10. The role of fibroblast growth factors and related oncogenes in tumor growth

Harold Brem and Michael Klagsbrun

The FGF family

Introduction

The fibroblast growth factors (FGF) constitute a family of seven mitogenic and structurally homologous polypeptides found in a variety of cells and tissues [for reviews see 1–9]. The FGF family includes acidic FGF (aFGF), basic FGF (bFGF), *int*-2, *hst*/K-*fgf*, FGF-5, FGF-6, and keratinocyte growth factor (KFGF) (Table 1). A simplified nomenclature has been proposed in which the FGF family members are named FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, and FGF-7, respectively. Structurally, the homologies between the seven FGF family members is 35–45%, with the homologies being greatest in the internal regions of these proteins. Their molecular weights range from 18 to 30 kDa. They all share with aFGF and bFGF the 3 exon-2 intron structure and the conservation of two cysteine residues. An important structural difference between the FGF family members is that, unlike the others, aFGF and bFGF lack signal peptide sequences and are not secreted proteins. Members of the FGF family, in particular bFGF [10], are also characterized by their strong affinity for heparin. The affinity of bFGF for heparin is manifested in its ability to bind to cell surface heparan sulfate proteoglycan (HSPG), an activity that is required for binding to high-affinity FGF receptors [9]. A schematic representation of the FGF family members portraying domains of sequence homology and signal peptides is shown in Fig. 1.

The biological significance of cell-associated aFGF and bFGF is still a matter of conjecture, while the other five FGFs are thought to be involved in typical paracrine and autocrine growth mechanisms. An important biological distinction is that aFGF, bFGF, and KGF do not transform the cells that produce them, while *int*-2, *hst*/K-*fgf*, FGF-5, and FGF-6 are oncogenes. Unlike aFGF and bFGF, which are found ubiquitously in adult tissue, these oncogenes are expressed primarily during embryogenesis, neonatal development, and in many tumors. KGF is associated mostly with the epithelium.

In this chapter, we will review the structural and biological properties of

Christopher C. Benz and Edison T. Liu (eds.), ONCOGENES AND TUMOR SUPPRESSOR GENES IN HUMAN MALIGNANCIES. *ISBN 0-7923-1960-5*

Table 1. Members of the FGF family

	Common name	MW	Originally found in
FGF-1	acidic FGF (HBGF-1)	18,000	Adult tissue (neural)
FGF-2	basic FGF (HBGF-2)	18,000	Most adult tissue
FGF-3	*int*-2	27,000	Site of MMTV integration, breast carcinoma
FGF-4	*hst*/K-*fgf*	23,000	Human stomach tumor (*hst*), Kaposi's sarcoma (K*FGF*)
FGF-5	*FGF* 5	29,000	Bladder carcinoma, hepatoma
FGF-6	FGF 6	?	Homologous to *hst*
FGF-7	Keratinocyte Growth factor (KGF)	28,000	Epithelial tissue stromal cells

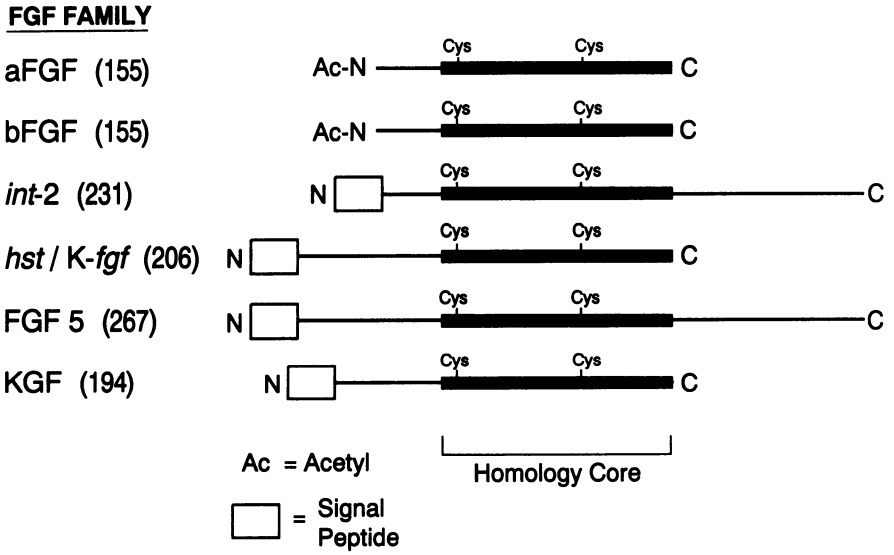

Figure 1. Schematic representation depicting FGF family structural features. The numbers in parentheses represent the numbers of amino acids in the open reading frames. Also depicted are the presence or absence of signal peptide sequences, the N-terminal amino acids blocked by acetylation, the regions of structural homology, and the presence throughout the family of two homologous cys residues.

the individual FGF family members, with an emphasis on their perceived role in tumorigenesis.

aFGF and bFGF (FGF-1 and FGF-2)

Acidic FGF and basic FGF will be discussed together, since only subtle physiological differences have been found between these two well-charac-

terized proteins. Acidic FGF and bFGF have a 53% protein sequence homology [11]. Both are single-chain polypeptides of 154 amino acids with molecular weights of about 18 kDa, as predicted from their cDNA nucleotide sequence. The gene for aFGF is located on chromosome 5 [12], while that of bFGF is located on chromosome 4 [13]. They also have other important structural differences [6,13–15]. Acidic FGF is an anionic protein with a pI of 5.6 [15], while bFGF is very cationic with a pI of about 10 [16]. In addition, there are forms of bFGF containing more than 154 amino acids that have molecular weights of 22–25 kDa [17–19]. These higher molecular weight forms of bFGF are generated by an unusual mechanism in which synthesis is initiated on the CUG start codons, rather than the typical AUG codon that initiates the 18 kDa form [18,19].

An important property of aFGF and bFGF is their interaction with heparin [20,21]. Both bind tightly to columns of immobilized heparin, a property that has facilitated their purification [1,10,15,20]. In addition, heparin stabilizes aFGF and bFGF, and protects them from heat, acid [21], and proteolytic degradation [22]. Acidic FGF and bFGF also bind to heparin-like molecules that are associated with cells. These include heparan sulfate proteoglycans (HSPG) in extracellular matrix and on cell surfaces. It has been suggested that aFGF and bFGF are sequestered or "stored" in the extracellular matrix [23–25] as part of a highly stable FGF-HSPG complex and are released during injury by a combination of proteases and heparinases [23]. The binding of bFGF to cell surface HSPG is a prerequisite for FGF's ability to bind to the FGF high-affinity receptor [9,26] as well as for bFGF mitogenic activity. Thus, heparin binding is an important property that modulates FGF structure, stability, *and* function. Interestingly, bFGF also maintains a high affinity for betacyclodextrin-tetradecasulfate, which structurally resembles heparin [27].

The biological activities of aFGF and bFGF are very similar. Both are important components of endothelial cell growth and differentiation, and stimulate new blood vessel growth, i.e., they are angiogenic in vivo [1]. Their angiogenic activity stems from the ability to stimulate many components in the formation of new blood vessels, such as (1) endothelial cell migration [28], (2) endothelial cell proliferation [29], (3) protease production [30], (4) matrix degradation [31], (5) plasminogen activator activity [32], and (6) capillary tube formation [33].

Acidic FGF and bFGF also stimulate the proliferation of a variety of other cell types in vitro, including fibroblasts [34], vascular smooth muscle cells [35], granulosa cells [36], osteoblasts [37], ovarian epithelial cells [38], oligodendrocytes [39], and keratinocytes [40].

There has been considerable effort to analyze the activities of aFGF and bFGF in vivo. One fruitful area has been the role of these growth factors in modulating wound healing. Since endothelial cells and fibroblasts are critical components of wound healing, mitogens of these cell types, such as aFGF and bFGF, might be expected to accelerate wound repair. In one of the first

studies to elucidate the role of fibroblast growth factor in wound healing, it was demonstrated that bFGF administered to a wound stimulates the formation of a highly vascular granulation tissue [41]. Subsequently, it was found that topical application of bFGF increased tensile strength in sutured linear incisions in rats [42] and accelerated the rate of closure of partial-thickness wounds in pigs [43]. If bFGF is blocked in vivo by local application of antibodies, the wound will not heal, suggesting that bFGF has a natural critical role in wound healing [44].

Folkman and colleagues hypothesized that duodenal ulcers are similar to surgical wounds that require bFGF to heal. Therefore they administered an acid-resistant oral form of bFGF to rats and found that angiogenesis was stimulated in the ulcer bed and the ulcers healed significantly more rapidly [45].

Acidic FGF is also active in vivo. When applied in Gelfoam implants in the peritoneal cavities of rats [46], aFGF stimulates angiogenesis.

One potential important therapeutic use for aFGF, bFGF, and KFGF in patients may be to reverse impaired wound healing. In rodents, the application of topical bFGF has been demonstrated to reverse wound healing impairments produced by systemic steroids and genetic obesity [47], genetically induced diabetes [48], and local bacterial contamination [49]. These results may be applicable to humans as well.

Fibroblast growth factor may also be useful in stimulating a variety of regenerative processes in the central nervous system. For example, bFGF increases neuronal preservation [50] and nerve regeneration [51].

int-*2 (FGF-3)*

int-2 was the first FGF-like oncogene to be described [52–55]. It was so named because of the initial discovery that it was induced to become transcriptionally active after integration (*int*) of the mouse mammary tumor virus into the mouse genome [53]. The *int*-2 gene is expressed in very specific time periods and locations from midgestation until birth in amphibia [56–58]. The *int*-2 gene induces mesoderm in *Xenopus laevis* animal pole cells and stimulates DNA synthesis in mammalian fibroblasts [59]. Based on these studies and others, it has been designated a developmental control gene [56].

Once the structure of *int*-2 was determined its homology to bFGF became apparent. The *int*-2 gene encodes for a protein of 231 amino acids that has a 46% homology to bFGF [60]. As expected for a secreted protein with a signal peptide, the *int*-2 protein can be detected in the endoplasmic reticulum of transfected cells [61]. However, the *int*-2 protein has not, to date, been shown to be an active mitogen and its mechanism in transforming cells is unclear.

As an oncogene induced by mammary tumor virus, *int*-2 would be expected to be expressed by breast tumors. Expression of *int*-2 in transgenic

mice results in epithelial cell hyperplasia in mammary and salivary glands, as well as prostatic epidermal hypertrophy, which results in male sterility [62]. Amplification of the *int*-2 gene has been found in a variety of human tumors, particularly in breast carcinomas [63–66] and squamous cell carcinomas of the head and neck region [66–69]. The clinical implications of these findings appear to be relatively insignificant, since they occur in less than a majority of patients with these tumors and have not been shown to have a significant correlation with outcome in patients in whom *int*-2 is expressed. In breast carcinoma, for example, the percentage of patients who show *int*-2 amplification is usually less than 30% [63–66].

Int-2 is expressed during embryogenesis and tumorigenesis; however, further work is needed to understand its function in oncogenically transforming cells. Since it is rarely found in adult preneoplastic cells, one possible function for *int*-2 is as a clinical prognosticator. Furthermore, its diagnostic significance may be amplified when it is found in the presence of other oncogenes [70–72]. The coamplification of oncogenes may be important for many oncogenes that have not yet had significant clinical impact when expressed in and of themselves. One exception is the expression of N-*myc* in neuroblastomas, which has clinical significance when it is singularly expressed [73].

hst/*K*-fgf *(FGF-4)*

The *hst*/K-*fgf* oncogene was isolated from two sources simultaneously. One source was NIH-3T3 cells transfected with the Kaposi sarcoma DNA, hence the name Kaposi FGF (K-fgf) [74,75]. The other source was NIH-3T3 cells transfected with DNA from a human stomach tumor, hence the name *hst* [76–78]. *hst*/K-*fgf* is located on chromosome 11 band q13 [77], approximately 40–50 kb from the *int*-2 gene. The *hst*/k-*fgf* has 43%, 38%, and 40% sequence homologies to aFGF, bFGF, and *int*-2, respectively.

hst/K-*fgf* has similar biological activities to aFGF and bFGF, but it has different structural features. In particular, the *hst*/K-*fgf* gene encodes for a 206 amino acid primary translation product that contains a hydrophobic signal peptide sequence. In distinction to aFGF and bFGF, the mature 23-kDa 176 amino acid protein of *hst*/k-*fgf* is glycosylated and secreted [75]. The gene for *hst*/K-*fgf* is rarely expressed in adult cells or in adult tissues [79]. It is expressed, however, in embryogenesis, specifically during mid-stage mouse embryogenesis. *hst*/k-*fgf* also stimulates DNA synthesis in mammalian fibroblasts [59]. As expected for an oncogene, *hst*/K-*fgf* synthesis has been demonstrated in a variety of solid tumors, including germ cell [80], esophageal [81], gastric [82], and breast tumors [83]. On the other hand, it is not expressed in hematopoietic tumors, such as leukemias [83]. Interestingly, although *hst*/K-*fgf* was first isolated from cells transfected with Kaposi sarcoma DNA, it has not been detected in the secreted material from cultured Kaposi sarcoma cells [84,85].

It has been suggested that *hst*/K-*fgf* induces the transformed phenotype by binding to cell surface receptors, thereby creating an autocrine closed loop [75]. No specific receptor has been identified for the *hst*/K-*fgf* protein, but it is thought to bind to the same receptor as aFGF and bFGF [85].

FGF-5

FGF-5 was originally isolated by transfection of a human bladder tumor DNA into NIH-3T3 cells [86]. Its gene sequence has 40–50% homology to aFGF and bFGF [87]. The FGF-5 gene is found on human chromosome number 4 [88] and encodes for a 267 amino acid protein with a signal sequence. There is some evidence that FGF-5 synthesis is dramatically increased if there is a deletion or a point mutation in an upstream open reading frame [89].

The FGF-5 protein is secreted as glycoprotein molecules of heterogeneous sizes [89]. It is a potent mitogen for endothelial cells and fibroblasts [87]. Messenger RNA transcripts for FGF-5 are found in nearly all phases of embryogenesis [90] and in the neurons of adult brains [91]. The protein for FGF-5 is also secreted from bladder carcinoma, endometrial carcinoma, and human hepatoma cell lines [87].

FGF-6

FGF-6 is an oncogene originally isolated from a mouse plasmid library by screening with the *hst*/K-*fgf* gene [92]. The FGF-6 gene is found on chromosome 12 band p13, unlike *int*-2 and *hst*/K-*fgf*, which are localized on chromosome 11 band q13 [93]. Transfection of NIH-3T3 cells with the FGF-6 gene transforms them. The amino acid sequence for FGF-6 is 70% identical to the aFGF product at the C terminus. FGF-6 is the least characterized of the FGF family members and there are very little data to date on expression of the FGF-6 protein.

KGF

The keratinocyte growth factor (KGF) has a 39% homology to bFGF [94]. The KGF gene encodes for a primary translation product of 194 amino acids, and the mature protein does have a signal peptide and is secreted. KGF is present in stromal cells (i.e., fibroblasts) derived from epithelial tissues and is present in embryonic and adult tissue [94]. It has not to date been identified in tumor cells. Unlike other FGF family members, it is neither an endothelial cell growth/angiogenesis factor nor is it an oncogene. Instead, it is a highly specific mitogen for epithelial cells, in general, and for keratinocytes in particular [94]. Thus it differs from aFGF and bFGF, which do not show such target cell specificity.

Table 2. Presence of fibroblast growth factors in human tumors

Tumor	aFGF	bFGF	*int*-2	*hst*/K-*fgf*	FGF-5	References
Adrenal carcinoma		+				175
Basal cell carcinoma		+				177
Bladder carcinoma		+			+	86,147
Brain tumors						
Gioblastoma	+	+				170–173
Meningioma		+				171
Acoustic neuroma		+				150
Pituitary tumors		+				151
Astrocytoma		+				174
Breast carcinoma		+	+	+		63–66,83, 152–155 169
Cervical carcinoma		+				160
Chollangiocellularcarcinoma		+				156
Colon carcinoma		+				78,179
Embryonal carcinoma		+		+		157,163
Endometrial carcinoma					+	87
Esophageal squamous cell carcinoma			+	+		67–69,81 72,158,159
Gastric adenocarcinoma				+		72,77,78,82
Hepatoma		+				87,160–162
Kaposi sarcoma		+				164,181
Laryngeal squamous cell carcinoma			+			67
Melanoma		+	+	+		70,160,177
Neuroblastoma	+	+				165,177
Oral cavity & tongue			+			67
Osteosarcoma		+				177
Ovarian carcinoma			+			166
Pancreatic adenocarcinoma		+				167
Renal cell carcinoma		+				147,149,168
Rhabdomyosarcoma	+	+				178,180
Teratocarcinoma		+	+	+		80,176
Tonsil squamous cell carcinoma			+			67

The FGF family and tumor growth

Members of the FGF family are expressed in animal and human tumors (for human tumors see, Table 2). The four FGF oncogenes, *int*-2, *hst*/K-*fgf*, FGF-5, and FGF-6 are involved in autocrine transformation of cells. The role of aFGF and bFGF is less clear, since they are found in both normal and tumor cells. Furthermore, tumorigenicity has not been directly correlated with aFGF or bFGF expression. Normal endothelial cells synthesize more bFGF than is expressed in many tumor cell lines [24,95]. The normal phenotype of these endothelial cells is maintained, even though these cells have FGF receptors and could in theory participate in autocrine transformation. Secondly, aFGF and bFGF are not secreted. Thus, even if expressed by tumor cells they may not be able to iduce autocrine trans-

formation, which typically requires interaction of a *secreted* growth factor with its receptor on the same cell type. Lack of active secretion also limits possible paracrine activity, unless aFGF or bFGF are released by alternative mechanisms, such as cell death.

There are conditions, however, in which aFGF or bFGF might induce autocrine cell transformation; for example, acquisition by FGF of a signal peptide. Cells transfected with native bFGF cDNA and overexpressing bFGF acquire an enhanced proliferation rate and a higher saturation density, evidence of a transformed phenotype. However, they remain density arrested and are nontumorigenic in syngeneic mice [96], suggesting that transformation in vitro is not necessarily correlated with tumorigenicity in vivo. Cells transfected with native aFGF cDNA are similarly nontumorigenic [97]. One possible explanation is that cells overexpressing the FGFs might release small but sufficient amounts of growth factor for stimulating autocrine growth in vitro but that in vivo this material diffuses away. Alternatively, aFGF and/or bFGF transform cells in culture by some type of internal autocrine mechanism in which FGFs are not released but interact with intracellular FGF receptors. This might occur in vitro to stimulate transformation but for some unknown reason may be insufficient to induce tumorigenicity in vivo.

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 [96,98]. 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 downregulation of the FGF receptor. Most importantly, the spbFGF-transformed cells are highly tumorigenic and metastatic. It has been suggested 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.

The molecular mechanism by which a signal peptide-bearing bFGF leads to transformation is yet to be determined. It is possible that a structural, posttranslational modification of FGF, being processed through the endoplasmic reticulum and golgi apparatus, may lead to an atypical interaction with the FGF receptor. The localization of bFGF-receptor interaction might play an important role in autocrine transformation. While native bFGF can interact with the FGF receptor only at the cell surface, spbFGF 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.

In summary, the four FGF oncogenes and their respective proteins are

the most likely to stimulate tumor growth. The reason for their oncogenic potential could be that, unlike aFGF and bFGF, these four oncogenes have naturally occurring signal sequences and encode for secreted proteins that appear to be involved in the autocrine transformation of cells possessing FGF receptors. In this regard, the distribution of *int*-2, *hst*/K-*fgf*, and FGF-5 is quite different than that of aFGF or bFGF. The oncogenes are rarely found in normal adult tissue. Rather, they appear to be mostly expressed during embryogenesis and in tumors. It is possible that FGF-related oncogenes are the forms of FGF preferentially expressed during periods of intense proliferation.

The FGF family and tumor vascularization

Proliferation of blood vessels is necessary for the normal growth and development of tissue. In the adult, angiogenesis occurs infrequently. Exceptions are found in the female reproductive system, where angiogenesis occurs in the follicle during its development, in the corpus luteum during ovulation, and in the placenta during pregnancy. These periods of angiogenesis are relatively brief and tightly regulated. Normal angiogenesis also occurs as part of the body's repair processes; for example, in the healing of wounds and fractures. By contrast, uncontrolled angiogenesis is usually pathological. For example, the ability of tumors to stimulate angiogenesis or new capillary blood vessel growth allows them to grow in an exponential manner [1,5,99–109]. The corollary of this principle is that without the ability of tumors to stimulate new blood vessels, the tumors will remain in a small, avascular state. Vascularization of a tumor also enhances metastatic potential. It has been recently demonstrated that in breast cancer patients their is a strong correlation between the number and density of microvessels in the primary tumor and the incidence of breast cancer metastases [110].

A number of growth factors have been shown to be angiogenic, including aFGF, bFGF, angiogenein, platelet-derived endothelial cell growth factor, vascular endothelial growth factor, tumor necrosis factor, and transformig growth factors-α and -β [1,4,7,111–114]. Acidic FGF and bFGF are the best characterized of all the angiogenesis factors. These FGFs stimulate angiogenesis in the classical bioassays, such as the normally avascular cornea and the chick chorioallantoic membrane [115, 116]. Acidic FGF and bFGF modulate endothelial cell activity in vitro in a manner consistent with being stimulators of angiogenesis in vivo. For example, in culture FGF stimulates endothelial cell chemotaxis [28] and proliferation [29] for endothelial cells. Endothelial cells themselves secrete substantial amounts of bFGF [29,95], most of which is associated with the subendothelial cell extracellular matrix [23–25,117–120]. FGF found in the endothelial cell extracellular matrix is an essential component required for blood vessel growth. It has been suggested that capillary growth is regulated locally by bFGF stored in

capillary basement membrane that is released to stimulate capillary endothelial cells in an autocrine manner [25,117]. Basic FGF has been shown to support tumor growth by stimulating blood vessel growth [121–123]. For example, colon carcinoma can be stimulated by the application of bFGF in vivo [121]. Since FGF receptors do not appear on the colon cancer cells themselves but are found on the endothelial cells in the tumor, it appears that colon carcinoma growth is mediated by bFGF-induced neovascularization. Neutralizing FGF antibodies significantly reduce the tumor volume, further suggesting an endothelial cell-stimulating paracrine role for FGF in the growth of some tumors [121,122]. Furthermore, when fibroblasts were transfected with a gene posessing a signal sequence fused to bFGF, large tumors grew in nude mice [123]. Antibodies to bFGF administered systemically resulted in approximately a 75% decrease in the size of these tumors [123].

An important question remains: How can bFGF, which is not normally secreted, become a paracrine vascularization factor? Several possible mechanisms of FGF release by tumors have been postulated, including tumor necrosis, tumor cell leakiness, acquisition of signal peptide, and induction of multidrug resistant genes that encode for proteins that are involved in FGF export. A possible mechanism involving differential bFGF export by normal and tumor cells has been reported [124]. In these studies, transgenic mice carrying the bovine papilloma virus genome (BPV-1) at first produced benign avascular dermal fibromatoses. Eventually, there was a transition from the avascular tumors to the formation of highly vascular malignant fibrosarcomas. The *switch* from avascular to vascular tumors was accompanied by a change in bFGF release profiles. Basic FGF was expressed in both normal dermal fibroblasts and in benign fibromatoses but was cell associated, a typical property of bFGF that has no signal peptide for secretion. In contrast, the fibrosarcoma cells had very little cell-associated bFGF and a substantial amount of exported bFGF-like activity, which was neutralized by anti-bFGF antibodies. Since bFGF is angiogenic, it may be that its export by fibrosarcoma cells results in a paracrine stimulation of blood vessel growth in the tumors. Tumor angiogenesis might not occur in the avascular fibromatoses because bFGF is not released by these tumor cells. The mechanism by which the fibrosarcoma cells export bFGF is not understood. The cells might have special pathways for exporting proteins, or alternatively, the bFGF in these cells might be altered structurally and exported. The precise nature of this mechanism is not understood to date.

Conclusions and future directions

Members of the FGF family are important modulators of tumor growth. The four FGF oncogenes — *int*-2, *hst*/K-*fgf*, FGF-5, and FGF-6 — are the most likely candidates to be involved in stimulating autocrine tumor growth and

tumor neovascularization because they are secreted. The role of aFGF and bFGF in tumor growth is less clear. Since they are not secreted proteins, it is not clearly understood how they could be involved in cell transformation. Recent studies have suggested some possible mechanisms that allow aFGF and bFGF to be involved in tumor growth. For example, internal autocrine loops may occur in which aFGF and bFGF interact with their receptors within cells. Another possible mechanism is the alteration of FGF structure or of the tumor cell that allows specific FGF export, resulting in the stimulation of tumor angiogenesis.

Given the possibility that FGF is involved in tumor growth, anti-bFGF therapy might have therapeutic value. Several strategies have been attempted. These include the following: (1) the use of neutralizing antibodies that would inhibit exported members of the FGF family [123], (2) the use of antisense oligonucleotides that inhibit FGF synthesis. Basic FGF antisense has been used to inhibit melanoma growth [125]. (3) The use of drugs that inhibit the interaction of FGF with its receptor. Suramin has been shown to inhibit FGF-FGF receptor interactions and to revert the phenotype of tumors dependent on FGF production [126,127]. (4) The use of reagents that degrade cell surface heparan sulfate proteoglycans (HSPG). It has been shown that bFGF needs to bind to cell surface HSPG in order to be mitogenic [9,26,128]. Inactivating cell surface HSPG with heparinase or with specific peptides that bind to HSPG might be a way to block the mitogenic activity of FGF family members in a tumor. (5) The use of angiogenesis inhibitors. These compounds include a synthetic laminin peptide [129], AGM 1470 [130–133]), minocycline [134], thrombospondin [135,136], cartilage-derived inhibitor [137], penicillamine [138,139], platelet-factor 4 [140], and modulators of collagen metabolism [141]. Angiostatic steroids with or without heparin [142,143] or with betacyclodextrin-tetradecasulfate [144] have also been found to be potent angiogenesis inhibitors. The extent to which these angiogenesis inhibitors interfere with FGF expression remains to be elucidated.

The presence of FGF family members in tissues and biological fluids [145–147] may have diagnostic value. Methods that could be used to detect these growth factors include immunocytochemistry of tumor tissue, in situ hybridization to detect FGF family transcripts, and ELIZA analysis [148,149].

There is a great deal still to be learned about the FGF family, including questions of structure, localization, biosynthesis, regulatory mechanisms, and involvement in angiogenesis. More detailed information regarding these FGF properties might continue to provide information that can be translated into further advances in the diagnosis and therapy of benign and malignant diseases.

Acknowledgments

We are very grateful to Dr. Judah Folkman for many fruitful and insightful discussions and for his continued support. We thank Ms. Wendy Evanko and Ms. Janet Fox for preparation of this manuscript. We also thank Dr. Alexander Soutter and Dr. Rosalind Rosenthal for review of this manuscript. This work was supported by USPHS CA 37392 (MK). Support by the Association for Academic Surgery Research Award and The Leon Hirsch Fellowship to Harold Brem are also appreciated.

References

1. Folkman J, Klagsbrun M: Angiogenic factors. Science 235:442–447, 1987.
2. Rifkin DB, Moscatelli D: Recent developments in the cell biology of basic fibroblast growth factor. J Cell Biol 109:1–6, 1989.
3. D'Amore PA: Modes of FGF release *in vivo* and *in vitro*. Cancer Met Rev 9:227–238, 1990.
4. Klagsbrun M, Folkman J: Angiogenesis. In: Sporn MB, Roberts AB (eds): Handbook of Experimental Pharmacology, Peptide Growth Factors and their Receptors, Part II, Vol. 95. Springer-Verlag, Berlin, 1990, pp 549–586.
5. Folkman J: The angiogenic activity of FGF and its possible clinical applications. In: Sara VR, Hall K, Low H (eds): Karolinska Institute Nobel Conference Series on Growth Factors, Growth Factors: From Genes to Clinical Application. Karolinska Institute Nobel Conference Series. Raven Press, New York, 1990, pp 201–216.
6. Klagsbrun M: The fibroblast growth factor family: Structural and biological properties. Progr Growth Factor Res 1:207–235, 1989.
7. Klagsbrun M, D'Amore PA: Regulators of angiogenesis. Annu Rev Physiol 53:217–239, 1991.
8. Klagsbrun M: The affinity of fibroblast growth factors (FGF's) for heparin; FGF-heparan sulfate interactions in cells and extracellular matrix. Curr Opin Cell Biol 2:857–863, 1990.
9. Klagsbrun M, Baird A: A dual receptor system is required for basic fibroblast growth factor activity. Cell 67:229–231, 1991.
10. Shing Y, Folkman J, Sullivan R, Butterfield C, Murray J, Klagsbrun M: Heparin affinity: Purification of a tumor-derived capillary endothelial cell growth factor. Science 223:1296–1299, 1984.
11. Esch F, Baird A, Ling N, Ueno N, Hill F, Denoroy L, Klepper R, Gospodarowicz D, Bohlen P, Guillemin R: Primary structure of bovine pituitary basic fibroblast growth factor (FGF) and comparison with the amino-terminal sequence of bovine brain acidic FGF. Proc Natl Acad Sci USA 82:6507–6511, 1985.
12. Jaye M, Howk R, Burgess W, Ricca GA, Chiu I-M, Ravera MW, O'Brien SJ, Modi WS, Maciag T, Drohan WN: Human endothelial cell growth factor: Cloning, nucleotide sequence, and chromosome localization. Science 233:541–545, 1986.
13. Abraham JA, Mergia A, Whang JL, Tumolo A, Friedman J, Hjerrild KA, Gospodarowicz D, Fiddes JC: Nucleotide sequence of a bovine clone encoding the angiogenic protein, basic fibroblast growth factor. Science 233:545, 1986.
14. Thomas KA, Rios-Candelore M, Fitzpatrick S: Purification and characterization of acidic fibroblast growth factor from bovine brain. Proc Natl Acad Sci USA 81:357–361, 1984.
15. Burgess W, Maciag T: The heparin-binding (fibroblast) growth factor family of proteins. Ann Rev Biochem 58:575–606, 1989.

16. Gospodarowicz D: Fibroblast growth factor. In: Pimental E, Perucho M (eds): Critical Reviews in Oncogenesis. CRC Press: Boca Raton, Fl, 1989, pp 1–26.
17. Moscatelli D, Joseph-Silverstein J, Manejias R, Rifkin DB: M_r 25,000 heparin-binding protein from guinea pig brain is a high molecular weight form of basic fibroblast growth factor. Proc Natl Acad Sci USA 84:5778–5782, 1987.
18. Prats H, Kaghad M, Prats AC, Klagsbrun M, Lelias JM, Liauzun P, Chalon P, Tauber JP, Amalric F, Smith JA, Caput D: High molecular mass forms of basic fibroblast growth factor are initiated by alternative CUG codons. Proc Natl Acad Sci USA 86:1836–1840, 1989.
19. Florkiewicz RZ, Sommer A: Human basic fibroblast growth factor gene encodes four polypeptides: Three initiate translation from non-AUG codons. Proc Natl Acad Sci USA 86:3978–3981, 1989.
20. Klagsbrun M, Shing Y: Heparin affinity of anionic and cationic capillary endothelial cell growth factors: Analysis of hypothalamus-derived growth factors and fibroblast growth factors. Proc Natl Acad Sci USA 82:805–809, 1985.
21. Gospodarowicz D, Cheng J: Heparin protects basic and acidic FGF from inactivation. J Cell Physiol 128:475–484, 1986.
22. Sommer A, Rifkin DB: 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.
23. 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 heparitinase and heparin-like molecules. Biochemistry 28:1737–1743, 1989.
24. Vlodavsky I, Folkman J, Sullivan R, Fridman 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.
25. Folkman J, Klagsbrun M, Sasse J, Wadzinski M, Ingber D, Vlodavsky I: A heparin-binding and angiogenic protein — basic fibroblast growth factor — is stored within basement membrane. Am J Pathol 130:393–400, 1988.
26. Yayon A, Klagsbrun M, Esko JD, Leder P, Ornitz DM: Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. Cell 64:841–848, 1991.
27. Shing Y, Folkman J, Weisz PB, Joullie MM, Ewing WR: Affinity of fibroblast growth factors for beta-cyclodextrin tetradecasulfate. Anal Biochem 185:108–111, 1990.
28. Stokes CL, Rupnick MA, Williams SK, Lauffenburger DA: Chemotaxis of human microvessel endothelial cells in response to acidic fibroblast growth factor. Lab Invest 63:657–668, 1990.
29. Schweigerer L, Neufeld G, Friedman J, Abraham JA, Fiddes JC, Gospodarowicz D: Capillary endothelial cells express basic fibroblast growth factor, a mitogen that promotes their own growth. Nature 325:257–259, 1987.
30. Gross JL, Moscatelli D, Rifkin DB: Increased capillary endothelial cell protease activity in response to angiogenic stimuli *in vitro*. Proc Natl Acad Sci USA 80:2623–2627, 1983.
31. Mignatti P, Tsuboi R, Robbins E, Rifkin DB: *In vitro* angiogenesis on the human amniotic membrane: Requirement for basic fibroblast growth factor-induced proteinases. J Cell Biol 108:671–682, 1989.
32. Presta M, Maier JA, Rusnati M, Moscatelli D, Ragnotti G: Modulation of plasminogen activator activity in human endometrial adenocarcinoma cells by basic fibroblast growth factor and transforming growth factor beta. Cancer Res 48:6383–6389, 1988.
33. Montesano R, Vassalli J-D, Baird A, Guillemin R, Orci L: Basic fibroblast growth factor induces angiogenesis in vitro. Proc Natl Acad Sci USA 83:7297–7301, 1986.
34. Gospodarowicz D: Localization of a fibroblast growth factor and its effect alone and with hydrocortisone on 3T3 cell growth. Nature 249:123–129, 1974.
35. Weich HA, Iberg N, Klagsbrun M, Folkman J: Expression of acidic and basic fibroblast

growth factors in human and bovine vascular smooth muscle cells. Growth Factors 2:313–320, 1990.
36. Gospodarowicz D, Ill CR, Birdwell CR: Effects of fibroblast and epidermal growth factors on ovarian cell proliferation *in vitro*. I. Characterization of the response of granulosa cells to FGF and EGF. Endocrinology 100:1108–1120, 1977.
37. Rodan SB, Wesolowski G, Thomas K, Rodan GA: Growth stimulation of rat calvaria osteoblastic cells by acidic fibroblast growth factor. Endocrinology 121:1917–1923, 1987.
38. Gospodarowicz D, Plouet J, Fujii DK: Ovarian germinal epithelial cells respond to basic fibroblast growth factor and express its gene: Implications for early folliculogenesis. Int J Dev Neuro 7:401–409, 1989. Endocrinology 125:1266–1276, 1989.
39. Besnard F, Perraud F, Sensenbrenner M, Labourdette G: Effects of acidic and basic fibroblast growth factors on proliferation and maturation of cultured rat oligodendrocytes.
40. Shipley GD, Keeble WW, Hendrickson JE, Coffey RJ Jr., Pittelkow MR: Growth of normal human keratinocytes and fibroblasts in serum-free medium is stimulated by acidic and basic fibroblast growth factor. J Cell Physiol 138:511–518, 1989.
41. Davidson JM, Klagsbrun M, Hill KE, Buckley A, Sullivan R, Brewer PS, Woodward SC: Accelerated wound repair, cell proliferation, and collagen accumulation are produced by a cartilage-derived growth factor. J Cell Biol 100:1219–1227, 1985.
42. McGee GS, Davidson JM, Buckley A, Sommer A, Woodward SC, Aquino AM, Barbour R, Demetriou AA: Recombinant basic fibroblast growth factor accelerates wound healing. J Surg Res 45:145–153, 1988.
43. Hebda PA, Klingbeil CK, Abraham JA, Fiddes JC: Basic fibroblast growth factor stimulation of epidermal wound healing in pigs. J Invest Dermatol 95:626–631, 1990.
44. Broadley KN, Aquino AM, Woodward SC, Buckley-Sturrock A, Sato Y, Rifkin DB, Davidson JM: Monospecific antibodies implicate basic fibroblast growth factor in normal wound repair. Lab Invest 61:571–575, 1989.
45. Folkman J, Szabo S, Stovroff M, McNeil P, Li W, Shing Y: Duodenal ulcer: Discovery of a new mechanism and development of angiogenic therapy that accelerates healing. Ann Surg 214:414–427, 1991.
46. Thompson JA, Haudenschild C, Anderson KD, DiPietro JM, Anderson WF, Maciag T: Heparin-binding growth factor 1 induces the formation of organoid neovascular structures *in vivo*. Proc Natl Acad Sci USA 86:7928–7923, 1989.
47. Klingbeil CK, Cesar LB, Fiddes JC: Basic fibroblast growth factor accelerates tissue repair in models of impaired wound healing. In Barbul A, Caldwell MD, Eaglstein WH, Hunt TK, Marshall D, Pines E, Skover G (eds): Clinical and Experimental Approaches to Dermal and Epidermal Repair: Normal and Chronic Wounds. Wiley-Liss, New York, 1991, pp 443–458.
48. Greehalgh DG, Sprugel KH, Murray MJ, Ross R: PDGF and FGF stimulate wound healing in the genetically diabetic mouse. Am J Pathol 136:1235–1246, 1990.
49. Steinberg BD, Phillips LG, Hokanson JA, et al.: Effect of bFGF on the inhibition of contraction caused by bacteria. J Surg Res 50:47–50, 1991.
50. Abe K, Takayanagi M, Saito H: Effects of recombinant human basic fibroblast growth factor and its modified protein CS23 on survival of primary cultured neurons from various regions of fetal rat brain. Jpn J Pharmacol 53:221–227, 1990.
51. Otto D, Frotscher M, Unsicker K: Basic fibroblast growth factor and nerve growth factor administered in gel foam rescue medial septal neurons after fimbria fornix transection. J Neurosci Res 22:83–91, 1989.
52. Peters G, Brookes S, Smith R, Placzek M, Dickson C: The mouse homolog of the hst/k-FGF gene is adjacent to int-2 and is activated by proviral insertion in some virally induced mammary tumors. Proc Natl Acad Sci USA 86:5678–5682, 1989.
53. 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.
54. Dickson C, Acland P, Smith R, Dixon M, Deed R, MacAllan D, Walther W, Fuller-Pace

F, Kiefer P, Peters G: Characterization of *int*-2: A member of the fibroblast growth factor family. J Cell Sci 13(Suppl.):87–96, 1990.
55. Muller WJ, Lee FS, Dickson C, Peters G, Pattengale P, Leder P: The *int*-2 gene product acts as an epithelial growth factor in transgenic mice. EMBO J 9:907–913, 1990.
56. 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.
57. Wilkinson DG, Peters G, Dickson C, McMahon AP: Expression of the FGF-related proto-oncogene *int*-2 during gastrulation and neurulation in the mouse. EMBO J 7:691–695, 1986.
58. Wilkinson DG, Bhatt S, McMahon AP: Expression pattern of the FGF-related proto-oncogene *int*-2 suggests multiple roles in fetal development. Development 105:131–136, 1989.
59. Paterno GD, Gillespie LL, Dixon MS, Slack JM, Heath JK: Mesoderm-inducing properties of INT-2 and kFGF: Two oncogene-encoded growth factors related to FGF. Development 106:79–83, 1989.
60. Dickson C, Peters G: Potential oncogene product related to growth factors. Nature 326:833, 1987.
61. Dixon M, Deed R, Acland P, Moore R, Whyte A, Peters G, Dickson C: Detection and characterization of the fibroblast growth factor-related oncoprotein INT-2. Mol Cell Biol 9:4896–4902, 1989.
62. Ornitz DM, Moreadith RW, Leder P: Binary system for regulating transgene expression in mice: Targeting *int*-2 gene expression with yeast *GAL4/UAS* control elements. Proc Natl Acad Sci USA 88:698–702, 1991.
63. Tsuda H, Hirohashi S, Shimosato Y, Hirota T, Tsugane S, Yamamoto H, Miyajima N, Toyoshima K, Yamamoto T, Yokota J, Yoshida T, Sakamoto H, Terada M, Sugimura T: Correlation between long-term survival in breast cancer patients and amplification of two putative oncogene-coamplification units: *hst*-1/*int*-2 and c-*erb*B-2/*ear*-1. Cancer Res 49:3104–3108, 1989.
64. Meyers SL, O'Brien MT, Smith T, Dudley JP: Analysis of the *int*-1, *int*-2, c-*myc*, and *neu* oncogenes in human breast carcinomas. Cancer Res 50:5911–5918, 1990.
65. Machotka SV, Garrett CT, Schwartz AM, Callahan R: Amplification of the proto-oncogenes *int*-2, c-*erb* B-2 and c-*myc* in human breast cancer. Clin Chim Acta 184:207–218, 1989.
66. Zhou DJ, Casey G, Cline MJ: Amplification of human *int*-2 in breast cancers and squamous carcinomas. Oncogene 2:279–282, 1988.
67. Merritt WD, Weissler MC, Turk BF, Gilmer TM: Oncogene amplification in squamous cell carcinoma of the head and neck. Arch Otolaryngol Head Neck Surg 116:1394–1398, 1990.
68. Kitagawa Y, Ueda M, Ando N, Shinozawa Y, Shimizu N, Abe O: Significance of *int*-2/*hst*-1 coamplification as a prognostic factor in patients with esophageal squamous carcinoma. Cancer Res 51:1504–1508, 1991.
69. Somers KD, Cartwright SL, Schechter GL: Amplification of the *int*-2 gene in human head and neck squamous cell carcinomas. Oncogene 5:915–920, 1990.
70. Adelaide J, Mattei M-G, Marics I, Raybaud F, Planche J, De Lapeyriere 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.
71. Ranzani GN, Pellegata NS, Previdere C, Saragoni A, Vio A, Maltoni M, Amadori D: Heterogeneous protooncogene amplification correlates with tumor progression and presence of metastases in gastric cancer patients. Cancer Res 50:7811–7814, 1990.
72. Tahara E: Growth factors and oncogenes in human gastrointestinal carcinomas. J Cancer Res Clin Oncol 116:121–131, 1990.
73. Hiyama E, Hiyama K, Yokoyama T, Ishii T: Immunohistochemical analysis of N-myc protein expression in neuroblastoma: Correlation with prognosis of patients. J Pediat Surg 26:838–843, 1991.

74. Delli-Bovi P, Curatola AM, Kern FG, Greco A, Ittman 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.
75. 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.
76. Yoshida T, Miyagawa K, Odagiri H, Sakamoto H, Little PFR, 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.
77. Yoshida MC, Wada M, Satoh H, Yoshida T, Sakamoto H, Miyagawa K, Yokota J, Koda T, Kakinuma M, Sugimura T, Terada M: Human *HST1* (*HSTF1*) gene maps to chromosome band 11q13 and coamplifies with the *INT2* gene in human cancer. Proc Natl Acad Sci USA 85:4861–4864, 1988.
78. Koda T, Sasaki A, Matsushima S, Kakinuma M: A transforming gene, *hst*, found in NIH 3T3 cells transformed with DNA from three stomach cancers and a colon cancer. Jpn J Cancer Res 78:325–328, 1987.
79. Delli-Bovi P, Mansukhani A, Ziff EB, Basilico C: Expression of the K-fgf protooncogene is repressed during differentiation of F9 cells. Oncogene Res 5:31–37, 1989.
80. Strohmeyer T, Peter S, Hartmann M, Munemitsu S, Ackermann R, Ullrich A, Slamon DJ: Expression of the *hst*-1 and c-*kit* protooncogenes in human testicular germ cell tumors. Cancer Res 51:1811–1816, 1991.
81. Tsuda T, Nakatani H, Matsumura T, Yoshida K, Tahara E, Nishihira T, Sakamoto H, Yoshida T, Terada M, Sugimura T: Amplification of the *hst*-1 gene in human esophageal carcinomas. Jpn J Cancer Res 79:584–588, 1988.
82. 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.
83. Theillet C, Le Roy X, De Lapeyriere O, Grosgeorges J, Adnane J, Raynaud SD, Simony-Lafontaine J, Goldfarb M, Escot C, Birnbaum D, Gaudray P: Amplification of FGF-related genes in human tumors: Possible involvement of *HST* in breast carcinomas. Oncogene 4:915–922, 1989.
84. Ensoli B, Nakamura S, Salahuddin SZ, Biberfeld P, Larsson L, Beaver B, Wong-Staal F, Gallo RC: AIDS-Kaposi's sarcoma-derived cells express cytokines with autocrine and paracrine growth effects. Science 243:223–226, 1989.
85. Basilico C, Newman KM, Curatola AM, Talarico D, Mansukhani A, Velcich A, Delli-Bovi P: Expression and activation of the K-*fgf* oncogene. Ann NY Acad Sci 567:95–103, 1989.
86. Zhan X, Culpepper A, Reddy M, Loveless J, Goldfarb M: Human oncogenes detected by a defined medium culture assay. Oncogene 1:369–376, 1987.
87. 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.
88. Dionne CA, Kaplan R, Seuranez H, O'Brien SJ, Jaye M: Chromosome assignment by polymerase chain reaction techniques: Assignment of the oncogene FGF-5 to human chromosome 4. Biotechniques 8:190–194, 1990.
89. Bates B, Hardin J, Zhan X, Drickamer K, Goldfarb M: Biosynthesis of human fibroblast growth factor-5. Mol Cell Biol 11:1840–1845, 1991.
90. Hébert JM, Basilico C, Goldfarb M, Haub O, Martin GR: Isolation of cDNAs encoding four mouse FGF family members and characterization of their expression during embryogenesis. Dev Biol 138:454–463, 1990.
91. Haub O, Drucker B, Goldfarb M: Expression of the murine fibroblast growth factor 5 gene in the adult central nervous system. Proc Natl Acad Sci USA 87:8022–8026, 1990.
92. Marics I, Adelaide J, Raybaud F, Mattei M-G, Coulier F, Planche J, de Lapeyreire O,

Birnbaum D: Characterization of the HST-related FGF-6 gene, a new member of the fibroblast growth gene family. Oncogene 4:335–340.
93. de Lapeyriere O, Rosnet O, Benharroch, Raybaud F, Marchetto S, Planche J, Galland F, Mattei M-G, Copeland NG, Jenkins NA, Coulier F, Birnbaum D: Structure, chromosome mapping and expression of the murine FGF-6 gene. Oncogene 5:823–831, 1990.
94. Finch PW, Rubin JS, Miki T, Ron D, Aaronson SA: Human KGF is FGF-related with properties of a paracrine effector of epithelial cell growth. Science 245:752–755, 1989.
95. Vlodavsky I, Fridman R, Sullivan R, Sasse J, Klagsbrun M: Aortic endothelial cells synthesize basic fibroblast growth factor which remains cell associated and platelet-derived growth factor-like protein which is secreted. J Cell Physiol 131:402–408, 1987.
96. Rogelj S, Weinberg RA, Fanning P, Klagsbrun M: Basic fibroblast growth factor fused to a signal peptide transforms cells. Nature 331:173–175, 1988.
97. Blam SB, Tischer E, Abraham JA, Aaronson S: Expression of acidic fibroblast growth factor in NIH/3T3 cells with and without the addition of a secretion signal sequence. J Cell Biochem (Suppl. 13b):152, 1989.
98. 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, New York, 1989, pp 19–27.
99. D'Amore PA, Klagsbrun M: Angiogenesis: Factors and mechanisms. In: Sirica AE (ed): The Pathobiology of Neoplasia. Plenum, New York, 1989, pp 513–531.
100. Folkman J: What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst 82:4–6, 1990.
101. Folkman J: Successful treatment of an angiogenic disease. N Engl J Med 320:1211–1212, 1989.
102. Folkman J, Waston K, Ingber D, Hanahan D: Induction of angiogenesis during the transition from hyperplasia to enoplasia. Nature 339:58–61, 1989.
103. Paku S, Paweletz N: First steps of tumor-related angiogenesis. Lab Invest 65:334–346, 1991.
104. Brem H, Guerin C, Tamargo R, Brem S, Brem H: Brain tumor angiogenesis. In: Kornblith PL (ed): Advances in Neuro-oncology. Futura, Mount Kisco, NY, 1988, pp 89–101.
105. Blood CH, Zetter BR: Tumor interactions with the vasculature: Angiogenesis and tumor metastasis. Biochem Biophys Res Acta 1032:89–118, 1990.
106. Zetter BR: Angiogenesis: State of the art. Chest 93:159S–166S, 1988.
107. Moses MA, Langer R: A metalloproteinase inhibitor of neovascularization. J Cell Biochem 47:1–6, 1991.
108. Zagzag D, Brem S, Robert F. Neovascularization and tumor growth in the brain: A model for experimental studies of angiogenesis and the blood-brain barrier. Am J Pathol 131:361–372, 1988.
109. Bouck N: Tumor angiogenesis: The role of oncogenes and tumor suppressor genes. Cancer Cells 2:179–184, 1990.
110. Weidner N, Semple JP, Welch WR, Folkman J: Tumor angiogenesis and metastasis — correlation in invasive breast carcinoma. N Engl J Med 324:1–8, 1991.
111. Levy AP, Tamargo R, Brem H, Nathans D: An endothelial cell growth factor from the mouse neuroblastoma cell line NB41. Growth Factors 2:9–19, 1989.
112. Conn SC, Kamen R: Amino acid and cDNA sequences of a vascular endothelial cell mitogen that is homologous to platelet-derived growth factor. Proc Natl Acad Sci USA 87:2628–2632, 1990.
113. Connolly DT, Heuvelman DM, Nelson R, Olander JV, Eppley BL, et al.: Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. J Clin Invest 84:1478–1489, 1989.
114. Rosenthal RA, Megyesi JF, Henzel WJ, Ferrara N, Folkman J: Conditioned medium from mouse sarcoma 180 cells contains vascular endothelial growth factor. Growth Factors 4:53–59, 1990.

115. Shing Y, Folkman J, Haudenschild C, Lund D, Crum R, Klagsbrun M: Angiogenesis is stimulated by a tumor-derived endothelial cell growth factor. J Cell Biochem 29:275–287, 1985.
116. Lobb RR, Alderman EM, Fett JW: Induction of angiogenesis by bovine brain derived class I heparin-binding growth factor. Biochemistry 24:4969–4973, 1985.
117. Klagsbrun M, Vlodavsky I: Biosynthesis and storage of basic fibroblast growth factor (BFGF) by endothelial cells: Implication for the mechanism of action of angiogenesis. In: Barbul A, Pines E, Caldwell M, Hunt TK (eds): Progress in Clinical and Biological Research; Growth Factors and Other Aspects of Wound Healing.
118. Ishai-Michaeli R, Eldor A, Vlodavsky I: Heparanase activity expressed by platelets, neutrophils, and lymphoma cells releases active fibroblast growth factor from extracellular matrix. Cel Regul 1:833–842, 1990.
119. Voldavsky I, Fuks Z, Ishai-Michaeli R, Bashkin P, Levi E, Korner G, Bar-Shavit, Klagsbrun M: Extracellular matrix-resident basic fibroblast growth factor: Implication for the control of angiogenesis. J Cell Biochem 45:167–176, 1991.
120. Presta M, Maier JAM, Rusnati M, Ragnotti G: Basic fibroblast growth factor is released from endothelial extracellular matrix in a biologically active form. J Cell Physiol 140:68–74, 1989.
121. Gross JL, Herblin WF, Dusak BA, Czerniak P, Diamond M, Dexter DL: Modulation of solid tumor growth in vivo by bFGF. Proc Am Assoc Cancer Res, 31:79, 1990.
122. Tanaka A, Matsumoto K, Nishizawa Y, Lu J, Yamanishi H, Maeyama M, Nonomura N, Uchida N, Sato B: Growth stimulation by androgens, glucocorticoids or fibroblast growth factors and the blocking of the stimulated growth by antibody against basic fibroblast growth factor in protein-free culture of shionogi carcinoma 115 cells. J Steroid Biochem Mol Biol 37:23–29, 1990.
123. Hori A, Sasada R, Matsutani E, Naito K, Sakura Y, Fujita T, Kozai Y: Suppression of solid tumor growth by immunoneutralizing monoclonal antibody against human basic fibroblast growth factor. Cancer Res 51:6180–6184, 1991.
124. Kandel J, Bossy-Wetzel E, Radvanyi F, Klagsbrun M, Folkman J, Hanahan D: Neovascularization is associated with a switch to the export of bFGF in the multistep development of fibrosarcoma. Cell 66:1095–1104, 1991.
125. Becker D, Meier CB, Herlyn M: Proliferation of human malignant melanomas is inhibited by antisense oligodeoxynucleotides targeted against basic fibroblast growth factor. EMBO J 8:3685–3691, 1989.
126. Yayon A, Klagsbrun M: Autocrine transformation by chimeric signal peptide-basic fibroblast growth factor: Reversal by suramin. Proc Natl Acad Sci USA 87:5346–5350, 1990.
127. Moscatelli D, Quarto N: Transformation of NIH 3T3 cells with basic fibroblast growth factor or the hst/K-*fgf* oncogene causes downregulation of the fibroblast growth factor receptor: Reversal of morphological transformation and restoration of receptor number by suramin. J Cell Biol 109:2519–2527, 1989.
128. Rapraeger AC, Krufka A, Olwin BB: Requirement of heparan sulfate for bFGF mediated fibroblast growth and myoblast differentiation. Science 252:1705–1708, 1991.
129. Sakamoto N, Iwahana M, Tanaka NG, Osada Y: Inhibition of angiogenesis and tumor growth by a synthetic laminin peptide, CDPGYIGSR-NH_2. Cancer Res 51:903–906, 1991.
130. Ingber D, Fujita T, Koshitmoto S, Sudo K, Kanamaru T, Brem H, Folkman J: Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumor growth. Nature 348:555–557, 1990.
131. Kusaka M, Sudo K, Fujita T, Marui S, Itoh F, Ingber D, Folkman J: Potent anti-angiogenic action of AGM-1470: Comparison to the fumagillin parent. Biochem Biophys Res Commun 174:1070–1076, 1991.
132. Brem H, Ingber D, Blood C, Bradley BS, Urioste S, Folkman J: Suppression of tumor metastasis by angiogenesis inhibition. Surg Forum 17:439–441, 1991.
133. Brem H, Tsakayannis D, Folkman J: Time dependent suppression of wound healing with

the angiogenesis inhibitor, AGM 1470. J Cell Biol 115:403, 1991.
134. Tamargo RJ, Bok RA, Brem H: Angiogenesis inhibition by minocycline. Cancer Res 51:672–675, 1991.
135. Bagavandoss P, Wilks JW: Specific inhibition of endothelial cell proliferation by thrombospondin. Biochem Biophys Res Comm 170:867–972, 1990.
136. Good DJ, Polverini PJ, Rastinejad F, Le Beau MM, Lemons RS, Frazier WA, Bouck NP: A tumor suppressor-dependent inhibitor of angiogenesis is immunologically and functionally indistinguishable from a fragment of thrombospondin. Proc Natl Acad Sci USA 87:6624–6628, 1990.
137. Moses M, Sudhalter J, Langer R: Identification of an inhibitor of neovascularization from cartilage. Science 248:1408–1410, 1990.
138. Matsubara T, Saura R, Hirohata K, Ziff M: Inhibition of human endothelial cell proliferation in vitro and neovascularization in vivo by D-penicillamine. J Clin Invest 83:158–167, 1989.
139. Brem SS, Zagzag D, Tsanaclis AMC, Gately S, Elkouby M-P, Brien SE: Inhibition of angiogenesis and tumor growth in the brain: Suppression of endothelial cell turnover by penicillamine and the depletion of copper, an angiogenic cofactor. Am J Pathol 137:1121–1142, 1990.
140. Maione TE, Gray GS, Hunt AJ, Sharpe RJ: Inhibition of tumor growth in mice by an analogue of platelet factor 4 that lacks affinity for heparin and retains potent angiostatic activity. Cancer Res 51:2077–2083, 1991.
141. Ingber D, Folkman J: Inhibition of angiogenesis through modulation of collagen metabolism. Lab Invest 59:44–51.
142. Crum R, Szabo S, Folkman J: A new class of steroids inhibits angiogenesis in the presence of heparin or a heparin fragment. Science 230:1375–1378, 1985.
143. Tamargo RJ, Leong KW, Brem H: Growth inhibition of the 9L glioma using polymers to release heparin and cortisone acetate. J Neurol Oncol 9:131–138, 1990.
144. Folkman J, Weisz PB, Joullie MM, Li WW, Ewing WR: Control of angiogenesis with synthetic heparin substitutes. Science 243:1490–1493, 1989.
145. Sano H, Forough R, Maier JAM, Case JP, Jackson A, Engleka K, Maciag T, Wilder RL: Detection of high levels of heparin binding growth factor-1 (acidic fibroblast growth factor) in inflammatory arthritic joints. J Cell Biol 110:1417–1426, 1990.
146. Sivalingam A, Kenney J, Brown GC, Benson WE, Donoso L: Basic fibroblast growth factor levels in the vitreous of patients with proliferative diabetic retinopathy. Arch Ophthalmol 108:869–872, 1990.
147. Chodak GW, Hospelhorn V, Judge SM, et al.: Increased levels of fibroblast growth factor-like activity in urine from patients with bladder or kidney cancer. Cancer Res 48:2083–2088, 1988.
148. Watanabe H, Hori A, Seno M, Kozai Y, Igarashi K, Ichimori Y, Kondo K: A sensitive enzyme immunoassay for human basic fibroblast growth factor. Biochem Biophys Res Commun 175:229–235, 1991.
149. Fujimoto K, Ichimori Y, Kakizoe T, Okajima E, Sakamoto H, Sugimura T, Terada M: Increased serum levels of basic fibroblast growth factor in patients with renal cell carcinoma. Biochem Biophys Res Commun 180:386–392, 1991.
150. Murphy PR, Myal Y, Sato Y, Sato R, West M, Friesen HG: Elevated expression of basic fibroblast growth factor messenger ribonucleic acid in acoustic neuromas. Mol Endocrinol 3:225–231, 1989.
151. Silverlight JJ, Prysor-Jones RA, Jenkins JS: Basic fibroblast growth factor in human pituitary tumors. Clin Endocrinol 32:669–676, 1990.
152. Donovan-Peluso M, Contento AM, Tobon H, Ripepi B, Locker J: Oncogene amplification in breast cancer. Am J Pathol 138:835–845, 1991.
153. Roux-Dosseto M, Martin PM: A paradigm for oncogene complementation in human breast cancer. Res Virol 140:571–591, 1989.
154. Tang R, Kacinski B, Validire P, Beuvon F, Sastre X, Benoit P, dela Rochefordiere A,

Mosseri V, Pouillart P, Scholl S: Oncogene amplification correlates with dense lymphocyte infiltration in human breast cancers: A role for hematopoietic growth factor release by tumor cells? J Cell Biochem 44:189–198, 1990.
155. Theillet C, Adnane J, Szepetowski P, Simon M-P, Jeanteur P, Birnbaum D, Gaudray P: *BCL*-1 participates in the 11q13 amplification found in breast cancer. Oncogene 5:147–149, 1990.
156. Matsuzaki K, Yoshitake Y, Miyagiwa M, et al.: Production of basic fibroblast growth factor-like factor by cultured human cholangiocellular carcinoma cells. Jpn Cancer Res 81:345–354, 1990.
157. Tiesman J, Rizzino A: Expression and developmental regulation of the k-FGF oncogene in human and murine embryonal carcinoma cells. In Vitro Cell Dev Biol 25:1193–1198, 1989.
158. Wagata T, Ishizaki K, Imamura M, Shimada Y, Ikenaga M, Tobe T: Deletion of 17p and amplification of the *int*-2 gene in esophageal carcinomas. Cancer Res 51:2113–2117, 1991.
159. Tsuda T, Tahara E, Kajiyama G, Sakamoto H, Terada M, Sugimura T: High incidence of coamplification of *hst*-1 and *int*-2 genes in human esophageal carcinomas. Cancer Res 49:5505–5508, 1989.
160. Moscatelli D, Presta M, Joseph-Silverstein J, Rifkin DB: Both normal and tumor cells produce basic fibroblast growth factor. J Cell Physiol 129:273–276, 1986.
161. Presta M, Moscatelli D, Silverstein JJ, Rifkin DB: Purification from a human hepatoma cell line of a basic fibroblast growth factor-like molecule that stimulates capillary endothelial cell plasminogen activator production, DNA synthesis and migration. Molec Cell Biol 6:4060–4066, 1986.
162. Klagsbrun M, Sasse J, Sullivan R, Smith JA: Human tumor cells synthesize an endothelial cell growth factor that is structurally related to basic fibroblast growth factor. Proc Natl Acad Sci USA 83:2448–2452, 1986.
163. Miller WH Jr., Moy D, Li A, Grippo JF, Dmitrovsky E: Retinoic acid induces down-regulation of several growth factors and proto-oncogenes in a human embryonal cancer cell line. Oncogene 5:511–517, 1990.
164. Schulze-Osthoff K, Goerdt S, Sorg C: Expression of basic fibroblast growth factor (bFGF) in Kaposi's sarcoma: An immunohistologic study. J Invest Dermatol 95:238–240, 1990.
165. Huang SS, Tsai CC, Adams SP, Huang JS: Neuron localization and neuroblastoma cell expression of brain-derived growth factor. Biochem Biophys Res Commun 144:81–87, 1987.
166. Sasano H, Garrett CT, Wilkinson DS, Silverberg S, Comerford J, Hyde J: Protooncogene amplification and tumor ploidy in human ovarian neoplasms. Human Pathol 21:382–391, 1990.
167. Beauchamp RD, Lyons RM, Yang EY, Coffey RJ, Jr., Moses HL: Expression of and response to growth regulatory peptides by two human pancreatic carcinoma cell lines. Pancreas 5:369–380, 1990.
168. Nakamoto T, Usui A, Oshima K, Ikemoto H, Mitani S, Usui T: Analysis of growth factors in renal cell carcinoma. Biochem Biophys Res Commun 153:818–824, 1988.
169. Adnane J, Gaudray P, Dionne CA, Crumley G, Jaye M, Schlessinger J, Jeanteur P, Birnbaum D, Theillet C: *BEK* and *FLG*, two receptors to members of the FGF family, are amplified in subsets of human breast cancers. Oncogene 6:659–663, 1991.
170. Paulus W, Grothe C, Sensenbrenner M, Janet T, Baur I, Graf M, Roggendorf W: Localization of basic fibroblast growth factor, a mitogen and angiogenic factor, in human brain tumors. Acta Neuropathol 79:418–423, 1990.
171. Takahashi JA, Mori H, Fukumoto M, Igarashi K, Jaye M, Oda Y, Kikuchi H, Hatanaka M: Gene expression of fibroblast growth factors in human gliomas and meningiomas: Demonstration of cellular source of basic fibroblast growth factor mRNA and peptide in tumor tissues. Proc Natl Acad Sci USA 87:5710–5714, 1990.
172. Megyesi JF, Klagsbrun M, Folkman J, Rosenthal RA, Brigstock DR: 18.4 kDa basic

fibroblast growth factor (bFGF) alone is in the conditioned medium from human glioblastoma cells that synthesize four bFGF isoforms: Evidence for autocrine stimulation by bFGF. J Cell Biol 115:419a, 1991.
173. Morrison RS, Gross JL, Herblin WF, Reilly TM, LaSala PA, Alterman RL, Moskal JR, Kornblith PL, Dexter DL: Basic fibroblast growth factor-like activity and receptors are expressed in a human glioma cell line. Cancer Res 50:2524–2529, 1990.
174. Sato Y, Murphy PR, Sato R, Friesen HG: Fibroblast growth factor release by bovine endothelial cells and human astrocytoma cells in culture is density dependent. Mol Endocrinol 3:744–748, 1989.
175. Corin SJ, Chen LC, Hamburger AW: Enhancement of anchorage-independent growth of a human adrenal carcinoma cell line by endogenously produced basic fibroblast growth factor. Int J Cancer 46:516–521, 1990.
176. Brookes S, Smith R, Casey G, Dickson C, Peters G: Sequence organization of the human *int*-2 gene and its expression in teratocarcinoma cells. Oncogene 4:429–436, 1989.
177. Osthoff-Schulze K, Risau W, Vollmer E, Sorg C: *In situ* detection of basic fibroblast growth factor by highly specific antibodies. Am J Pathol 137:85–92, 1990.
178. Lobb RR, Rybak SM, St. Clair DK, Fett JW: Lysates of two established human tumor lines contain heparin-binding growth factors related to bovine acidic brain fibroblast growth factor. Biochem Biophys Res Commun 139:861–867, 1986.
179. Matsuura N, Seo M, Murata A, Ohmachi Y, Morikawa Y, Kakudo K: Immunohistochemical analysis of basic fibroblast growth factor in the human gastrointestinal cancer (abstracts). J Cell Biol 115:420, 1991.
180. Schweigerer L, Neufeld G, Mergia A, Abraham JA, Fiddes JC, Gospodarowicz D: Basic fibroblast growth factor in human rhabdomyosarcoma cells: Implications for the proliferation and neovascularization of myoblast-derived tumors. Proc Natl Acad Sci USA 84:842–846, 1987.
181. Xerri L, Hassoun J, Planche J, Guigou V, Grob J-J, Parc P, Birnbaum D, deLapeyriere O: Fibroblast growth factor gene expression in AIDS-Kaposi's sarcoma detected by *in situ* hybridization. Am J Pathol 138:9–15, 1991.