: An oncogene isolated by transfection of Kaposi's sarcoma DNA encodes a growth factor that is a member of the FGF family. Cell 50: 729–737, 1987
46. Delli-Bovi P, Curatola AM, Newman KM, Sato Y, Moscatelli D, Hewick RM, Rifkin DB, Basilico C: Processing, secretion and biological properties of a novel growth factor of the fibroblast growth family with oncogenic potential. Mol Cell Biol 8: 2933–2941, 1988
47. Yoshida T, Miyagawa K, Sagamoto H, Katoh O, Sugimura T, Terada M: *HSTI* oncogene encoding an FGF-related growth factor: its product, expression, clustering and coamplification with *int*-2. J Cell Biochem Supp 13b: 3, 1989
48. Hebert JM, Basilico G, Goldfarb M, Haub O, Martin GR: Isolation of cDNAs encoding four mouse FGF family members and characterization of their expression patterns during embryogenesis. Developmental Biol 138: 454–463, 1990
49. Neufeld G, Gospodarowicz D: The identification and partial characterization of the fibroblast growth factor receptor of baby hamster kidney cells. J Biol Chem 260: 13860–13868, 1985
50. Olwin BB, Hauschka SD: Identification of the fibroblast growth factor receptor of Swiss 3T3 cells and mouse skeletal muscle myoblasts. Biochem 25: 3487–3492, 1986
51. Friesel R, Burgess WH, Mehlman T, Maciag T: The characterization of the receptor for endothelial cell growth factor by covalent ligand attachment. J Biol Chem 261: 7581–7584, 1986
52. Huang SS, Huang JS: Association of bovine brain derived growth factor receptor with protein tyrosine kinase activity. J Biol Chem 261: 9568–9571, 1986
53. Moenner M, Chevallier B, Badet J, Barritault D: Evidence and characterization of the receptor to eye derived growth factor I, the retinal form of basic fibroblast growth factor on bovine epithelial cells. Proc Natl Acad Sci USA 83: 5024–5028, 1986
54. Neufeld G, Gospodarowicz D: Basic and acidic fibroblast growth factors interact with the same cell surface receptors. J Biol Chem 261: 5631–5637, 1986
55. Neufeld G, Gospodarowicz D: Identification of the fibroblast growth factor receptor in human vascular endothelial cells. J Cell Physiol 136: 537–542, 1988
56. Moscatelli D: High and low affinity binding sites for basic fibroblast growth factor on cultured cells: Absence of a role for low affinity binding in the stimulation of plasminogen activator production by bovine capillary endothelial cells. J Cell Physiol 131: 123–130, 1987
57. Moscatelli D: Metabolism of receptor-bound and matrix-bound basic fibroblast growth factor by bovine capillary endothelial cells. J Cell Biol 107: 753–759, 1988
58. Kan M, DiSorbo D, Hou J, Hoshi H, Mansson P-E, McKeehan W: High and low affinity binding of heparin-

binding growth factor to a 130-kDa receptor correlates with stimulation and inhibition of growth of a differentiated human hepatoma cell. J Biol Chem 263: 11306–11313, 1988
59. Vlodavsky I, Folkman J, Sullivan R, Friedman R, Ishai-Michaeli R, Sasse J, Klagsbrun M: Endothelial cell-derived basic fibroblast growth factor; synthesis and deposition into subendothelial extracellular matrix. Proc Natl Acad Sci USA 84: 2292–2296, 1987
60. Baird A, Ling N: Fibroblast growth factors are present in the extracellular matrix produced by endothelial cells *in vitro:* implications for a role of heparinase-like enzymes in the neovascular response. Biochem Biophys Res Commun 142: 428–435, 1987
61. Weiner H, Swain J: Acidic fibroblast growth factor mRNA is expressed by cardiac myocytes in culture and the protein is localized to the extracellular matrix. Proc Natl Acad Sci USA 86: 2683–2687, 1989
62. Globus RK, Plouet J, Gospodarowicz D: Cultured bovine bone cells synthesize basic fibroblast growth factor and store it in their extracellular matrix. Endocrinol 124: 1539–1547, 1989
63. Folkman J, Klagsbrun M, Sasse J, Wadzinski M, Ingber D, Vlodavsky I: Heparin-binding angiogenic protein – basic fibroblast growth factor – is stored within basement membrane. Amer J Pathol 130: 393–400, 1988
64. Casscells W, Speir E, Sasse J, Klagsbrun M, Allen P, Lee M, Calvo B, Chiba M, Haggroth L, Folkman J, Epstein SE: Isolation, characterization, and localization of Heparin-binding growth factors in the heart. J Clin Invest 85: 433–441, 1990
65. Gonzalez AM, Buscaglia M, Ong M, Baird A: Distribution of basic fibroblast growth factor in the 18-day rat fetus: localization in the basement membranes of diverse tissues. J Cell Biol 110: 753–765, 1990
66. Muesch A, Hartman E, Rohde K, Rubartelli A, Sitia R, Rapoport TA: A novel pathway for secretory proteins? TIBS 15: 86–88, 1990
67. Flaumenhaft R, Moscatelli D, Saksela O, Rifkin DB: The role of extracellular matrix in the action of basic fibroblast growth factor: matrix as a source of growth factor for long term stimulation of plasminogen activator production and DNA synthesis. J Cell Physiol 140: 75–81, 1989
68. Bashkin P, Doctrow S, Klagsbrun M, Svahn CM, Folkman J, Vlodavsky I. Basic fibroblast growth factor binds to subendothelial extracellular matrix and is released by heparinase and heparin-like molecules. Biochem 28: 1737–1743, 1989
69. Saksela O, Rifkin DB: Release of basic fibroblast growth factor-heparan sulfate complexes from endothelial cells by plasminogen activator-mediated proteolytic activity. J Cell Biol 110: 767–775, 1990
70. Friesel R, Burgess WH, Maciag T: Heparin-binding growth factor stimulates tyrosine phosphorylation in NIH 3T3 cells. Mol Cell Biol 9: 1857–1865, 1989
71. Coughlin SR, Barr P, Cousens L, Fretto L, Williams LT: Acidic and basic fibroblast growth factors stimulate tyrosine kinase activity *in vivo*. J Biol Chem 263: 988–993, 1988
72. Nanberg E, Morris C, Vara F, Higgins T, Rozengurt E: FGF utilizes a novel signal transduction pathway in Swiss 3T3 fibroblasts. J Cell Biochem Supp 13b: 156, 1989
73. Bikfalvi A, Dupuy E, Inyang AL, Fayein N, Leseche G, Courtois Y, Tobelem G: Binding, internalization, and degradation of basic fibroblast growth factor in human microvascular endothelial cells. Exp Cell Res 181: 75–84, 1989
74. Lee PA, Johnson DE, Cousens L, Fried V, Williams LT: Purification and the cDNA cloning of basic FGF receptor. Science 245: 57–60, 1989
75. Ruta M, Burgess W, Givol D, Epstein J, Neiger N, Kaplow J, Crumley G, Dionne C, Jaye M, Schlessinger J: Receptor for acidic fibroblast growth factor is related to the tyrosine kinase encoded by the fms-like gene (FLG). Proc Natl Acad Sci USA 86: 8722–8726, 1989
76. Kornbluth S, Paulson KE, Hanafusa H: Novel tyrosine kinase identified by phosphotyrosine antibody screening of cDNA libraries. Mol Cell Biol 8: 5541–5544, 1988
77. Burrows LW, Olwin B: Isolation of a receptor for acidic and basic fibroblast growth factor from embryonic chick. J Biol Chem in press, 1990
78. Bouche G, Gas N, Prats H, Baldin V, Tauber JP, Tessie J, Almaric F: Basic fibroblast growth factor enters the nucleolus and stimulates the transcription of ribosomal genes in ABAE cells in G_0–G_1 transition. Proc Natl Acad Sci USA 84: 6770–6774, 1987
79. Baldin V, Roman AM, Bosc-Bierne I, Amalric F, Bouche G: Translocation of bFGF to the nucleus in G_1 phase cell cycle specific in bovine aortic endothelial cells. EMBO J in press 1990
80. Burwen SJ, Jones AL: The association of polypeptide hormones and growth factors with the nuclei of target cells. TIBS 12: 159–162, 1987
81. Fedarko NS, Conrad HE: A unique heparan sulfate in the nuclei of hepatocytes: Structural changes with the growth state of the cells. J Cell Biol 102: 587–599, 1986
82. Masayuki I, Fedarko NS, Conrad HE: Transport of heparan sulfate into the nuclei of hepatocytes. J Biol Chem 261: 13575–13580, 1986
83. Gospodarowicz D, Cheng J: Heparin protects basic and acidic FGF from inactivation. J Cell Physiol 128: 475–484, 1986
84. Sommer A, Rifkin D: Interaction of heparin with human basic fibroblast growth factor: protection of the angiogenic protein from proteolytic degradation by a glycosaminoglycan. J Cell Physiol 138: 215–220, 1989
85. Saksela O, Moscatelli D, Sommer A, Rifkin DB: Endothelial cell-derived heparan sulfate binds basic fibroblast growth factor and protects it from proteolytic degradation. J Cell Biol 107: 743–751, 1988
86. Rogelj S, Weinberg RA, Fanning P, Klagsbrun M: Characterization of tumors produced by signal-peptide-bFGF transformed cells. J Cell Biochem 39: 13–23, 1989
87. Jaye M, Lyall RM, Mudd R, Schlessinger J, Sarver N:

Expression of acidic fibroblast growth factor cDNA confers growth advantage and tumorigenesis to Swiss 3T3 cells. EMBO J 7: 963–969, 1988

88. Dotto PG, Moellmann G, Ghosh S, Edwards M, Halaban RJ: Transformation of murine melanocytes by basic fibroblast growth factor cDNA and oncogenes and selective suppression of the transformed phenotype in a reconstituted cutaneous environment. J Cell Biol 109: 3115–3128, 1989
89. Iberg N, Rogelj S, Fanning P, Klagsbrun M: Purification of 18 kDa and 22 kDa forms of basic fibroblast growth factor from rat cells transformed by the ras oncogene. J Biol Chem 264: 19951–19955, 1989
90. Garret JS, Cuughlin SZR, Niman HL, Trmble PM, Giels GM, Williams LT: Blockade of autocrine stimulation in simian sarcoma virus-transformed cells reverses down regulation of platelet-derived growth factor receptors. Proc Natl Acad Sci USA 81: 7466–7470, 1984
91. Betsholtz C, Johnson A, Heldin CH, Wastermark B: Efficient reversion of simian sarcoma virus-transformation and inhibition of growth factor-induced mitogenesis by suramin. Proc Natl Acad Sci USA 83: 6440–6444, 1986
92. Wilson EJ, Wormall A: Studies on suramin. Biochem J 45: 224–231, 1949
93. Bookstein R, Shew J, Chen P, Scully P, Lee W: Suppression of tumorigenicity of human prostate carcinoma cells by replacing a mutated RB gene. Science 247: 712–725, 1990
94. Weissman BE, Saxon PJ, Raspuale SR, Jones GR, Geiser AG, Stanbridge EJ: Introduction of a normal human chromosome 11 into a Wilms' tumor cell line controls its tumorigenic expression. Science 236: 175–180, 1987

Address for offprints:
A. Yayon,
Children's Hospital,
300 Longwood Avenue,
Boston, MA 02115, USA